The XLIX

Report of The

BEAN IMPROVEMENT COOPERATIVE

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Coordinating Committee

Jim Beaver
Jim Kelly (President)
Phil Miklas
Soon Jai Park
Howard F. Schwartz (Ex officio)
Bert Vandenberg
Antonio de Ron
Ken Kmiecik
Jim Myers
Ron Riley
Ron Shellenberger

Please address correspondence about BIC membership and BIC annual reports to:

Dr. James D. Kelly, BIC President
Department of Crop & Soil Sciences
Michigan State University
East Lansing, MI 48824
U. S. A.
Tele: 517-355-0271 extension 1181 // FAX: 517-353-3955
Email: kellyj@msu.edu

http://www.css.msu.edu/bic

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BIC ONLINE

The National Agricultural Library (NAL) is working to provide on line access to Volumes 1-48 of the BIC Annual Reports, which date back to 1957 and to the 1973, 1977, 1979, and 1982 Conference Proceedings. When completed, the BIC collection will be hosted on NAL's Digital Repository Web site (http://naldr.nal.usda.gov/.). To further increase accessibility and visibility for the BIC publications, each article will be indexed in the AGRICOLA database. The AGRICOLA records will link to the full text article for easy access from the database. Digitizing and indexing the BIC publications is expected to be completed by August 2006. NAL will also investigate the requirements to create a routine process to index and archive future issues. Additional information regarding these activities will be forthcoming.

THE 49th ANNUAL REPORT OF THE BEAN IMPROVEMENT COOPERATIVE -BIC

The Bean Improvement Cooperative enjoyed a stimulating meeting at the 2005 Biennial Meeting in Newark, Delaware. The meeting had approximately 100 registered participants and featured 29 oral presentations and 37 poster presentations. The quality of both the oral and poster presentations was excellent. The focus of the National Dry Bean Symposium on genetics of bean nutritional quality was very topical given the current interest in subject and the international activities directed toward the biofortification of major food crops. The meeting began with the Frazier-Zaumeyer Distinguished Lecture, entitled: '*Plant Breeding and Genomics – Staying in Contact*.' The lecture was presented by Dr. Perry Cregan, Soybean Geneticist, Research Leader of the Soybean Genomics and Improvement Laboratory, USDA-ARS, Beltsville, MD. The meeting received excellent and generous support from the following organizations: Basin Seed; Harris Moran Seed Company; Seminis Vegetable Seeds; United States Dry Bean Council; University of Delaware Vegetable Crops Program. The strong support of these organizations allowed this meeting to succeed. On behalf of the BIC, I wish to acknowledge the very substantial assistance of the organizing committee, particularly Dr. Ed Kee and Ms. Emmalea Ernest and I wish to thank the sponsors and the participants for making the meeting a success.

Two student awards were presented for both oral and poster presentations at the BIC meeting.

The outstanding student oral presentation was entitled: '*Comparison of marker-assisted selection and direct selection for introgression of common bacterial blight in dry bean*' presented by Robert Duncan, University of California, Davis – Robert Gilbertson, advisor.

The outstanding poster presentation was entitled: '*Dry bean transformation to enhance white mold resistance'* presented by Ann Armenia, Michigan State University – Jim Kelly, advisor.

On behalf of the BIC, I would like to recognize Chet Kurowski for his years of dedicated service on the BIC Coordinating Committee and I wish to welcome Ron Shellenberger as the newest member of the coordinating committee. I also would like to recognize Jim Beaver who served as acting chair of the BIC Genetics Committee and Tim Porch who assumed the role as chair of the Genetics Committee. I wish to welcome Carlos Urrea as a new member on the Genetics Committee.

The BIC is looking forward to celebrating its 50th anniversary in 2007. Details of the 2007 BIC meeting in Madison, WI are provided in this issue or can be found at the BIC Web page www.css.msu.edu/bic. Madison was the venue for the first BIC meeting and is the most appropriate to celebrate our 50th anniversary. Members are asking to check the web page periodically for upcoming events and deadlines related the BIC.

Finally, the BIC mourns the passing of a friend and colleague Dr. Shigemi Honma from Michigan State University. Shig was awarded the BIC Meritorious Service Award in 1975 and he is best known for the early work on the introgression of common blight resistance from tepary bean. The BIC recognizes him for his significant achievements to bean research.

Dr. James D. Kelly, BIC President

BIC COMMITTEE MEMBERSHIP - 1957 TO 2006

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

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
2006 Hosfield, Schwartz, Singh

GENETICS COMMITTEE

2005: James S. Beaver (Acting Chair), Matthew W. Blair, Paul Gepts, Phil McClean, Phil Miklas, Tim Porch, Molly Welsh (ex officio).
2006: Tim Porch (Chair), James S. Beaver, Matthew W. Blair, Paul Gepts, Phil McClean, Phil Miklas, Carlos Urrea, Molly Welsh (ex officio).

REPORT OF THE BIC GENETICS COMMITTEE

(Minutes submitted by Tim Porch – abstracted)

The Genetic Committee met in Newark, DE on November 2, 2005 at 3:30pm. Decisions were made on six of the 10 topics discussed. Only decision items are shown below. A full version of the minutes can be found at www.css.msu.edu/bic
Topic: New anthracnose locus, *Co-11*

Decision

1. Accepted symbol of *Co-11* for new anthracnose resistance gene from 'Michelite'. Celeste Vidigal presented allelism testing results from the manuscript "Characterization of anthracnose resistance in Michelite" that shows that the gene from 'Michelite' represents a new locus, accepted by the committee as *Co-11*.

Topic: New anthracnose allele in 'Widusa', *Co-1*^{5}
Decision

2. Jim Kelly and Celeste Vidigal presented allelism testing results for 'Widusa' showing that anthracnose resistance from 'Widusa' is conferred by a new allele of *Co-1*^{5}. The committee accepted the designation of *Co-1*^{5}.

Topic: Designation of *Co-7*
Decision

3. Decision that the 1998 paper, "Marker-assisted dissection of the oligogenic anthracnose resistance in the common bean cultivar, G2333,"(Theor. Appl. Genet. 96:87-94) has precedence for the naming of the *Co-7* locus. A note will be sent on behalf of the Genetics Committee to the French group indicating that the 1998 paper has precedence over their later publication.

Topic: Phaseolin variants
Decision

4. Manuscript describing and naming new phaseolin variants in manuscript "Genetic diversity and origin of Slovene common bean (*Phaseolus vulgaris* L.) germplasm as revealed by AFLP markers and phaseolin analysis" will be sent to Daniel Debouck for review. His recommendations will then be sent to the Genetics Committee for final decision regarding the naming presented in the manuscript.

Topic: New membership in Genetics Committee
Decision

5. Carlos Urrea was nominated and accepted as a new member of the Genetics Committee.

Topic: Chairmanship of Genetics Committee
Decision

6. Tim Porch was nominated and accepted as the new chairman of the Genetics Committee to replace Jim Beaver.

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).

2005 BIC AWARD RECIPIENTS

2005 FRAZIER - ZAUMEYER DISTINGUISHED LECTURESHIP AWARD

PERRY B. CREGAN

Dr. Perry Cregan, Research Geneticist, and Research Leader of Soybean Genomics and Improvement Laboratory, USDA-ARS, located at Beltsville, MD. Perry is an outstanding scientist and leader in the area of soybean genetics. Dr. Cregan was trained as a classical wheat breeder at North Dakota State University, but changed crops when he had the opportunity to join USDA-ARS program at Beltsville. Before beginning his research career Dr. Cregan spent two years in the Peace Corps in Nicaragua as a dry bean and corn extension agent. At Beltsville he continued to carry out a traditional program with soybean genetics as it related to nodulation and nitrogen fixation. In the late 1980's and early 1990's, Dr. Cregan became convinced that developing molecular markers could speed up the breeding program. After much experimentation, he started to concentrate on simple sequence repeat (SSR) DNA length polymorphisms and their relationship to quantitative trait loci (QTL). He was the first to demonstrate the heritability of SSR's in a plant species. He organized team of scientist that developed the SSR based Integrated Genetic Linkage Map of Soybean Genome that is consensus map used by essentially all public and private soybean geneticists and breeders. More recently he has targeted DNA markers for genes controlling soybean cyst nematode resistance, used SSR profiling to assist in identity protection of soybean cultivars and developed single nucleotide polymorphism (SNP) markers in more than 1,300 soybean genes. A SSR/SNP-based consensus map is currently under construction in soybean.

Dr. Cregan has authored or co-authored 114 peer reviewed publications. His leadership in the area of soybean genomics was recognized by his peers and he was elected Chair of the Cellular Biology and Molecular Genetics section of the Crop Science Society of America, Fellow of Crop Science Society of America and Fellow of the American Society of Agronomy. His technology transfer ability was also recognized when he received the Beltsville Area Technology Transfer Award for 2000; the ARS National Technology Transfer Award for 2000; and the Federal Laboratory Consortium Technology Transfer Award for 2001.

As a principal or co-principal investigator, Dr. Cregan has brought in over $3.1 million dollars into his research program from non-ARS sources, and he has led a very diverse research program. Other examples of his leadership are the following: Associate Editor of Crop Science, Cell Biology and Molecular Genetics Division; Representation of the Crop Science Society of America on the "Scientific Forum on Plant Pesticides" (This Forum, with representatives from 12 scientific Professional societies, formulated a response from the scientific community to proposed EPA regulations of genetically engineered plants); member of the joint ARS, CSREES panel to plan and write the draft "USDA Agricultural Genome Initiative; participated in collaborative development with the AMS Plant Variety Protection Office of a legally acceptable DNA profiling protocol for use in Plant Variety Protection; representative to the joint USDA, ARS/INRA (French equivalent to ARS) meeting to develop plans for joint US/French collaborative legume genomics research. Finally, in association with the University of Maryland he has served on graduate committees of 11 students.

A. (BERT) VANDENBERG

Dr. Bert Vandenberg, University of Saskatchewan, Saskatoon, Canada has worked on dry beans for nearly 15 years after obtaining his Ph.D. in the Department of Crop Science and Plant Ecology, Plant Breeding and Genetics. He was appointed Associate Professor and Pulse Crop Research Chair in May of 1998 and has been a full Professor at the University of Saskatchewan since March 2000.

Bert is tireless in his promotion of the Pulse Industry in Saskatchewan and in Canada. Although the lentil and pea crops are much more important to SK agriculture, Bert has also promoted dry bean as an option in appropriate growing regions. When he started working on beans in the early 1990s, they were hopelessly unadapted to our short growing season and very few producers were willing to take the risk. An aggressive crossing program coupled with a few seasons of selection with frost in August allowed him to shorten the days to maturity sufficiently to offer producers bean cultivars that would mature and yield well in our climate. CDC Pintium, a short season pinto, saved a few Manitoba (and probably North Dakota) producers last year when they couldn't get into the field to plant until mid June – far too late for their usual varieties.

Bean agronomy is also an important aspect of his research that has helped make the crop a viable option in SK. Bert and his field crew probably have the most experience growing beans in the province and he is called upon regularly for advice. Bert has been involved in the development of over 80 varieties of pulse crops, including more than 20 beans in several different market classes. He has co-authored more than 50 refereed publications, 13 specifically on bean, and has given countless presentations on pulse crop research to students, other researchers and the industry.

Bert shares his enthusiasm for dry bean research with everyone, especially his students. He encourages both his undergraduate and graduate students to actively participate in research activities outside of their thesis project by offering them opportunities to participate in day-to-day aspects of running the breeding program. Dr. Vandenberg has supervised or co-supervised 10 M.Sc. and 3 Ph.D. students and has served on academic committees of 12 graduate students.

Bert has served on the executive of numerous pulse crop related committees and organizations including the Bean Improvement Committee. He has been on the BIC Coordinating Committee since 1998 and was co-chair of the NAPIA-BIC meetings in Calgary in 1999. He has advised Producer Groups as well as Food Processing Groups on trends in the Pulse Industry and travels regularly to meet with importers of Canadian pulse crops to keep the breeding program in tune with current and emerging market demands.

JORGE ALBERTO ACOSTA GALLEGOS

Dr. Jorge Alberto Acosta Gallegos received his B.S. degree in Plant Breeding from the Escuela Superior de Agricultura Antonio Narro, at Buenavista, Saltillo, Méx., in 1972. He earned his M.S. degree in Plant Breeding from the Universidad Autónoma Agraria Antonio Narro (UAAAN) in 1978. Dr. Acosta earned a Ph. D. degree in Plant Breeding and Genetics under the supervision of Dr. M. Wayne Adams at Michigan State University in 1988.

Dr. Acosta has achieved many professional awards and technical successes throughout his career, developing more than 21 common bean varieties in Pinto, Bayo, Flor de Mayo, Black and Flor de Junio commercial classes. He released Pinto Villa, the drought-resistant dry bean cultivar, which was grown in 170,000 hectares in 1997 in the Mexican highlands yielding 35% more than traditional pinto cultivars. Since the early 90's Pinto Villa has been sown on thousands of hectares in Mexico, is considered an important gene source for drought and multiple disease resistance, and is widely used as a parent in bean breeding programs in several countries. Dry bean varieties released by Dr. Acosta and his INIFAP's team permitted bean producers to obtain greater economical benefits, contributed to solve several social and production problems and contributed to alleviate the hunger in Mexico and the world in a sustainable and natural way.

Dr. Acosta established and reinforced several collaborative research projects between Mexican and international institutions for germplasm collection and studies in crop evolution, breeding and genetics, physiology, phenology and agronomy. He participated for more than 15 years as the Host Country Principal Investigator in Mexico with the Bean/Cowpea CRSP Collaborative Project between Michigan State University and INIFAP, from which important advances were obtained in nitrogen fixation, plant breeding, phenological plasticity, disease resistance and detecting bean adaptation mechanisms under drought. Dr. Acosta served on the Technical Committee of the CRSP for five years and as Chair in 1998. He has also collaborated with international researchers to collect more than 465 wild, weedy, landraces and bred accessions in *Phaseolus* species and contributed to better representation of bean species in germplasm banks in México and other countries. Wild relatives for common bean has been used by Dr. Acosta and other researchers to detect allele *arc 7* of Arcelin, an insecticidal lectin-like protein present in wild bean seeds that confers resistance to bean bruchids.

Dr. Acosta has published over 170 national and international refereed and non refereed scientific and technical papers, which resulted from inter-institutional collaborative research and represents important advances in common bean knowledge. He also participated in field training and thesis direction for more than 16 undergraduate and postgraduate students from different Mexican universities. Throughout his 30 productive years of research, Dr. Acosta has constantly reinforced INIFAP's dry bean and other legume programs across production areas in the Mexican highlands and the tropics. He is a paragon because of his intimate knowledge of *Phaseolus* species and his creativity and successes on several research fronts. Dr. Acosta's enthusiasm and work ethic continues to inspire his colleagues and friends alike.

PHILLIP N. MIKLAS

Dr. Phil Miklas, USDA-ARS, Prosser, Washington first worked on dry bean in the summer of 1982 at the Colorado State University Agricultural Research Center at Fruita, Colorado. He planted and harvested yield trials for Dr. John Keenan and inspected bean seed fields for Dr. Mark Brick in fulfillment of an externship requirement for his B.S. degree at nearby Mesa State College in Grand Junction. After completion of the Ph.D. in breeding and genetics with Dr. Ken Grafton at North Dakota State University in 1991, Phil worked as a Post-Doctoral Fellow with Dr. Jim Kelly at Michigan State University for one year. He accepted a job with the USDA-ARS in Mayaguéz, Puerto Rico in 1992 with a mandate for dry bean germplasm enhancement. In 1996, Phil replaced Dr. Matt Silbernagel at Prosser upon his retirement.

Dr. Miklas has gained national and international prominence with his outstanding work on generating resistance-linked markers and developing different marker-assisted selection strategies. He had a direct hand in developing and applying markers for *Ur-3*, *Ur-4*, *Ur-5*, and *Ur-11* genes for rust resistance, the *bgm-1* gene and a major QTL conditioning resistance to *Bean golden yellow mosaic virus*, the *bc-1*^{2} gene for *Bean common mosaic virus* resistance, *Bct-1* gene for *Beet curly top virus* resistance, *Pse-1* gene for halo blight resistance, and QTL for resistance to white mold and common bacterial blight. Along the way new marker systems (RAPDs, SCARs, and TRAPs) were adopted for bean, and novel marker-assisted selection strategies including selective mapping of QTL, bulked segregant analysis combined with near-isogenic lines to tag genes, recombination-facilitated marker-assisted selection to overcome gene pool specificity of resistance-linked markers, multiplex PCR for three SCAR markers linked with independent QTL governing resistance to common bacterial blight, and co-dominant interpretation of dominant markers using a real-time quantitative PCR approach.

Dr. Miklas is an excellent universal collaborator with both private and public researchers nationally and internationally. He has been exceptionally productive in developing enhanced bean germplasm and his record is truly admirable by any standards. He has been a lead scientist or major collaborator to more than 100 improved bean germplasm lines and cultivars. His most recent releases include kidney and cranberry with *I* + *bc-3* genes for resistance to BCMV, pinto USPT-CBB-1, USPT-ANT-1 and USPT-WM-1 with resistance to common bacterial blight, anthracnose, and white mold, respectively, and the dark red kidney USDK-CBB-15 with a high level of resistance to common bacterial blight. Many of the recent releases incorporated MAS as an integral component of the breeding process.

Dr. Miklas is an active member of the BIC Coordinating, Bean Genetics, Phaseolus Crop Germplasm, W-150 regional project, and WSU cultivar release committees. He currently serves as Associate Editor for Crop Science, PI for the Bean/Cowpea Breeding Project for East and Southern Africa, and is an active participant in the National Sclerotinia Initiative, Western Regional Bean Trials, National Cooperative Dry Bean Nursery, and National White Mold Nursery.

DAVID M. WEBSTER

Dr. David Webster developed a long lasting obsession with all things - *Phaseolus* - in early childhood in North Carolina; his earliest memories are of a yellow blow-up beach toy, a sea dragon he named "Captain Kidney Bean". After fifty years and 25 PVP'd dry and garden bean cultivars, David still counts among life's great pleasures looking at the seed, working with the plant, and, above all else, eating beans.

At Kalamazoo College David took a Bachelors degree in chemistry, magna cum laude, in 1973. He then enrolled as a student with Professor Luis Sequeira at the University of Wisconsin. Dr. Sequeira saw the degree in chemistry and determined to make a physiological plant pathologist out of him but was broad minded enough to relent when David began to show inclinations toward breeding plants for disease resistance. It so happened that his model species was the *Phaseolus* bean. Thoughts of a real job after his Master's degree were dispelled by an opportunity to do work on his Ph.D. dissertation at CIAT on a project related to breeding for resistance to common bacterial blight. His work at CIAT established the importance of adaptation in the expression of disease resistance. It was at CIAT that he amazed a visiting Dermot Coyne with questions about how the farmers in Nebraska put the bamboo stakes in the ground when they were growing great northern beans!

David's career since graduation from Wisconsin has been all out of the same office at Filer, Idaho, owned variously since he started by Asgrow Seed, in turn owned by Upjohn, then by Seminis Vegetable Seeds, and, likely by the time of this reading, by Monsanto. It was with Asgrow under the tutelage of John Atkin that David learned how to manage a seriously big, streamlined, efficient breeding program. Although a bit of a technophobe regarding things digital, he introduced the first use of a database in the breeding programs at Filer; anything was better than long nights copying over pedigree planting and harvest lists. He has emphasized disease resistance in his breeding programs and has developed cultivars and germplasm with resistance to halo blight, bacterial brown spot, common bacterial blight, anthracnose, rust, BCMV, BYMV, BCTV, and various soil-borne fungi; not always by design but he at least knows when he's been lucky. Many of his cultivars have a blend of horticulture, quality, yield and resistance to diseases. Among the 19 dry bean cultivars developed by David are 'Etna, Avanti, Buster, Cabernet and Pink Panther'. Among the 6 garden beans are 'Opus, Gold Mine, Tema, and Lodi'. They are grown in North America and also have significant market shares in Italy, Spain, Hungary, France, and Greece where he has an extensive collaborative work.

Dr. Webster recognizes the collaborations and exchange with members of the BIC as a key ingredient to any success he's enjoyed, as well as the opportunity to contribute to the success of his colleagues. Although training students has not been a major part of his career, he has happily hosted local and foreign students and visitors. He served as chairman of the *Phaseolus* crop advisory committee from 1989 to 1991.

IN MEMORY OF SHIGEMI HONMA

Shigemi Honma, Emeritus Professor of Horticulture at Michigan State University, died in East Lansing, Mich. on May 30, 2005. Prof. Honma was born February 14, 1920 in Haina, Hawaii. He served for 3 ½ years with the famed 442nd Regimental Combat Team of Nisei soldiers in World War II, and was awarded the Bronze Star and the Purple Heart. He earned a B.A. at Cornell University in 1949, and a Ph.D. from the University of Minnesota in 1953. He then took a position as assistant horticulturist at the University of Nebraska, where he made the first successful interspecific hybrid between common bean (*Phaseolus vulgaris*) and tepary bean (*P. acutifolius*). This permitted the transfer of the tepary bean's resistance to common bacterial blight to the hybrid. The work was published in the *Journal of Heredity* 47: 217 (1956). The paper is one of the most cited references in the bean literature, and won Dr. Honma the Meritorious Service Award from the Bean Improvement Cooperative in 1975. The hybrid was subsequently used by Dr. Dermot Coyne of Nebraska and other breeders to incorporate this resistance into *P. vulgaris*. This pioneering work encouraged others to explore the potential of interspecific hybridization in bean improvement.

Dr. Honma moved to Michigan State University as an assistant professor in 1955, and was named full professor in 1966. He retired in 1986 after a distinguished career as a breeder of vegetable crops, during which he released four cauliflower, two celery, three lettuce, nine pepper, and six tomato cultivars. Much of his effort was directed toward improving resistance to fungal, bacterial and viral diseases. Two of the cauliflower cultivars, Self-Blanch and Stovepipe, were designed to reduce the labor required to tie up the leaves to shade the head and avoid greening. He co-authored, with Prof. Sylvan Wittwer, the book *Greenhouse Tomatoes - Guidelines for Successful Production*, as well as over 100 scientific and popular articles on vegetable cultivar improvement.

Dr. Honma received invitations from both U.S. and international institutions to present lectures on his work. The Department of Horticulture at the University of Minnesota invited him to present the Distinguished Alumni Lecture there in April 1981. During 1981-82, he represented the USDA and Michigan State University in establishing cooperative exchanges of scientific information with Bulgarian scientists, and was invited to lecture on vegetables for the Royal Project in Thailand. Dr. Honma is survived by his wife, Isao, and two children Alan and Valerie.

2007 BIENNIAL BIC/USDBC MEETING

The 2007 BIC meeting will take place in Madison, Wisconsin. The tentative date under consideration for the BIC, United States Dry Bean Council (USDBC) and related meetings is Monday Oct 29 - Wednesday Oct 31, 2007. The North American Pulse Improvement Association (NAPIA) meeting date being explored is Thursday and Friday Nov. 1st & 2nd. Several venues for the meeting are being considered. As this is the 50th Anniversary of the BIC we are looking for any suggestions to mark this event in a special way. Please take some time to set aside some of those photos, memories, etc that might be shared. Further information will be forthcoming through the BIC web site (http://www.css.msu.edu/bic , the 2006 annual report, and individual mailings to local committee members. For further information contact Jim Nienhuis nienhuis@calshp.cals.wisc.edu or Ken Kmiecik ken.kmiecik@seminis.com

PLANT BREEDING AND GENOMICS: STAYING IN TOUCH

Perry Cregan
Soybean Genomics and Improvement Laboratory
USDA, ARS, Beltsville, MD 20705, USA.

My first professional contact with agriculture came as a Peace Corps Volunteer working in the small village of Pueblo Nuevo in Estelí province in northwestern Nicaragua. I served as an agronomist working with subsistence farmers who cultivated mainly maize and common bean. The preferred market class in Nicaragua was small red beans which had a number of problems including susceptibility to curly top virus (and other viruses) as well as a trailing growth habit. Working with the local extension agent I attempted to introduce a disease resistant black bean with an erect growth habit. Despite these improved characteristics, the small farmers with whom I worked were not receptive to a black bean. I was fortunate to obtain large samples of five small red bean cultivars with good virus resistance and a desirable growth habit from Dr. Matt Silbernagel, USDA-ARS Dry Bean Project at Prosser, WA. The susceptibility to rust of the small red beans from the USDA would not have surprised a trained agronomist but it certainly was a surprise to me and allowed me to realize how little I knew about agronomy, plant pathology and agricultural science.

As a result of my Peace Corps experience with common bean I determined to obtain a degree in agriculture to complement my B.A. degree. I was fortunate to find a program at Oregon State University for ex-Peace Corps Volunteers who had worked in agriculture and who wanted to obtain a B.S. degree in agriculture. My intention was to study plant pathology and plant breeding with the goal of eventually working with common bean improvement. However, I became a student employee in the large small grain breeding program of Dr. Warren Kronstad and was eventually convinced that graduate study in small grain breeding and genetics was an excellent path to follow in terms of career opportunities both in the U.S. and abroad. Soon after completing my studies at Oregon State I moved to North Dakota State University and became a graduate research assistant in the barley breeding project. I completed my M.S. in barley breeding and genetics and my Ph.D. in wheat breeding and quantitative genetics. Shortly thereafter I began work as a Research Geneticist in the Nitrogen Fixation and Soybean Genetics Laboratory at the USDA, ARS, Beltsville, MD.

The research assignment at the USDA, Beltsville was closely associated with the USDA National Rhizobium Culture Collection. Among other activities I embarked upon was a project to discover soybean genotypes that excluded soybean-nodulating rhizobia (*Bradyrhizobium japonicum*) present in U.S. soils but that would allow nodulation by specific strains of rhizobia known to be highly effective N-fixers. This research resulted in the discovery of a number of exotic soybean germplasm lines that stopped nodulation by different sub-populations of the indigenous *B. japonicum* population. At this time RFLP genetic markers were beginning to be used in soybean and we determined to attempt the use of marker assisted selection to combine into a single soybean genotype the genes that excluded different subpopulations of *B. japonicum*. I quickly realized that from this experience with RFLP technology that required 1) Southern blotting, 2) probe labeling and cleanup, 3) hybridization and washing as well as the use of radiolabeled phosphorus was not a procedure that would work well in the context of most plant breeding programs. It was at this time that I first began to become aware of the larger field of human genetics and genomics research and of certain basic research advances that might have applicability to plant genetics. Specifically, the work of Weber and May (1989) describing microsatellite or simple sequence repeat (SSR) DNA markers in human convinced me that some basic research discoveries could be quickly translated into useful tools for plant improvement. It was shortly thereafter that we discovered a number of soybean sequences in GenBank, the DNA sequence database which contained SSR sequences that could be converted into highly polymorphic genetic markers that were inherited in a Mendelian fashion (Akkaya *et al*. 1992). We subsequently developed and mapped more than 1000 soybean SSRs that are broadly used in both public and private sector soybean breeding and genetics

research (Cregan *et al*. 1999a; Song *et al*. 2004). The availability of expressed sequence tags (EST) developed by basic soybean genomics research served as one source of sequence for SSR marker development. Bacterial artificial chromosome (BAC) libraries that are used for the creation of physical genome maps were also made available by genomics researchers and served as a tool for the development of SSR markers targeted to specific positions in the genome that were of interest to soybean breeders for purposes of marker assisted selection (Cregan *et al*. 1999c). Using selected BAC clones, our laboratory developed markers targeted to the major gene that provides resistance to the soybean cyst nematode (Cregan *et al*. 1999b).

In April of 1999 the SNP Consortium Ltd. was created with the objective of discovering 400,000 human single nucleotide polymorphisms (SNPs). These useful DNA markers were to be made freely available to public and private researchers to for numerous purposes such as gene discovery, diagnostics, the application of genetic association analysis and the prediction of response to drug treatments. The rapid development of inexpensive and high throughput systems for SNP analysis suggested SNPs as a useful alternative DNA marker system in plants. By 200l soybean genomics researchers had deposited more than 200,000 soybean EST sequences in GenBank and these had been analyzed to create a set of unique genes or "unigenes". The unigenes provided the basis for the discovery of SNP DNA markers in soybean genes that began in our laboratory at Beltsville in 2002. The discovery process was greatly aided by rapid DNA sequence analysis and software such as PolyBayes (Marth *et al*. 2001) designed by human genomicists for the identification of sequence polymorphisms (SNPs) in DNA sequence alignments. In January 2006 we will report on the first SNP-based genetic map of soybean that will be based on SNPs in approximately 1500 soybean genes. Interestingly, about 15% of the polymerase chain reaction (PCR) primers used to detect SNPs in soybean genes amplify a PCR fragment from common bean and more than half of these contain a SNP that is polymorphic in the BAT93 x Jalo EEP558 mapping population. Clearly, the common bean research community is in a position to take advantage of genomics projects proposed by researchers such as Dr. Paul Gepts at the Univ. of CA, Davis. If funded, a research proposal recently submitted to the National Science Foundation by Dr. Gepts and others will produce results of great value to common bean breeders and geneticists. These results will include a large suite of SSR markers, BAC libraries, and a set of SNP markers. Genomics research has much to offer more traditional common bean breeding and genetics research just as such research has greatly benefited the soybean breeding and genetics community.

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THE BEANS FOR HEALTH ALLIANCE: A PUBLIC-PRIVATE SECTOR PARTNERSHIP TO SUPPORT RESEARCH ON THE NUTRITIONAL AND HEALTH ATTRIBUTES OF BEANS.

Widders, I.E., Bean/Cowpea CRSP, Michigan State University, East Lansing, MI.

Both developing and developed countries around the world continue to be confronted with major crises in public health and malnutrition. As was recognized by Dr. B.H. Brundland, previous Director General of the World Health Organization, "it is impossible to achieve long-term social, economic, and environmental development without addressing the basic issues of human health and nutrition."

Clearly agriculture provides food-based solutions to many of the global health and malnutrition concerns. This is reinforced by a recent IFPRI study which concluded that: (1) improved nutrition and sanitation play a larger role in improving societal health than developments in medical technology; (2) preventative approaches cost societies dramatically less than medical treatment, and (3) food-based approaches contribute to sustainable development.

Examples of global scale health problems which are dietary related include under-nutrition, obesity, type-2 diabetes, chronic diseases, and HIV/AIDS. Under-nutrition due to protein, vitamin and micronutrient deficiencies in diets contribute to widespread infant mortality, stunted growth, reduced cognition, and lower productivity. Obesity has been declared by the WHO to be a rapidly growing global epidemic, affecting both developed and developing countries. Malnutrition weakens the immune systems of HIV positive individuals thus increasing the rate of its progression to AIDS.

Obesity, over nutrition, is perhaps the "new" global health concern with over 1 billion people being overweight. Within the U.S., over 60% of the population in many states are currently classified as either overweight or obese. The fundamental problem is that obesity is a leading causal factor of many chronic diseases, including type-2 diabetes, cardiovascular disease and cancers. In addition, it contributes to dramatic increases in health care costs, losses in labor productivity which are causing a "drag" to the growth and competitiveness of the U.S. economy.

Consumption of beans and related pulses is a viable solution to these global health problems. Considered by many to be the perfect food, beans are nutrient dense with high contents of protein, micronutrients and vitamins, high in dietary fiber, and have a low glycemic index**.**

In 2003, the **"**International Alliance to Promote the Health Benefits of Beans and Other Pulses" (Beans for Health Alliance) was established with funding from USAID. The BHA is a "Global Development Alliance" involving private industry leadership and investment. It currently has over 80 members including food processing companies, grain traders/exporters, grower associations, public health organizations, NGOs, universities and health research institutions from both the U.S. and foreign countries including Canada and Mexico. The global mission of the BHA is to educate consumers with science-based information on the health benefits of eating beans and related edible pulses so as to increase global consumption and address strategic global health concerns in an economical and sustainable manner. The foundational beliefs of the BHA are that the consumption of nutritious bean-based foods are essential for good health and nutrition, that the marketing of nutritious foods is vital to the survival and growth of food industries, that food industries play a major role in the dissemination

of nutrition information to consumers, and that bean producers and processors provide food-based solutions to global health and malnutrition problems.

Major activities of the BHA include: (1) the establishment of a Bean Health Research Program, (2) the development of a website with nutrition information to increase consumer awareness of the importance of "beans" in human health and nutrition (<www.beansforhealth.org>), (3) obtaining federal approval for a Dietary Guideline for beans, (4) sponsoring and organizing a symposium, workshop and poster session at the International Nutrition Congress in Durban, South Africa, on September 19-23, 2005, (5) participating in the American Dietetic Association Food and Nutrition Conference and Expo in St. Louis on October 22-25, 2005, (6) and influencing research program priorities and leveraging funds from federal agencies and private organizations/foundations for health/nutrition research on beans.

The Bean Health Research Program, administered by Michigan State University, is currently funding four health research projects on beans involving human or animal feeding studies. Such research was considered to be of high priority and necessary for the industry to potentially obtain dietary guidelines or health claims for beans and related dry grain pulses in the future.

A study at Arizona State University, *Impact of Pinto and Cowpea Consumption on Heart Disease and Type-2 Diabetes,* lead by Drs. Donna Winham, Carol Johnston and Andrea Hutchins, is testing the hypothesis that long-term pinto bean or black-eyed pea (cowpea) ingestion as compared to placebo will reduce biomarkers associated with risk for cardiovascular disease and type-2 diabetes in apparently healthy subjects with moderately raised fasting serum insulin. The study involved a total of 20 human subjects. Participants consumed ½ cup daily of canned pinto beans, black-eyed peas or carrots for eight weeks each as a supplement to their usual diet. Preliminary findings indicate that eating beans reduces total and LDL cholesterol by 8.1 and 7.2%, respectively. There were no significant differences in the HDL cholesterol, triglycerides, hs-CRP, weight, BMI, or blood pressure among the subjects.

A research project at the USDA/ARS Human Nutrition Research Center, entitled *Impact of Pinto Bean Consumption on Colon Health in Humans*, lead by Dr. Philip Reeves, tested the hypothesis that the consumption of a single meal of beans per day effectively alters *in-vivo* fermentation patterns in a manner that is associated with resistance to colon cancer. A total of 80 persons participated. Each participant was required to consume ½ cup daily of cooked pinto beans or a chicken soup dish of similar nutrient composition for 12 weeks. Measurements included *in vitro* production of short chain fatty acids, changes in specific bacterial populations in the long intestine using DNA probes, in addition to blood analyses of total cholesterol, HDL, triglycerides, and glucose. No findings could be reported at the present time since much of the data remain to be analyzed.

A study by the Cancer Prevention Laboratory at Colorado State University, entitled *UnderstandingUnique Nutritional Attributes of Different Bean Market Classes,* lead by Drs. Mark Brick and Henry Thompson, is seeking to determine if bean market classes differ in *in vivo* antioxidant activity and glycemic index and in their potential to promote human health in the context of a pre-clinical model for breast cancer. Fourteen market classes of beans were compared in this study for total phenols, antioxidants activity (TEAC and ORAC), and *in vivo* activity in a rat model system to assess effects of consumption on mammary pathologies. Preliminary results confirm that market classes vary in their phenolic content and antioxidant activity. In general, the 14 bean samples grouped into three categories for both phenol content and antioxicant activity. Pink, small red, pinto, and dark red kidney beans ranked highest for

both phenol content and antioxidant activity, while black and light red kidney beans were intermediate and yellow, cowpeas and white beans were lowest. Phenol content and antioxidant activity were highly correlated ($r = 0.97$, $p < 0.01$). A comparison of a cooked bean product to raw beans indicated that the cooked beans had significantly reduced phenol content (by almost 1/3 in some classes), especially in the bean lines that had high phenolic content.

The fourth BHA supported study involves a collaborative research project between Michigan State University, the University of Botswana and Sokoine University of Agriculture in Tanzania; "*Evaluation of the Ability of Beans to Improve Nutrition and Immune Status in HIV+ African Children."* This study, being lead by Drs. Maurice Bennink and Lorraine Weatherspoon, Jose Jackson, and Theobald Mosha, is testing the hypotheses that eating beans provides protein and amino acids necessary for normal growth in HIV seronegative and seropositive children, as well as provides the necessary amino acids to rebuild lean tissue and strengthen immune systems in HIV infected children thus delaying the onset of AIDS**.** Approximately 50 children are participating in the Botswana study and 107 children in the Tanzanian study. Subjects are being fed either a bean protein/micronutrient fortified sorghum flour or a micronutrient fortified sorghum flour (the control). Measurements are being taken of growth parameters, fat, mass, muscle area, cognitive ability, motor performance, viral load and CD4%. Since these are double blind feeding studies that are continuing, no data are available yet.

The Beans for Health Alliance has had a major impact on the priorities and organizational structure of the U.S. bean industry. Because of their recognition of the importance of the different sectors working together, the U.S. bean industry is currently considering a major reorganization and consolidation. Unique attributes of this innovative private-public sector alliance include:

- **Networks** state grower associations, grain traders and processors with universities.
- Is both **domestic and international** in scope and membership
- Has attracted new **federal funding** for research
- Is contributing to the development of research capacity and is providing expertise to the industry in the areas of **nutrition and health**.

The justifications for a potential industry merger are to develop a unified mission, to achieve administrative economies, and to pursue a common agenda for the industry. The U.S. dry bean industry has determined that this agenda should include domestic promotion tied to health, international liaison and market development, and expanded research investments especially in nutrition.

PHENOLIC ACIDS PROFILES OF BEANS COMMONLY CONSUMED IN THE UNITED STATES

Devanand L. Luthria[1],*, Marcial A. Pastor-Corrales[2]
[1]Food Composition Laboratory, Beltsville Human Nutrition Research Center, BARC (East),
[2]Vegetable Laboratory, Plant Sciences Institute,
10300 Baltimore Avenue, ARS, USDA, Beltsville, MD 20705-3000

Introduction

Phenolic compounds are important phytonutrients that are widely distributed throughout the plant kingdom. These compounds are known to provide protection against certain types of cancers, cardiovascular, and other chronic diseases (1). The beneficial properties of phenolic compounds have been partially attributed to their antioxidant activity (2). Over 8000 different phenolic compounds have been reported from various plant sources (3). The common structural feature of all phenolic compounds is the presence of at least one 'phenol' (an aromatic ring possessing at least one hydroxyl group) moiety. Phenolic compounds can be broadly divided into two major categories, namely simple phenols and polyphenols. Polyphenols can be further subdivided into two main groups, tannins (polymers of phenolic acids, catechins or epicatechins) and flavonoids (flavones, isoflavones, anthocyanins, chalcones, flavonol, flavanones, etc.) Simple phenols can also be further classified into two categories, phenolic acids (cinnamic acid or benzoic acid and its derivatives) and coumarins (Figure 1)(4).
Phenolic compounds can either be found as a simple aglycon unit or in conjugation with sugars, acids, carbohydrates, proteins, or other cellular components (5). Solubility of phenolic compounds is influenced by its polarity, which in turn is determined from its structure, degree of polymerization, its interaction with other component of its matrix, particle size of the matrix, polarity of extraction solvent, extraction technique and conditions. Therefore it is important to optimize sample preparation procedures for accurate analysis of these compounds in different plant matrices (5, 6). We reported the determination of phenolic acid content in three black bean cultivars (T-39, Jaguar and Eclipse) (7). In continuation of our research, we have determined the phenolic acids content of nine additional bean classes commonly consumed in the United States.

Materials and Methods

As reported earlier, all bean samples (Pinto, Great Northern, Navy, Dark Red Kidney, Light Red Kidney, Red Mexican, Cranberry, Pink and Alubia) were provided by Dr. M.A. Pastor-Corrales of the vegetable Laboratory, USDA (Beltsville, Maryland) (7). Standards of phenolic acids (caffeic, ferulic, para-coumaric and sinapic) were purchased from Sigma (St. Louis, MO, USA).

Saponification and extraction of free phenolic acids

All bean samples were ground in a coffee grinder and sieved through a size 20 standard sieve to obtain a uniform particle size fraction (particle size < 0.825 mm). The ground material was stored at -60^{o}C in an inert nitrogen atmosphere until analyzed. All bean samples were saponified by the same hydrolysis procedure as reported previously (7). The liberated free phenolic acids were extracted with ethyl acetate (2 x 6.4 mL) and analyzed by HPLC. All extractions were carried out in triplicate and all identified phenolic acids were quantified with external standards by using HPLC analysis (7).

Results and Discussion

It is well documented in the literature that phenolic acids can occur in multiple forms (free, esterified, glycosylated or as a polymer) in different food matrices. Thus the polarity of phenolic acids vary significantly depending on its chemical structure and it is challenging to extract multiple forms with a single extraction solvent (3,5). Hence, saponification of dried bean samples was performed to generate free phenolic acids so that aglycon forms of each phenolic acid can be extracted and analyzed accurately by HPLC. Identification of phenolic acids in bean samples was carried out by comparison of UV spectra and retention times with authentic standards obtained from commercial sources (8).

The three predominant phenolic acids that were extracted from all cultivars were identified as ferulic acid, *p*-coumaric acid and sinapic acid. Ferulic acid was the most abundant phenolic acid in all bean cultivars. Intermediate levels of *p*-coumaric acid and sinapic acid were identified in all other cultivars. The total phenolic acid content for all bean cultivars ranged between 19mg/100g and 36mg/100g of dried bean sample. Highest amount of total phenolic acid (48mg/100g) was extracted from T-39 cultivar of Black bean class as reported previously. Minimum (19mg/100g) amount of total phenolic acid were extracted from Cranberry bean class. The phenolic acid content of the other bean classes varied between these two extreme values.

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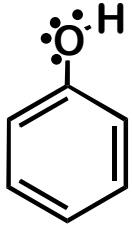
Figure 1. Chemical classification of phenolic compounds



*** These compounds can occur in free form (aglycon) or conjugated form with sugar or acid units**

EFFECT OF ISOLATES AND CONCENTRATIONS OF *XANTHOMONAS CAMPESTRIS* PV. *PHASEOLI* ON DRY BEAN GENOTYPES

Margarita Lema, Henry Terán, and Shree P. Singh.
University of Idaho, Kimberly, ID.

Introduction

Common bacterial blight (CBB), caused by *X. campestris* pv. *phaseoli* Smith (Dye), is a important seed-borne disease of common bean in many parts of the world. The CBB causes 20-60% yield losses and adversely affects seed quality. Leyna and Coyne (1985) Lienert and Schwartz (1994) and Pompeu and Crowder (1973) reported significant effects of cultivar, isolate and concentration. Different concentrations beginning from 10^5 are used for CBB screening and some cannot discriminate between susceptible and resistant genotypes. An appropriate concentration of inoculum may depend on the isolate utilized (Aggour et al., 1988). Our objective was to study the effect of isolates and concentrations of *Xcp* on different dry bean genotypes.

Materials and Methods

Twenty-nine dry bean genotypes with different levels of resistance to CBB and two susceptible checks ("ICA Pijao" and "UI 114") were evaluated. *Xanthomonas* isolates from Colorado and Wisconsin and bacterial concentrations of $5x10^7$ and $5x10^9$ cfu/ml were used. Sequential inoculations on the primary and first trifoliolate leaves were realized 10 and 20 days after sowing, respectively. About 14 and 21 days after each inoculation, disease evaluations were made on a 1 to 9 disease severity scale, where 1 = no visible symptoms, and 9 = severely diseased. The multiple-needles inoculation method and a randomized complete block design with three replications were used. A three-factor factorial was used to analyze data. Evaluations were carried out in the greenhouse at Kimberly, Idaho in 2005.

Results

Significant differences among isolates (I), inoculum concentration (C), and reaction of dry bean genotypes (G) were found on the primary and first trifoliolate leaves, respectively (Table 1). There was a significant interaction between I x C, I x G and C x G on primary leaf and all interactions were significant on trifoliolate leaf. The mean disease scores on the primary leaf were lower than on the trifoliolate leaf (Table 2). On trifoliolate leaf, Wisconsin isolate was more virulent than Colorado isolate and in all cases $5x10^9$ effected higher disease scores than $5x10^7$. Both *Xcp* at $5x10^7$ on the primary and Colorado isolate at $5x10^7$ and Wisconsin isolate at $5x10^9$ cfu/ml on the trifoliolate leaf did not separate dry bean genotypes. Both *Xcp* at $5x10^9$ on the primary and Colorado isolate at $5x10^9$ and Wisconsin isolate at $5x10^7$ cfu/ml on the trifoliolate leaf separated susceptible, intermediate and resistant genotypes. In each case, VAX 3 and Wilkinson 2 showed the lowest disease scores.

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Table 1. Analysis of variance for 31 genotypes evaluated with *Xcp* in the greenhouse at Kimberly, Idaho in 2005.

		Mean Square			
Source	df	Primary leaf		First trifoliolate	
Replication	2	329.9	**	144.6	**
Isolate (I)	1	27.9	*	2833.6	**
Concentration (C)	1	3873.9	**	2603.9	**
Genotype (G)	30	35.4	**	31.3	**
I x C	1	33.6	*	1021.0	**
I x G	30	7.8	*	4.2	**
C x G	30	23.2	**	4.7	**
I x C x G	30	6.9		9.1	**
Error	1000	5.2		1.1	

*,**: significant at 5 and 1%, respectively.

Table 2. Mean CBB reaction in the primary and first trifoliate leaves for two concentrations of Colorado and Wisconsin *Xcp* isolates for 29 resistance sources and two susceptible dry beans, evaluated in greenhouse at Kimberly, Idaho in 2005.

Genotype	Primary Leaf				First Trifoliate			
	Colorado		Wisconsin		Colorado		Wisconsin	
	$5x10^7$	$5x10^9$	$5x10^7$	$5x10^9$	$5x10^7$	$5x10^9$	$5x10^7$	$5x10^9$
A 493	1.0	5.6	1.0	6.8	1.0	8.1	7.8	8.9
Colima 9	1.0	4.4	1.1	6.3	1.1	6.8	5.4	8.7
G 1320	1.0	4.9	1.2	6.4	1.1	8.2	7.6	8.0
G 17341	1.0	4.3	1.0	6.0	1.1	4.8	5.6	7.6
Ica Pijao	1.0	8.1	1.9	6.3	2.0	8.7	8.3	9.0
ICB 3	1.0	7.3	1.9	7.2	1.0	8.0	6.6	9.0
ICB 6	1.2	6.4	1.9	6.3	1.2	8.7	8.7	8.7
ICB 8	1.3	8.4	1.6	5.2	1.1	8.3	8.0	8.3
ICB 10	1.0	8.3	2.8	9.0	1.9	7.2	7.2	8.7
ICB 12	1.0	6.8	1.4	7.9	1.2	7.8	7.9	8.7
Montana 5	1.0	7.0	1.7	6.6	2.0	7.8	7.0	8.4
Montcalm	1.3	4.9	2.4	6.0	2.4	8.6	7.8	9.0
OAC 88-1	1.0	4.4	1.0	6.8	1.5	7.8	7.8	8.2
Pinto UI 114	1.2	3.8	2.0	6.0	2.8	8.7	7.2	9.0
Tamaulipas 9-B	1.0	9.0	3.7	6.3	1.0	7.3	7.7	9.0
TARS VCI-4	1.0	5.6	3.0	7.0	1.6	7.6	8.2	8.3
USDK CBB-15	1.0	5.2	2.0	5.7	1.6	8.0	7.7	8.7
USPT 72	1.0	6.7	3.0	7.0	2.7	8.7	7.8	8.7
USPT 73	1.0	5.4	2.7	6.2	3.1	8.0	8.0	9.0
USPT CBB-1	1.0	3.9	3.1	4.6	1.3	8.4	7.8	8.7
VAX 1	1.0	3.1	1.2	4.5	1.0	6.5	6.8	8.3
VAX 2	1.0	6.4	1.0	5.2	1.1	7.6	6.6	8.0
VAX 3	1.1	4.1	1.0	1.0	1.0	3.6	3.4	6.6
VAX 4	1.5	3.1	1.0	2.6	1.1	4.8	6.6	7.9
VAX 5	1.0	4.0	1.0	1.2	1.0	4.9	7.0	7.9
VAX 6	1.0	3.4	1.0	1.0	1.0	4.6	5.3	7.4
Wilkinson 2	1.0	1.0	1.0	2.9	1.0	2.1	4.8	6.1
XAN 91	1.0	6.5	3.3	8.5	1.5	6.3	8.7	8.8
XAN 112	1.0	8.4	1.0	6.3	5.4	9.0	8.7	8.5
XAN 159	1.1	3.6	1.0	3.3	1.0	3.6	6.9	8.7
XAN 309	1.0	3.9	1.2	2.0	1.0	5.8	6.1	9.0
Mean	1.1	5.4	1.7	5.3	1.5	6.9	7.0	8.4
LSD(0.05)[1]	2.1	2.1	2.1	2.1	1.0	1.0	1.0	1.0
LSD(0.05)[2]		0.4				0.2		

[1] To compare between genotypes within isolate and concentration; [2] To compare between means of isolate and concentration.

COMPARISON OF MARKER-ASSISTED AND DIRECT SELECTION FOR INTROGRESSION OF COMMON BACTERIAL BLIGHT RESISTANCE IN COMMON BEAN

Robert W. Duncan[1], Henry Terán[2], Shree P. Singh[2], Robert L. Gilbertson[1].
[1]University of California Davis, Davis, CA, [2]University of Idaho, Kimberly, ID.

Common Bean (*Phaseolus vulgaris* L.) is highly susceptible to common bacterial blight (CBB) caused by *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye (*Xcp*). CBB resistance has been introduced into *P. vulgaris* from interspecific hybridization with *P. acutifolius* and *P. coccineus* (reviewed by Singh and Muñoz, 1999). Recent research has demonstrated that marker-assisted selection (MAS) can be used to follow the introgression of CBB resistance outside of the original mapping populations (Kelly et al. 2003). The objective of this research is to compare direct phenotypic selection and MAS for selection of CBB resistant progenies in a wide double-cross population having Andean and Middle American parents.

Marker-assisted selection for CBB resistance was performed with the following SCAR markers linked with CBB resistance (marker name in bold and linkage group [LG] and the source of resistance are in parenthesis): **SAP6** (LG B10, GN No. 1 sel. 27), **SU91** (LG B8, XAN159), and **BC420** (LG B6, XAN159). MAS and direct selection for CBB resistance was compared in a population of 707 F_1 progenies derived from a VAX3 (SAP6 and SU91) and Wilkinson2 (BC420) by dark red kidney double-cross. The 707 F_1 progeny (plants) were inoculated with *Xcp* ($1x10^8$ cfu/ml) with the razor blade method. All 707 F_1 plants were screened for the presence of each of the three SCAR markers. This methodology allowed for direct comparison of both selection techniques on individual F_1 plants.

Using a 1 – 9 rating scale (1<4 = resistant, 4<7 = intermediate, 7-9 = susceptible) for direct selection, 73 F_1 plants were resistant, 257 were intermediate, and 377 were susceptible (Table 1). The 73 resistant plants had a mean disease severity index (DSI) of 2.9, with a range of 2.0 - 3.7. Using the results from the direct selection, we were able to evaluate the effectiveness of each marker individually (Table 2) and in all possible combinations (Table 3) for identifying CBB resistant progenies. Each individual marker identified a high proportion of the 73 resistant F_1 plants; however, hundreds of intermediate and susceptible plants also were identified with each marker. There was no significant difference in CBB DSI for plants with or without the SAP6 marker, whereas using the presence of SU91 or BC420 resulted in a significant reduction of the DSI (6.0 vs. 7.2 and 7.0, respectively). Interestingly, the most effective individual marker for selecting CBB resistance in this population was the phenotypic marker of lilac flower color (DSI = 5.8) (Table 2).

Similar results were obtained when the three SCAR markers were analyzed in all combinations (Table 3). Again, many of the CBB resistant plants were identified, but so were a high proportion of intermediate and susceptible plants. The most effective marker combination was SU91 together with BC420, identifying plants with a mean DSI of 5.4. The addition of SAP6 to SU91 and BC420 did not improve the mean DSI value. Of the 707 F_1 plants, 192 had all three SCAR markers, and those also had a mean DSI of 5.4.

In conclusion, for this double-cross population, direct selection (DSI = 2.9) was superior to MAS (DSI = 5.4) for selection of CBB resistance. In fact, 27% of the plants with all three SCAR markers were rated as susceptible. Selection of these plants in a MAS CBB resistance breeding program would increase the time and the cost of the development of a resistant line. Conversely, 4% of the CBB resistant plants did not contain any of these markers, further demonstrating the quantitative nature of CBB resistance, and the need for the development of additional molecular markers linked with these QTL.

Table 1. The total number, the mean common bacterial blight disease severity index (DSI) and DSI range for 707 F_1 plants identified by direct selection from a VAX3, Wilkinson2, and dark red kidney double-cross population.

	Resistant	**Intermediate**	**Susceptible**
# of Plants	73	257	377
Mean DSI	2.9	5.5	8.0
Range	2.0 – 3.7	4.0 – 6.7	7.0 – 9.0

Table 2. The number of plants and the mean disease severity index (DSI) for the presence and absence of CBB resistance SCAR markers (SAP6, SU91 and BC420) or flower color markers (white and lilac) according to DSI classes determined by direct selection.

CBB Score	**SAP6+**	**SAP6-**	**SU91+**	**SU91-**	**BC420+**	**BC420-**	**Lilac**	**White**
Resistant	64	9	63	10	60	13	53	5
Intermediate	218	39	184	73	150	107	91	93
Susceptible	302	75	165	212	149	228	97	154
Mean DSI	6.5	6.7	6.0	7.2	6.0	7.0	5.8	6.9

Table 3. The number of plants and the mean disease severity index (DSI) for the presence and absence of CBB resistance SCAR markers (SAP6, SU91 and BC420) in all possible combinations according to DSI classes determined by direct selection.

CBB Score	**SAP6+ SU91+**	**SAP6- SU91-**	**SAP6+ BC420+**	**SAP6- BC420-**	**SU91+ BC420+**	**SU91- BC420-**	**SAP6+ SU91+ BC420+**	**SAP6- SU91- BC420-**
Resistant	54	4	52	5	50	4	43	3
Intermediate	166	21	128	17	109	32	97	11
Susceptible	141	51	126	52	61	124	52	37
Mean DSI	6.0	7.2	6.0	7.2	5.4	7.4	5.4	7.3

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MOLECULAR MARKERS USED TO IMPROVE THE LEVEL OF RESISTANCE TO COMMON BACTERIAL BLIGHT IN DRY BEAN.

Ibarra-Perez, F.J.[1], J.A. Acosta-Gallegos[1], R. Navarrete-Maya[2], R. Zandate-Hernandez[1], P. Fernandez-Hernandez[1] and J.D. Kelly[3]. [1]INIFAP, Serapio Rendón 83, Col. San Rafael, México, D.F. 06770. [2] FES-Cuatitlan, UNAM, México D.F. [3] 370 PSSB, Michigan State University, E. Lansing, MI 48824 (ibarra.francisco@inifap.gob.mx)

Over the years, traditional common bean production in the highlands of Mexico has allowed for the broad distribution of seed transmitted diseases, among them common bacterial blight (CBB) caused by *Xanthomonas campestris* pv. *Phaseoli*. The problem arises since the use of clean seed is not a common practice among farmers, and none of the landrace or improved cultivars have an acceptable level of resistance to CBB (Singh and Munoz, 1999. There is also evidence for genetic diversity and pathogenic variation of CBB in the region (Mkandawire et al., 2004). In this research opaque black seeded bean lines carrying SCAR markers SU91 and SAP6 were tested and rated for CBB reaction across locations in the Mexican highlands.

A trial set up as a 7X7 simple lattice design was established after the onset of the rainy season (july) at several locations in the highlands of Mexico (Table 1) during 2005. The trial included 47 advanced lines plus two checks: Negro 8025 (susceptible) and VAX 6 (resistant). VAX 4 and Negro Tacana (DOR 390) were included for comparison in two locations. All lines included carry the SCAR markers SU91 and SAP6 (1). Plots were two-six m rows spaced at 30 inches. CBB reaction was scored on the foliage during the reproductive stage (different Days After Planting – DAP) using the 1 to 9 scale (2). Only in one location scores were also taken on the incidence of CBB on pods.

The weather was particularly dry across all locations in 2005 during the reproductive stage of growth (data no shown). Across locations, Durango displayed the highest incidence of CBB, while lower incidence was registered in Celaya at 42 DAP and in Madero at 62 DAP (Table 1)., VAX 6 displayed less susceptibility to CBB than Negro 8025 across all test sites. This was expected since VAX6 was specifically bred for CBB resistance (Singh and Munoz, 1999) and is important to point out that the resistance in this cv was effective throughout the region. In general, improved lines displayed lower CBB average values than the parental cv Negro 8025 and within each group of lines there were some with the same or higher level of resistance as VAX 6, the resistant donor. On the pod readings small differences among the groups of lines were observed, the population derived from (DOR 500/8025)//VAX 4 displayed the highest score.

All improved lines before being scored for the SCAR markers (Ibarra et al., 2005), were selected on the basis of seed yield and commercial seed quality, thus in addition to adaptation, important progress is being made on the resistance to CBB, particularly if it is considered that high levels of CBB resistance have been not found in common bean (Singh and Munoz, 1999). In this research emphasis is being made on the improvement of opaque black seeded cultivars due to its high demand by consumers, however, beans in other important market classes such as pinto, pink and yellow, also need enhanced resistance to CBB. For this new sources of resistance are needed with adaptation to temperate and semiarid highland environments since the VAX lines are mainly adapted to the humid tropics.

Table 1. Field reaction to CBB on leaves of 47 bred lines carrying the SU91 and SAP6, SCAR markers. Test sites in the highlands of Mexico during the 2005 rainy season.

Population	# of lines	Durango 77 DAP[1]	Madero 62 DAP	Chihuahua 56 DAP	Celaya 42 DAP	Celaya 77 DAP	Celaya[2] 81 DAP	Popn. Ave.
8025//8025/VAX6	5	5.3 (5-6)[3]	3.5 (3-5)	4.1 (3-6)	2.1 (1-3)	4.5 (3-6)	3.0 (2-4)	3.4 (2-5)
Tacaná/VAX6// 8025/VAX6	11	5.3 (4-7)	2.9 (2-4)	4.2 (3-6)	2.1 (1-4)	4.1 (3-6)	2.9 (2-4)	2.9 (2-4)
DOR500//8025/VAX4	5	6.3 (4-7)	4.3 (3-4)	5.7 (3-6)	2.5 (1-3)	5.3 (4-6)	4.3 (2-5)	3.2 (1-6)
8025/VAX6	26	4.9 (3-7)	3.2 (2-4)	4.2 (3-7)	2.0 (1-3)	4.2 (3-6)	3.3 (2-5)	2.8 (1.5)
VAX6		5.5	3.0	5.0	2.2	3.7	3.3	5.8
VAX4		4.0	4.0					
Negro 8025		7.0	4.0	7.0	3.3	5.7	3.0	5.0
Negro Tacaná		5.0	4.0					
Site average		5.3	3.3	4.6	2.2	4.4	3.2	3.8
Standard deviation		0.830	0.510	0.995	0.483	0.668	0.681	0.428

[1] DAP = Days after planting.
[2] Reaction recorded on pods.
[3] Numbers in parenthesis are the minimum and maximum values

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DEVELOPMENT OF RACES OF Phytophthora phaseoli, THE CAUSAL AGENT OF DOWNY MILDEW OF LIMA BEAN (*Phaseolus lunatus*) AND DEVELOPMENT OF RESISTANCE.

T. A. Evans, R. P. Mulrooney and L. Santamaria.

Department of Plant and Soil Sciences, University of Delaware, Newark, DE.

Introduction

Over the past five years two new races of *Phytophthora phaseoli,* the causal agent of downy mildew, have been detected in Delmarva lima bean fields. During the 2000 growing season race E of *P. phaseoli* caused in excess of two million dollars damage to the lima bean crop. The 2003 growing season was similar climatologically to the 2000 season and a new phenotype of *P. phaseoli* was dominant, race F, yet losses were minimal. These losses were lessened in part due to the timely application of copper or Ridomil-Gold fungicides and to varietal selection based on a number of years of evaluation by our laboratory. Varietal resistance is the most economical control measure for downy mildew and development of two new races of this pathogen in such a short period of time has caused a problem for breeders providing resistant germplasm for the use of growers.

Materials and Methods

Lima bean varieties were tested during the 2004 and 2005 seasons for resistance to the recently identified races E and F of *Phytophthora phaseoli,* separately, at the University of Delaware's Research and Education Center near Georgetown and the University of Delaware Agricultural Experiment Station, Newark. Results from 2004 and 2005 were similar and the data from 2004 is reported herein. Plots were arranged in a randomized complete block design with four replications. Each plot consisted of one fifteen-ft row. Rows were spaced 30 inches apart and the middle ten feet of each row was evaluated for the percent of plants infected at approximately 2 weeks after inoculation and percent of pods infected at harvest. The soil types for the two sites were Falsington loamy sand and Matapeake silt loam, respectively. Irrigation was supplied as needed throughout each growing season. Varieties were planted 10 July (Georgetown) and 15 July (Newark). A susceptible variety (either M-15 or Cypress) was planted every five rows to facilitate disease spread. Sporangial suspensions of race E and race F of *P. phaseoli* were applied, individually, on 3 Sept (Georgetown) and 16 Sept (Newark). A first inoculation took place on 29 Aug for both locations and a second inoculation on 11 Sept. In all cases, inoculations were carried out in the evening using a backpack sprayer and after inoculation plants were misted nightly to increase humidity and leaf wetness. Percent of plants and pods infected were determined 2-3 weeks after inoculation and as plots reached approximately 10% dry pods.

USDA and CIAT (International Center of Tropical Agriculture) germplasm of *Phaseolus lunatus* was evaluated for resistance to races E and F of *P. phaseoli*. An evaluation of 219 accessions from different geographic regions of the world for their horticultural characteristics and resistance was carried out. In April 2003 we received 109 accessions from the germplasm collection of CIAT and 110 from USDA-Germplasm Resources. These sources had 763 accessions from which we selected 219 accessions representative of different geographic regions with approximately 75 % baby and 25% Fordhooks types.

During the 2003 season a field evaluation of horticultural characteristics was made at the University of Delaware's Agricultural Experiment Station, Newark, DE. The characteristics

observed included plant architecture, days to flowering, flower color, pod set, germination and stand count. Five seeds of each accession were planted on 30 June. The 219 accessions were planted in 30-inch rows and 2 foot spacing within rows. Observations were initiated on 7 Aug at the onset of flowering and was continued until 27 Aug. Of the 219 accessions, 87% were determined to be vine types and 13% bush types. Twenty-five varieties determined to be horticulturally acceptable were evaluated in the field for resistance to *P. phaseoli* races E and F during the summers of 2004 and 2005 using the methods previously described..

Results and Discussion

We can conclude that the varieties 184-85, Cypress and C-Exp 122 are resistant to race E and M-15 and 8-78 are resistant to race F (Table 1). The cultivar Sussex was determined to be moderately susceptible to both race E and F and no variety tested was resistant to both race E and F. It should be noted that the variety Cypress, which was determined to be susceptible to race F in these trials, was determined to have a "slow mildewing" characteristic. When large plots of only the variety Cypress were inoculated with race F it performed like a resistant variety, presumably because of reduced levels of secondary inoculum.

From the USDA and CIAT disease resistance trial two accessions were identified that as highly resistant to race E (PI-189403) and PI-549456) and one with resistance to race F (PI-549456). No single accession was resistant to both races tested. These three accessions may serve as new sources of resistance for breeders.

Table 1. Evaluation of lima bean varieties used in the Mid-Atlantic States for resistance to races E and F of *P. phaseoli*.

Location and cultivar	Type	Plants infected 28 Sep, %[z,x]	Pods infected 12-19 Oct, %[y,x]	Yield, lb/A	Rating by race
NEWARK					Race E
122-03-205	Baby	2.2 c[w]	0.00 e[w]	5248.6 a[w]	R
C-elite-Select	Baby	2.5 c	0.75 de	4828.1 a	R
Cypress	Baby	4.0 c	1.00 de	5138.2 a	R
184-85	Baby	4.2 c	1.25 d	3781.4 b	R
Sussex	Fordhook	50.7 b	41.00 c	1294.4 cd	S
Henderson Bush	Baby	98.0 a	38.50 c	1387.6 c	S
Dixie Butter Pea	[v]	99.0 a	43.00 c	1199.3 cd	S
M-15	Baby	99.2 a	51.50 c	467.7 de	S
Eastland	Baby	100.0 a	69.75 b	138.3 e	S
8-78	Baby	100.0 a	100.0 a	0.0 e	S
GEORGETOWN					Race F
122-03-205	Baby	76.5 ab	23.0 a	1787.5 cd	S
C-elite-Select	Baby	97.5 a	20.2 a	2144.8 bc	S
Cypress	Baby	55.5 b	12.5 a	3046.2 ab	S
184-85	Baby	85.2 ab	22.2 a	2120.9 bc	S
Sussex	Fordhook	17.7 c	13.7 ab	866.0 d	S
Henderson Bush	Baby	61.7 b	16.2 a	3128.7 ab	S
Dixie Butter Pea	[v]	65.2 b	17.5 a	2863.9 abc	S
M-15	Baby	9.7 c	3.0 b	1991.1 bcd	R
Eastland	Baby	5.0 c	3.2 b	3867.3 a	R
8-78	Baby	8.5 c	1.0 b	2831.4 abc	R

[z] Includes infection of any plant part including racemes, petioles or pods.

[y] At harvest at 10% dry pods.

[x] Arcsin transformed percentage data were analyzed, Fisher's Protected LSD (P=0.05) was used to compare means, and detransformed means are presented.

[w] Means followed by the same letter are not significantly different (Fisher's LSD, P=0.05).

[v] Neither baby or Fordhook seed type.

EPIDEMIOLOGICAL STUDIES OF DOWNY MILDEW OF LIMA BEAN CAUSED BY *Phytophthora phaseoli.*

L. Santamaria, T.A. Evans and R.P. Mulrooney,
Department of Plant & Soil Sciences, University of Delaware, Newark, Delaware, 19716.

Downy mildew of lima bean, caused by *Phytophthora phaseoli* Thaxt., is the number one disease associated with lima bean production on the east coast. One aspect of the management of downy mildew is predicting when it may occur. Forecasting systems are typically based on weather factors like temperature, rainfall, leaf wetness and relative humidity. We believe that timing and duration of leaf wetness is a major factor in the development of downy mildew of lima bean. There is one existing forecasting system based on weather monitoring for downy mildew (Hyre, 1959). This model was developed prior to the existence of the current races of *P. phaseoli.* The Hyre model used rainfall and temperature to predict disease development. The objective of this research was to determine if leaf wetness duration and timing is a principal environmental condition, which predicts the development of downy mildew, and to consider if other environmental factors such as temperature, humidity, and rainfall contribute to disease development.

Materials and Methods

Field experiments were carried out in the summers of 2003, 2004 and 2005 to evaluate the effect of leaf wetness duration and timing on disease development and spread. The field plot was established at the Experiment Station of the University of Delaware, Newark. The cultivar Eastland, susceptible to race E of *P. phaseoli,* was used in these experiments. Variables monitored were leaf wetness, rainfall, relative humidity, temperature, wind speed and direction. Data was recorded with WatchDog data logger (model 450 - Spectrum Technologies, Inc. Plainfield, IL) installed in each of the study plots. Leaf wetness sensors, Tipping-Bucket Rain Gauges were placed in the center of each experimental plot, and one Wind Speed and Direction Weather Station for all the experimental area. Irrigation and mist: a low pressure/low volume misting system was installed. RainBird MicroSpinners controlled by battery-operated timers were used to provide the desired leaf wetness. Supplemental irrigation was applied as required to lima beans by trickle irrigation. Sporangia release pattern: a Burkard 7-Day Recording Spore Trap was placed in a plot of each treatment. At the end of 7-days, the tape of each spore trap was removed, cut into sections representing daily periods, and then examined microscopically for sporangia. Spore trap data was taken over the course of three weeks. Leaf wetness treatments**:** to test the effect of different leaf wetness durations on disease development, four treatments with different misting schedules were established with three replications during Summer 2003. 1) No mist; 2) 10 ON/ 5 OFF min; 3)30 ON/ 20 OFF min; 4) 1 hour ON/ 1hour OFF. The treatments were applied between 12 am to 6 am. Based on the 2003 results and supplemental greenhouse and growth chamber experiments a different misting timing was applied for Summer 2004 and 2005. 1) No mist – Natural conditions; 2) 4 PM – 8 PM; 3) 8 PM – 12 AM with four replications. Plots of 20x20 ft were inoculated in the center area of each one (3x3 ft) with 200 ml of a sporangial solution of $2x10^4$ sporangia/ml of *P. phaseoli* (race E). Inoculum was applied with a hand atomizer, and obtained by inoculating Concentrated Fordhook seedlings in the greenhouse. Disease Evaluation and Development**:** plots were evaluated from the first day of infection (day 7) and every three days thereafter for development of typical symptoms of downy mildew on pins, pods and racemes. For the development and spread of disease, newly infected plants were marked with a different color flag and mapped. Horsfall-Barratt Disease Severity Ratings were taken at the same time. Disease progress was monitored over a three-week period.

Results and Discussion

Four hours of leaf wetness was determined to be the minimum to establish infection and significant sporulation. The 2003 spore trap data demonstrated an early afternoon release of sporangia for *P. phaseoli.* Data for three spore traps were similar over the course of the experiment. Burkard spore trap data indicated the release of sporangia between 11AM to 3PM daily (Fig. 1). This suggested that leaf wetness in the afternoon could be beneficial for epidemic disease development. Field experiments during

summer 2004 showed a significant difference between treatments with mist and natural conditions. The same experiment was repeated during the summer 2005, with similar results (Fig. 2). Disease incidence increased significantly about day 12 after inoculation. The number of infected plants increased rapidly in the mist treatments. Data was collected until the disease reached the plot border (Fig. 3).

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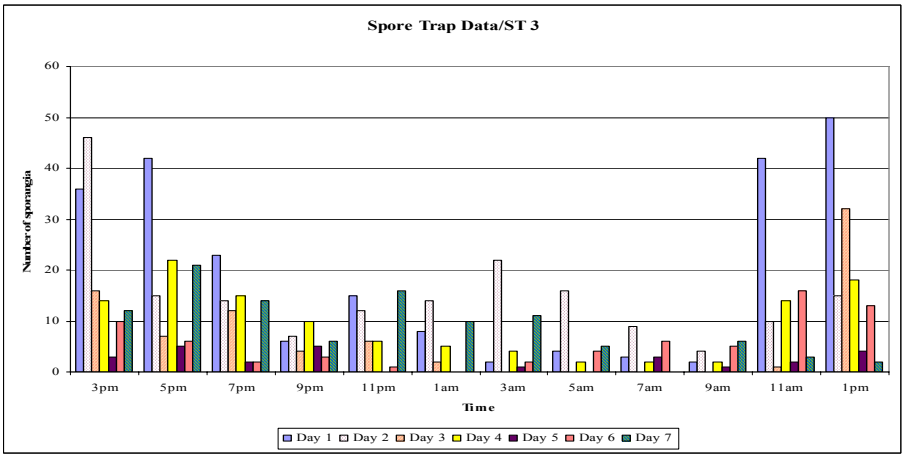

Figure 1.- Release pattern of sporangia of *P. phaseoli*. Data collected from tape of Burkard Spore Traps. Week data (9/5 -9/12, 2003) of the number of sporangia

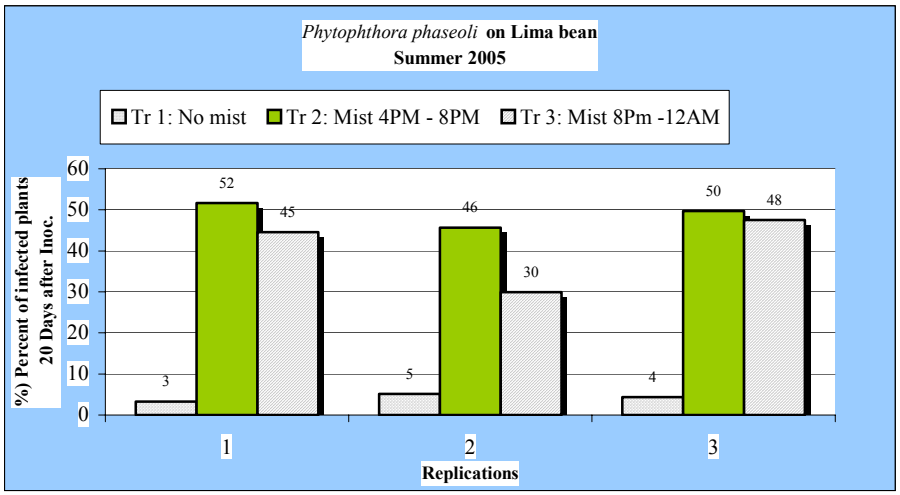

Figure 2.- Disease Incidence –Summer 2005

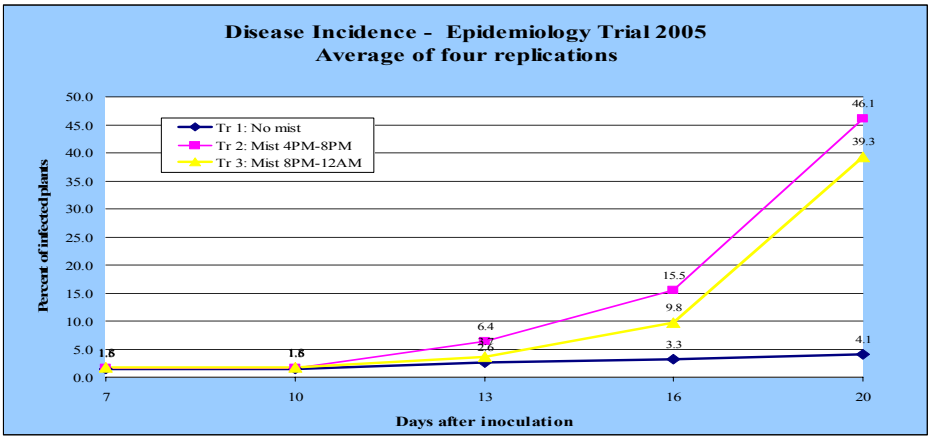

Figure 3 - Area Under the Disease Progress Curves for Various Leaf Wetness Duration and Timing

THE 'MAFFEI 15' LIMA BEAN COMPENSATES FOR REDUCED PLANT STAND

Pill, Wallace G., Thomas A. Evans, Michael W. Olszewski, Robert P. Mulrooney, and Walter E. Kee Jr.

Department of Plant and Soil Sciences, University of Delaware, Newark, DE 19717-1303

Abstract. 'Maffei 15' baby lima beans seeds were sown every 6 cm in rows 76 cm apart to yield a nominal stand of 215,000 plants/ha at two locations in Delaware over two years. Seedlings were thinned within two weeks of planting to provide 0, 16.7, 33.3 and 50.0 % stand reduction at two in-row spacing patterns to determine subsequent effects on vegetative and reproductive growth. Shoot fresh weight/m^2 was decreased only in 2003 by 21% and bean fresh weight/m^2 was decreased only in 2004 by 13.8% when plant stand decreased to 50%. This disproportional vegetative and reproductive growth response to stand reduction resulted from a compensatory linear increase in shoot fresh weight, usable pod number, and bean fresh weight of individual plants. Thus, the 'Maffei 15' lima bean tolerates a considerable loss of plant stand with little or no effect on yield.

The study was conducted during June – September on Kalmia loamy sand (fine loamy silicaceous, thermic Typic Hapludult) near Georgetown, Del. (lat. 38.7° N, long. 75.3° W) in 2003, and on Matapeake silt loam (fine silty, mixed mesic Typic Hapludult) in Newark, Del. (lat. 37.3°N, long. 75.5°W) in 2004. Seeds of 'Maffei 15' lima bean were machine planted every 6 cm in rows 76 cm apart to provide a nominal stand of 215,000 seeds/ha. Plant population densities were created by hand–removal of plants within two weeks of planting. In addition to the full stand (0% stand reduction), three percentages of stand reduction were created at two in-row spacings (gaps) by removing plants. The percentages and gaps were: 16.7% (one plant out of every consecutive six, or two consecutive plants out of every twelve); 33.0% (one plant out of every consecutive three, or two consecutive plants out of every six; and 50% (every other plant, or two consecutive plants out of every four). Each treatment consisted of four 6 m-long rows.

The 4 (stand reduction) by 2 (gaps) factorial experiment was arranged in randomized block design with four replications. Blocks consisted of four 6m long rows per treatment with two border rows on each side. Plots received 90 kg N/ha from 14N-3P-12K (14-7-14) on the day of planting. Imazethapur herbicide was incorporated preplant at 36g a.i./ha. Manual cultivation subsequently controlled weeds. Other pest control measures followed Univ. of Delaware recommendations (Univ. of Delaware, 2003). Plots received at least 50 mm of water each week from rain or irrigation from planting to harvest.

At the time of harvest, plants from the central 3m of the two inner rows of each treatment were pulled out of the ground, counted and weighed. Pods were manually stripped from plants and separated and counted as flat (immature), usable (green), and dry (overly mature). The green pods were threshed mechanically and the seed fresh weight (economic yield) determined. All data were recorded on a per plant and per unit area (m^2) basis. In 2004, the numbers of nodes and branches on 10 plants from each treatment-replicate combination were counted.

Since in-row gaps had no effect on any variable in either year, only the results of percentage stand reduction are reported. The 17.8 and 20.0 plants per m^2 achieved in 2003 and 2004, respectively, with no stand reduction represented 83% and 93% of the potential stand of 215, 000 plants/ha. The nominal 16.7, 33.3 and 50.0% nominal stand reductions were, respectively, 23.6, 35.9 and 49.4% in 2003, and 19.5, 31.5 and 49.5% in 2004.

As stand decreased from 100% to 50%, shoot fresh weight/m^2 decreased only 21% in 2003 and was unaffected in 2004; while bean fresh weight/m^2 was unaffected in 2003 and decreased only 13.8% in 2004. This absence or less than proportional decrease in shoot or bean fresh weight in response to stand reduction reflects the ability of plants to respond vegetatively and reproductively in a compensating manner to the decreasing population density.

The numbers of flat, dry or usable pods/m^2 were unaffected by stand reduction in either year. In both years, the usable pods were 87% of the total pod number per plant, indicating that plant population density had no effect on crop maturation rate.

Linear increases in shoot fresh weight, number of usable pods and bean fresh weight of individual plants in response to decreasing stand during both years confirmed the ability of plants to respond positively to reduced population density. The positive relationship between the number of usable pods or bean fresh weight with the numbers of nodes and branches per plant in response to decreasing plant stand may indicate that the increase in branches supported the increase in reproductive structures.

The reproductive structures of lima beans are indeterminate racemes, with the first flowers produced on the early inflorescences being the most critical for fruit production. It remains unknown whether the greater pod set/retention and bean fresh weight of individual plants with decreasing plant stand was associated with more racemes borne on more branches. The greater leaf area per plant may have decreased the temperature and increased the relative humidity within the canopy, conditions known to favor pod set and retention.

The results of this study have shown that reducing ‘Maffei 15’ lima bean stand by up to 50% reduced bean fresh weight per unit area by only 16%, averaged over two years. This disproportional relationship resulted from increased vegetative growth and reproductive yield of individual plants which compensated for the reduced plant stand. It remains unknown whether indeterminate lima beans would compensate similarly for reduced plant stand.

SELECTION FOR DROUGHT RESISTANCE IN DRY BEAN LANDRACES AND CULTIVARS

Carlos German Muñoz-Perea[1], Henry Terán[1], Richard G. Allen[1], James L. Wright[2], Dale T. Westermann[2], and Shree P. Singh[1]
[1]University of Idaho, 3793 North 3600 East, Kimberly, Idaho; [2]USDA-ARS-NWISRL, 3793 North 3600 East, Kimberly, Idaho

INTRODUCTION

Drought is a worldwide production constraint affecting >60% area planted to dry bean. On the American continents, drought is endemic in northeastern Brazil (>1.5 million ha) and in the central and northern highlands of Mexico (>1.5 million ha) where drought is often intermittent, and complete crop failures are common. In Central America, moderate drought towards the end of the cropping season is also frequent. Moreover, in Chile, coastal Peru, and the western and intermountain U.S. characterized by inadequate summer rainfall (<75mm), terminal drought is even more likely and bean cannot be grown without supplemental irrigation.

The effects of drought vary depending upon the frequency, duration, and intensity of stress and growth stages affected. Moderate drought during vegetative and reproductive stages reduced yield by 20% and 50%, respectively. Excessive flower, ovule, and pod abortions occur due to drought during pre-flowering and fruiting periods. Moderate to severe drought stress reduces biomass yield, number of seeds and pods, days to maturity, harvest index, seed yield, and seed weight. Also, drought stress has reduced yield, nodulation and N_2-fixation, N harvest index, and N- and water-use efficiency. Root rots caused by *Macrophomina phaseolina* (Tassi) Goid., *Fusarium solani* f. sp. *phaseoli* (Burk.) Snyder & Hansen, and other fungi may aggravate drought stress. Similarly, drought-stressed cultivars are prone to damage by leafhoppers (*E. kraemeri*) in the tropics and subtropics.

In the western U.S., the ratio of agricultural use over total use of water is the highest in country. For example, this ratio is 98% in Idaho, 82% in Oregon, 92% in Colorado, and 95% in Wyoming. Moreover, the fresh water resource is becoming increasingly stretched between irrigated agriculture, endangered species, water quality needs of municipalities, and recreation. Water is routinely transferred away from agriculture, so that in many areas, irrigated agriculture must develop cultivars requiring lower inputs of water if a viable economic base is to be sustained. Moreover, reduced water, nutrient, and pesticide use efficiency, as well as increased production costs have become severe problems for bean growers.

Seed yield under drought-stressed (DS) and non-stressed (NS) conditions, reduction in seed yield due to drought stress, and drought susceptibility index are the most reliable integrated measures of cultivar response to drought. In common bean, the highest level of drought resistance is found in the race Durango cultivars that were domesticated in semi-arid Mexican highlands. Some cultivars of races Jalisco and Mesoamerica also carry moderate to high levels of drought resistance.

Materials and Methods

Sixteen dry bean landraces and cultivars of great northern, pinto, and red market classes were evaluated under NS and DS conditions at Kimberly, Idaho in 2003 and 2004. The NS treatment received seven irrigation in 2003 and five irrigation in 2004, and DS only four in 2003 and two

in 2004. Each plot consisted of 8 rows, 25 ft long with 4 replicates arranged in a randomized block design. The DS and NS plots were sown in the same field at the same time. Water use efficiency (WUE) was measured for six cultivars under NS and DS conditions. Hansen data-loggers and watermarks were installed at three depths (0.23, 0.46, and 0.92 m) to record water potential. In addition, gravimetric soil samples were taken at 11 points up to 2 m depth before and after each irrigation. Plant and seed uptake of nutrients, biomass and seed yield, harvest index, 100-seeds and days to maturity were evaluated.

RESULTS AND DISCUSSION

Large differences among years and dry bean cultivars were recorded for biomass and seed yield, harvest index, WUE, percent yield loss due to drought stress (PR), and drought susceptibility index (DSI). Water use was higher within the top 0.5 m in NS and DS environments. No significant water use was recorded below 0.5 m depth in both test environments. Drought stress was severe in 2003 (drought intensity index 0.62) than in 2004 (DII 0.27) probably due to delayed first irrigation, soil compaction, other management practices, and higher temperatures and solar radiation. Mean biomass and seed yield, harvest index, water and nutrient uptake, WUE, and nutrient harvest index reduced due to drought stress. All early maturing cultivars (Common Pinto, LeBaron, Topaz, UI 320, and US 1140) except Othello were drought susceptible. Severe drought stress in 2003 permitted identification of Common Red Mexican and Mesa having high level of drought resistance with high yield in DS and NS conditions, and Matterhorn and Othello with high yield in DS and moderate yield in NS. NW 63 and UI 239 had intermediate level of drought resistance. All drought resistant cultivars had high nutrient plant and seed uptake and Othello and Common Red Mexican had high WUE which decreased from the landrace to modern cultivars of red market class of race Durango.

Table 1. Mean seed yield (kg ha^{-1}) of drought resistant and susceptible dry bean cultivars evaluated in drought-stressed and non-stressed conditions at Kimberly, Idaho in 2003 and 2004.

Identification	2003				2004			
	Non-stressed	Drought-stressed	Percent reduction	DSI[1]	Non-stressed	Drought-stressed	Percent reduction	DSI[1]
Common Red Mexican	2162	1164	46	0.7	3671	2836	23	0.8
Matterhorn	1905	979	49	0.8	3458	2699	22	0.8
Mesa	2039	1312	36	0.6	4143	3040	27	1.0
Othello	1805	1199	34	0.5	3611	2762	24	0.9
UI 259	1850	471	75	1.2	4031	2928	27	1.0
UI 320	1407	502	64	1.0	3645	2415	34	1.2
LSD (0.05)	204	630			422	385		

[1]Drought Severity Index: values <1 indicate drought resistance and 1> drought susceptibility.

LOW PHOSPHORUS TOLERANCE IN AN ANDEAN BEAN POPULATION

Karen A. Cichy[1], Matthew Blair[2], and Sieg Snapp[1]
[1] Department of Horticulture, Michigan State University, East Lansing MI
[2] Biotechnology Unit CIAT (Centro Internacional de Agricultura Tropical)

Introduction

Acid weathered soils of the tropics and subtropics are often low in available phosphorus. Common bean is especially susceptible to low P soils in part due to phosphorus required for nodulation and N fixation. Low P soils are a major constraint to bean production in regions of Africa and Latin America where farmers lack access to sufficient P fertilizer (Wortmann et al 1998). Variability for tolerance to low P soils has been identified in the Andean gene pool (Yan et al 1995). Tolerance to low P soils can be achieved by two distinct routes: uptake efficiency and utilization efficiency. Uptake efficiency is the ability to extract more P from the soil and has been shown to be related root system traits that increase the root surface area and allow capture of more P form the soil, including specific root length, root hair density and mychorriaze (Gahoonia and Nielsen. 2003). Utilization efficiency relates to tissue P requirement and P remobilization. The objectives of this study were to determine the type of efficiency that explained variance in tolerance to low P soils in a recombinant inbred line population that was developed from a cross between low P tolerant line G19833 and low P susceptible AND696.

Materials and Methods

Seventy seven $F_{5:7}$ recombinant inbred lines (RILs) were developed from a cross between the low soil P tolerant Peruvian landrace G19833 (type III growth habit) and the low soil P susceptible CIAT improved line AND696 (type 1growth habit). The RILs were segregating for determinancy, with 56 determinate and 21 indeterminate lines.

In 2005, the 77 RILs, G19833, AND696, and two check varieties were planted in Darien, Colombia in a 9x9 lattice design with three replications at two soil P levels, low P (45 kg/ha triple super phosphate) and high P (300 kg/ha triple superphosphate). The soil of this site in an Andisol with a native soil P of 2 mg/kg based on bray II extraction method. Various plant measurements were taken to elucidate underlying factors involved in the parental genotypes differences in tolerance to low soil P, including shoot biomass and P concentration at mid podfill, seed yield, 100 seed weight, and P concentration.

Results and Discussion

Soil P level had a significant impact of shoot P concentration, P uptake, P utilization, seed yield, and seed P concentration (Table 1). There was not a significant genotype effect for P uptake. There was however a significant genotypic effect on P utilization. These data indicate the difference seen in seed yield among the genotypes was not due to P uptake differences, but to differences in P utilization within the plant. The correlations (Table 2) show a positive correlation between P uptake and seed yield in low P soil demonstrating that although genetic variation for the trait was not observed it remains an important determinant of yield. There was also a weak but significant negative correlation between seed P concentration and P utilization, indicating that P use efficiency contributes to yield variability.

Table 1: Means and ANOVA output for plant growth data in a split-plot design where soil phosphorus level is the whole plot and genotype is the split-plot.

Variable	Mean Values		Effect	Degrees of Freedom	F value	Significance at 0.05**
P in shoot (g/kg)	P treatment	mean	Phosphorus level	1	1806	**
	low P	2.10	Genotype	78	1.99	**
	high P	3.36	P level * genotype	77	1.12	NS
P uptake (mg P/g dw)	low P	170	Phosphorus level	1	1610	**
	high P	576	Genotype	78	1.05	NS
			P level * genotype	77	0.95	NS
P utilization (g seed/mgP)	low P	0.27	Phosphorus level	1	286.95	**
	high P	0.17	Genotype	78	2.51	**
			P level * genotype	77	1.00	NS
Seed Yield (g/meter)	low P	43.5	Phosphorus level	1	949	**
	high P	92.5	Genotype	78	2.86	**
			P level * genotype	77	1.53	**
Seed P (g/kg)	low P	3.14	Phosphorus level	1	1304	**
	high P	4.05	Genotype	78	3.70	**
			P level * genotype	77	1.36	**

Table 2: Correlation between seed yield and plant P levels under low soil P.

Variable	r value	Significance at 0.05**
Seed yield &P in shoot	0.33	**
Seed yield &P uptake	0.57	**
P utilization & Seed P	-0.22	**

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BEYOND PARTICIPATORY BEAN BREEDING: A CANDO (CLIENT, AGROECOLOGICAL NICHE AND DEVELOPMENT ORIENTED) APPROACH IN MALAWI

Sieg Snapp[1*], Anne Ferguson[2], Henry Mloza-Banda[3], Courtney Gallaher[1] and Karen Cichy[1], [1]Dept of Horticulture and Dept. of Crop and Soil Sciences, Michigan State University, East Lansing MI, [2]Dept of Anthropology, Michigan State University, East Lansing MI, [2]Dept of Crop Science, Bunda College of Agriculture, University of Malawi, Lilongwe, Malawi
*Presenter (snapp@msu.edu)

Introduction. Participatory and decentralized plant breeding strategies have been developed to improve the ability of plant breeders to select bean cultivars that are adapted to local conditions and to farmer preferences. Given the diversity of climate and edaphic constraints and limited resources of smallholder farmers, it is particularly challenging to develop appropriate cultivars and cropping system combinations for southern Africa bean systems. We report here on a project to promote participatory assessment and intensification of bean production in the fertile inland valleys of Malawi, using a 'CANDO' approach (Client, Agroecological Niche and Development Oriented). Agroecological analysis was combined with surveys to identify promising bean cultivars for diverse niches. This expands on participatory approaches to include consideration of equity, livelihoods and environmental sustainability of intensified bean systems.

The pilot study site chosen was the Chingale watershed along the Shire River, in southern Malawi. The site is representative of areas of intensive riverine and wetlands cultivation, areas which are undergoing intensification of cultivation, throughout southern and eastern Africa. Recently, national governments and donors have turned to dry season cultivation and informal irrigation as means to improve food security and cash incomes for the poor. These systems are ideal for bean seed and grain production.

In 2002 a multidisciplinary collaboration of anthropologists, agricultural economist, and crop and soil scientists was initiated with Michigan State University, Bunda College of Agriculture, University of Malawi, Chingale farmers and staff of World Vision-Zomba, a non government organization. This bean intensification, soil and water management study was funded by the Bean/Cowpea CRSP of the USAID. On-farm participatory trials evaluating improved bean lines and soil/water management systems were carried out with 27 farmers in conjunction with social science studies and training of farmers and farm advisors from World Vision and Malawi Extension. A comprehensive survey of 37 farmers was carried out in 2005 that investigated project impact and issues of equity and sustainability of bean intensification in vulnerable wetlands. The survey instrument included formal and semi-structured questions addressing topics of intensification of bean production, irrigation and soil management, and farmer knowledge gained from the Bunda/WVI projects. In combination with the interview, soil sampling was conducted of respondent bean fields (three samples per field, a random transect with four sub-samples composite per sample). Soil analysis was conducted at MSU to determine total carbon, nitrogen, available phosphorus (MehlichIII), pH and soil texture.

Results. To evaluate growth habitat and performance within different irrigation systems, the participatory trials included contrasting growth types, a bush type II and a climbing type IV. The type II bean line (BCMV-B2) and Type IV bean line (IZ 309-1) were evaluated under the five

water management systems described below in Table 1. Grain yield was higher for the type II line, presumably due to the adaptability of this genotype to the relatively late-season planting that is required at sites located near the Shire River which are flooded early in the season. The high-yield potential of hillside sites with gravity-fed irrigation is presumably due to early planting (allowing longer-duration pod fill) and access to water late into the season from the relatively abundant riverine water resources of this system.

Table 1. Yield data from on-farm trials carried out in Chingale, 2003 (n=27)

Treatment	Plant Height at Maturity		Grain Yield (kg/ha)	
	Type II	Type IV	Type II	Type IV
Motorized Pump	103.2	204.9	1292.0	947.0
Treadle Pump	54.1	167.3	1349.0	546.0
Well – Watering Can	49.2	141.0	1161.0	632.0
Gravity Irrigation	76.0	241.2	3124.0	1882.0
Residual Moisture	48.8	171.3	975.0	519.0
Mean	66.3	185.1	1580.0	905.0
LSD (0.05)	17.1	22.5	827.2	896.8

Upscaling of bean production using the recommended agronomic and water management techniques took place during 2004 involving 20 farmers, 55% women. The results demonstrated that women were as successful as men in bean production. Women produced a total of 7,428 kg versus 3,525 kg of beans produced by men, when provided with the same amount of initial seed.

The 2005 survey revealed that farmers had gained experience with different Bunda College-developed bean lines. Farmer assessment of varieties is presented in Table 2, which shows that farmers have a strong preference for large seed types (AND 659, Nanyati, Kalima and Maluwa). This is in support of previously identified quality traits preferred for home consumption and market sales (Ferguson 1994).

Table 2. Male and female farmer assessment of varieties preferred in 2005 (n=37)

	BCMV-B2	IZ 309-1	PC 490-D8	AND 659	Nanyati	Kalima	Maluwa
	Positive Response (%)						
Male	60	20	-	80	85	88	90
Female	46	36	100	66	91	91	100

Conclusions. We found that short-duration bean cultivars were ideal for a wide range of niches in the environment, including diverse irrigation systems. Climbing bean had high yield potential but require improved heat tolerance to perform well at lower altitudes over a long season. Farmer evaluation and on-farm performance indicated that root rot tolerance and seed quality traits (large size and light color) are top priority areas for genetic improvement. The improved bean lines evaluated by farmers in this study require further plant breeding attention to develop the preferred large seed type. The soil characterization undertaken was consistent with considerable investments in fertility by smallholders, and the long-term sustainability of intensified bean production over the dry season in inland valleys of southern Africa.

MANAGING WHITE MOLD OF LIMA BEANS WITH REDUCED RISK FUNGICIDES AND BIOFUNGICIDES.

Kathryne L. Everts[1, 2] and Xin-Gen Zhou[1]
[1]Natural Resource Sciences and Landscape Architecture, University of Maryland, Salisbury.
[2]Plant and Soil Science Department, University of Delaware, Newark.

INTRODUCTION

White mold (*Sclerotinia sclerotiorum*) of lima beans (*Phaseolus lunatus*) is an endemic disease in Delaware and Maryland. Acreage in coastal regions and along the Delaware Bay often experience yield losses of 20%. During the development of a pest management strategic plan in 2003, lima bean producers ranked white mold as the most important disease problem. At the same time, fungicide options for managing white mold were limited following the loss in availability of benomyl.

MATERIALS AND METHODS

Experiments on the efficacy of 1) reduced-risk fungicides and biofungicides and 2) the soil-applied biofungicide *Coniothyrium minitans* were conducted on growers' farms in Delaware in 2003 and 2004. All experiments were arranged in randomized complete block design with four replications. Fungicides were applied with a CO_2 backpack sprayer that delivered 420 l/ha at 3.0 kg/cm^2.

In 2003, the Fordhook lima bean cultivar 'Sussex' was seeded on 27 June to a grower's field near Harbeson, DE. Plots were 3.6 m wide and 12.2 m long. Fungicides were applied on 6 August. White mold severity was determined by counting the number of infected pods in two rows, which were 1 m in length, and infected pods on the soil surface surrounding the rows (in a 1.5 m x 1 m area) on 17 September. Pods were removed from stems manually, infected pods were counted, and pods with no visible white mold were threshed and weighed.

The cultivar 'Cypress' was seeded on 16 July 2004, to a grower's field near Lewes, DE. Lima beans began flowering on 19 August. Plots for evaluation of *C. minitans* were 12.2 m wide and 9.1 m long. Treatments were 1) *C. minitans* (Contans 5.3WG at 2.2 kg/ha) applied on 23 July or 2) 3 August, 3) at both dates, 4) Thiophanate methyl (Topsin M 4.5 F at 2.9 l/ha) or 5) nontreated (Table 1). A second experiment evaluated fungicide efficacy and plots were 3.6 m x 9.1 m. In both experiments, the center row of each plot was harvested with an OxBo one-row bean picker on 13 October. To evaluate inoculum that remained in the field, the number of sclerotia in three randomly selected areas that were each 0.3 m^2 was counted in each plot on 14 October.

RESULTS

White mold severity was high in 2003 and 2004. Although nontreated plots had the lowest yield and the most sclerotia remaining on the soil surface, neither application of *C. minitans* nor thiophanate methyl significantly increased yield or reduced the number of sclerotia/m^2 (Table 1). On 17 Sep 2003, plots sprayed with Endura alone or in combination with Penetrator Plus had significantly fewer pods infected with white mold than nontreated plots (Table 2). Endura plus HyperActive, Serenade, Switch at 0.8 kg/ha and 1.0 kg/ha and Omega had intermediate levels of pods infected with white mold that were not significantly different than the nontreated plots. Plots sprayed with Endura, alone, with HyperActive, or Penetrator Plus, and Pristine had significantly higher yield than nontreated plots. In 2004, Endura, Topsin M, Serenade in combination with Topsin M, Sonata, Switch and Omega reduced the number of sclerotia/m^2 compared to nontreated plots. Yield was highly variable across the field and there were no significant differences among treatments.

Table 1. Lima bean yield and number of sclerotia on the soil surface following application of *Coniothyium minitans* (Contans) or Thiophanate methyl (Topsin M) in Lewes, DE. 2004.

Treatment and rate/ha	Date of application	Yield (kg/ha)	No. sclerotia/m^2
Contans 5.3WG 2.2 kg	23 July	3744	21
Contans 5.3WG 2.2 kg	3 August	2130	3
Contans 5.3WG 2.2 kg	23 July and 3 August	2757	26
Topsin M 4.5F 2.9 l	24 August	2533	20
Nontreated	---	1995	40
LSD (*P*=0.05)		N.S.	N.S.

Table 2. Lima bean yield and number of infected pods following application of fungicides on 6 Aug in a commercial field near Harbeson, DE in 2003.

Treatment and rate/ha	Infected pods/m^2 17 Sep	Yield (kg/ha)
Endura 70WG 0.5 kg + Hyper Active 0.7 l	2.3 cd*	1771 a
Endura 70WG 0.5 kg + Penetrator Plus 3.2 l……..	1.6 d	1928 a
Endura 70WG 0.5 kg…….....	1.8 d	1928 a
Topsin M 70WP 2.24 kg…….....	5.1 ab	1592 ab
Topsin M 4.5F 3.6 l…….....	3.9 abcd	1592 ab
Serenade 10WP 6.7 kg……..……....	2.8 bcd	1390 ab
Sonata F 9.4 l…….....	3.9 abcd	1188 b
Switch 62WG 0.8 kg……..	2.6 bcd	1524 ab
Switch 62WG 1.0 kg……..	2.8 abc	1501 ab
Omega 4 SC 0.6 l……..	2.3 bcd	1614 ab
Pristine 38WG 1.5 kg..…….....	4.6 cd	1771 a
Serenade 10WP 4.5 kg + Topsin M 70WP 1.1 kg ……....	6.6 a	1211 b
Nontreated ……....	4.8 abc	1211 b
LSD (*P*=0.05)	2.6	538

* Mean values in each column followed by the same letter are not significantly different at (*P*=0.05) according to Fisher's protected least significant difference test.

Table 3. Lima bean yield and number of sclerotia on the soil surface following application of fungicides on 24 Sep in a commercial field near Lewes, DE in 2004.

Treatment and rate/ha	No. sclerotia/m^2*	Yield (kg/ha)
Endura 70WG 0.6 kg + Penetrator plus 1.2 l	14 c**	2533
Endura 70WG 0.4 kg + Penetrator plus 1.2 l.....	14 c	1972
Topsin M 4.5F 2.9 l.....	19 bc	2331
Serenade 10WP 6.7 kg.....	40 ab	2825
Serenade 10WP 4.5 kg + Topsin M 4.5F 1.5 l.....	11 c	2511
Sonata F 9.4 l	11 c	2242
Switch 62WG 1.0 kg.....	15 c	2399
Omega 45 C 0.6 l	0 c	2331
Nontreated.....	50 a	2174
LSD (*P* = 0.05)	24	N.S.

*Mean value of sclerotia counted in three randomly selected 0.3 m^2 areas on the soil surface per plot.

**Mean values in each column followed by the same letter are not significantly different (*P* = 0.05) according to Fisher's protected least significant difference test

CHEMICAL CONTROL STRATEGIES FOR DOWNY MILDEW (*PHYTOPHTHORA PHASEOLI*) OF BABY LIMA BEAN.

Mulrooney RP[1*], Davey JF, Dept. Plant Pathology, Cornell Univ., Riverhead,NY, Evans TA[1].
[1]Plant and Soil Science Dept, University of Delaware, Newark, DE.

Introduction:

Since the emergence of new races of the *Phytophthora phaseoli* that are pathogenic on many of the current varieties of baby lima beans, fungicide applications for the control of downy mildew are important for the successful production of lima beans in the Mid-Atlantic region where downy mildew is endemic.

Since the major proportion of baby lima beans in the DE, MD and NJ region are planted for fall harvest, this time of the year also provides ideal conditions for downy mildew infection most years. Downy mildew was controlled for years by plowing down debris from the previous crop and planting resistant varieties. In the mid-90's the race composition of *P. phaseoli* began to shift and many of the resistant varieties were not resistant to the new races. Fungicide applications were needed in seasons where environmental conditions were favorable for infection. Fungicide trials were initiated again to find the most effective products and how to use them for maximum economic return.

Materials and Methods:

Fungicides were tested from 2003-05 for control of downy mildew of baby lima bean at the University of Delaware's Experiment Station Farm in Newark, DE. The baby lima bean cultivar Eastland was planted on from Jul 7- Jul 17 with a commercial four-row Monosem planter. Dual Magnum 7.62E (1.75 pt/A) and Pursuit 2SC (1.0 oz/A) were applied pre-emergence for weed control. The soil type was a Matapeake silt loam soil and nitrogen (60 lb/A) was side-dressed after seedling emergence. Treatments were arranged in a randomized complete block design with four replications. Each plot consisted of three sprayed rows, 20 ft long and spaced 30 in. apart. A single border row separated each plot. The middle 10 ft of the center row of each plot was evaluated for percentage of infected pods, percentage of infected plants, total number of pods/10 ft, and yield. All plots were inoculated with a sporangial suspension (10^3) of *Phytophthora phaseoli*, race E, in the evening using a Solo backpack sprayer when the plants reached full flower. The plots were misted nightly with a low pressure misting system equipped with low volume misting nozzles. The system was operated intermittently from dusk to dawn daily to increase humidity and favor infection. Supplemental drip irrigation was provided when needed throughout the growing season. Fungicides were applied using a backpack CO_2 pressurized sprayer that delivered 30 gal/A at 52 psi. Applications were made with a broadcast boom equipped with hollow cone nozzles (D4 disks, no. 45 cores). For the curative trials fungicides were applied as soon as the first symptoms were observed in the plots. The middle ten feet of the center spray row was evaluated for percentage of infected plants (presence of infection on the raceme, petiole or pod). The plants were harvested in a timely manner and the percentage of infected pods, total number of pods/10 ft, and yield were determined.

Results:

The fungicides that have been tested included in Table 1.

Contact Fungicides	Systemic fungicides (translaminar)
Fixed coppers ■ **Kocide 2000 DF** ■ **Champ DP** ■ **Champ 2F** ■ **Tri-basic copper sulfate** ■ **Cuprofixx Disperss** **Acrobat** **Bravo** **Biologicals** ■ **Sonata** ■ **Serenade**	■ **Curzate** ■ **Gavel** ■ **Previcur Flex** ■ **Tanos** ■ **Quadris (Amistar)** ■ **Headline (Cabrio)** ■ **Reason** ■ **Ridomil Gold/Copper** ■ **Phostrol**

During the past three years of testing the best programs have incorporated the use of Ridomil Gold/Copper or Phostrol for control of downy mildew. Two applications have usually been better than a single preventative application. Both products also provide early curative control as well if applied at the first sign of disease. Both products have been granted 24 (c) special local need registrations for use in DE. Ridomil Gold/Copper also has a 24 (c) registration in MD as a result of our work. Effective rates of Ridomil Gold/Copper is 2.0 lbs./A and optimum Phostrol rates are still being investigated but two applications of 2 pts were as effective as two applications of 4 pts in 2005. In situations where disease pressure is considered light and preventative applications are made, 2.0 lb rates of fixed copper such as Champ or Kocide have also been effective and are inexpensive. Our goal has been to identify fungicides with excellent efficacy and timing their application for maximum control. Headline has also been promising. It has not always provided the best disease control but it does provide good control and yield increases have been observed that equal or exceed the yields of the products that produce the best control. Headline when labeled will give growers a fungicide that will also provide control of soybean rust if it becomes an important disease of lima beans. Progress has been made in identifying good fungicides and the next step will be in utilizing forecasting models to determine the best time of application.

REACTION OF COMMON CULTIVARS TO THE ASIAN SOYBEAN RUST PATHOGEN, *PHAKOPSORA PACHYRHIZI*, UNDER FIELD CONDITIONS IN SOUTH AFRICA AND BRAZIL

M. A. Pastor-Corrales[1], M. M. Liebenberg[2], Aloisio Sartorato[3], P. A. Arraes-Pereira[4]
[1]Vegetable Laboratory, USDA-ARS, Beltsville, MD, 20705-2350, USA, [2]ARC-Grain Crops Institute, Private Bag X1251, Potchefstroom, 2520, South Africa, [3]Embrapa Arroz e Feijao, C.P.179, CEP 75375-000, San Antonio de Goias, GO, Brazil, [4]Embrapa/Labex, USDA, ARS, OIP, Beltsville, MD 20705-5141

Introduction

Phakopsora pachyrhizi, the fungus that causes the Asian soybean rust (ASR) disease is unusual for its capacity to infect soybeans (*Glycine max*) and some 95 other leguminous species, including dry and snap beans (*Phaseolus vulgaris*). Many of the reported hosts are from host-range studies conducted in the greenhouse involving artificial inoculations of single cultivars.

ASR was reported occurring in soybeans for the first time in South Africa in 2001 and in Brazil in 2002. Subsequently, ASR has caused devastating epidemics in both countries. ASR was also reported occurring for the first time in the continental US in November 2004. The ASR pathogen has also been recently reported infecting a dry bean cultivar under field conditions in South Africa (du Preez et al, 2005). There are no reports indicating whether *P. pachyrhizi* occurs in other common bean cultivars under field conditions and whether the ASR pathogen can cause major yield losses on dry and snap beans. This is the first report of the reaction of common bean cultivars to the ASR pathogen under field conditions in South Africa and Brazil.

Material and Methods

Due to constraints in international seed movement, only a few common bean cultivars, that had been used extensively in breeding dry and snap beans for resistance to the common bean rust *Uromyces appendiculatus* were selected for evaluation in Brazil and South Africa. In these countries, as well as in some dry bean producing US states, dry beans are often planted in fields adjacent to soybean fields. In Brazil, the trials were planted in Goiania and Rio Verde in the state of Goias. The common bean cultivars were planted in a field in very close proximity to a field of soybeans naturally infected with ASR. Leaves from top, middle and lower parts of the common bean plants were collected and evaluated for ASR severity using a 0-100 % scale. In South Africa, the trial was planted at the Cedara Agricultural Research Station near Pietermaritzburg in KwaZulu-Natal. Soybeans were planted on December 9, 2004. Common beans were planted on January 17, 2005, in two rows left empty between the soybean rows. A 1-9 severity scale was used for severity evaluation where 1 was assigned to plants with no visible ASR symptoms and 9 to plants with very severe symptoms that resulted in severe premature defoliation.

Results and Discussion

The results from South Africa and Brazil were compared here with unpublished results obtained previously under greenhouse conditions in the US (Table 1). The soybeans planted in Brazil developed severe ASR symptoms. In Goiania, the soybeans had 70 % average severity while in Rio Verde the average severity was 60 %. In both locations, the common bean cultivars Aurora, CNC, and PI 181996 had no visible ASR symptoms, while the other cultivars had very mild symptoms.

In South Africa, soybeans had well established ASR symptoms by March 11, while the common bean cultivars only had isolated pustules on the foliage. By April 7, the soybeans were completely defoliated prematurely by the ASR pathogen, while low infection was observed on

the lower leaves of most common beans planted adjacent to the heavily infected soybeans. On April 7, the average infection on leaves of mature common beans plants located 3 to 5 meters from heavily infected soybeans was very mild. The mild ASR symptoms on common beans in South Africa compared to the severe symptoms on soybeans suggest that common beans are much less susceptible to ASR than soybeans. More research needs to be done to confirm these initial results. More importantly, several common bean cultivars, such CNC, Aurora, Early Gallatin and PI 181996 exhibited high levels of resistance to six isolates of the soybean rust pathogen from Taiwan, Thailand, Zimbabwe, Brazil and Paraguay when inoculated under greenhouse conditions in the US (data not shown). These were also the same common bean cultivars that were the most resistant to the ASR pathogen under field conditions in Brazil and South Africa. The results from the greenhouse in the US and field in Brazil and South Africa indicate that some common beans are highly resistant to the ASR pathogen. The gene or genes in common beans that control the resistance to the ASR pathogen can be utilized to develop ASR-resistant common beans through traditional breeding. It may be possible to clone the ASR resistance genes from common bean and express them in soybeans using transgenic methods.

Reference: du Preez, E. D., N. C. van Rij, K. F. Lawrance, M. R. Miles, and R. D. Frederick. 2005. First Report of Soybean Rust Caused by *Phakopsora pachyrhizi* on Dry Beans in South Africa**.** Plant Dis. 89:206.

TABLE 1. Comparison of the reaction of common beans to the Asian soybean rust pathogen under field conditions in South Africa and Brazil with unpublished results obtained previously under greenhouse conditions in the United States

		USA		South Africa*		Brazil		
		Greenhouse		Cedara		Goiania	Rio Verde	
SOYBEANS	R. GENES	Index(1-9)		Index (1-9)		Severity (0-100)		
PI 200492	Rpp1	7.2	S	Local checks		Checks	Checks	
PI 230970	Rpp2	7.6	S	9	VS	70.0	60.0	VS
Ina		8.6	VS					
PI 45925B	Rppr	9.0	VS					
COMMON BEANS Inoculated in the Greenhouse								
CNC	Ur-cnc1, -cnc2	3.9	R/I	4	I	0.0	0.0	R
Early Gallatin	Ur-4	4.7	I Low	6.5?	I?	0.0		
Aurora	Ur-3	5.0	I Low	?(>6)	I or S	0.0	0.0	R
PI 181996	Ur-11	5.4	I Low	4	I	0.0	0.0	R
BelMiNeb-RMR-5	Ur-4, -6, -11	5.4	I Low	4.25	I	0.6	0.0	
BelDak-RR-2	Ur-3, -6, -CNN	5.8	I	6	I/S	0.0	0.5	
BelMiNeb-RMR-7	Ur-3, -4, -11	5.8	I	6	I/S	0.3	0.5	
Pinto 114		5.8	I	?	?	0.6	0.0	
BelMiDak-RMR-10	Ur-4, -11	6.1	I	5.5	I			
	Ur-3, -4, -6, -					0.6	0.0	
BelDakMi-RMR-18	11	6.1	I	5.5	I			
BelDakMi-RMR-14	Ur-3, -6, -11	6.8	I/S	6	I/S			
PI 260418		6.8	I/S	4	I	2	0.0	
BelNeb-RR-1	Ur-5, -6, -7	7.2	S	5	I	0.9		
	Ur-3, -4, -6, -					0.6		
BelMiNeb-RMR-8	11	7.2	S	5	I			
Golden Gate Wax	Ur-6	7.2	S	?(>6)	I or S	0.0	0.5	
Mexico 309	Ur-5	7.9	S	5	I	0.3	0.0	

*South Africa: a "?": other diseases were very severe making evaluation very difficult or impossible.

*South Africa: 0-3 = R; 4-6 = I; 6-6.9 = I/S; 7 = S; 8-9 = VS. Ratings have been taken as an average of the complete canopy (which ranged from 0 to 8 and in others from 6 to 7), taking the amount of defoliation into account.

*South Africa: long-season lines had the advantage of new growth, and in April, the epidemic stood more or less still after the defoliation of the soybeans.

INHERITANCE AND ALLELISM OF ANTHRACNOSE RESISTANCE IN COMMON BEAN JALO VERMELHO CULTIVAR

M. C. Gonçalves-Vidigal, G.F. Lacanallo, P.S. Vidigal Filho, M. J. C. Nunes and J. P. Poletine
Departamento de Agronomia, Universidade Estadual de Maringá, Brazil. E-mail: mcgvidigal@uem.br

Introduction

Genetic resistance is the most effective method of controlling anthracnose in common bean, and as new resistance sources become available (Mahuku et al., 2002), their genetic characterization is essential to ensure novelty from previously characterized sources. At the moment, eleven anthracnose resistant loci have been characterized, ten of them *Co-2 to Co-11* are Mesoamerican, and only one locus is from Andean origin (*Co-1)* (Kelly and Vallejo, 2004). At the locus *Co-1* an allelic series was observed and it is present in all of the four differential Andean cultivars, Michigan Dark Red Kidney (*Co-1*), Kaboon (*Co-1*^{2}), Perry Marrow (*Co- 1*^{3}), and Widusa (*Co-1*^{5}) (Melotto and Kelly, 2000; Gonçalves-Vidigal et al., 2003). Therefore, new resistant Andean sources have been constantly sought. Jalo Vermelho possesses morphoagronomic characteristics similar to Andean cultivars; thus resistance spectrum studies were carried out. This cultivar showed to be resistant to races 9, 23, 31, 65, 81, 89, 95, 453 (Mesoamerican), and to the Andean race 55 (Vidigal Filho et al., 2004). Since Jalo Vermelho has different resistance spectrum of in relation to a small group of cultivars of Andean origin in the differential series, the objective of this study was to characterize the anthracnose resistant genes in the Andean cultivar Jalo Vermelho.

Material and Methods

The Andean common bean Jalo Vermelho, resistant to race 453, 89, 65, and 64 was crossed with Michigan Dark Red Kidney, Kaboon, Perry Marrow, Widusa, Michelite, Cornell 49-242, TU, AB 136, BAT 93, and PI 207262. The F_1 and F_2 generations were obtained under conditions of greenhouse at the NUPAGRI. Seedlings of the parents, F_1 and F_2, were spray-inoculated with a spore suspension (1.2×10^6 spore mL^{-1}) of each race of *C. lindemuthianum*. After a 48 hours incubation period in a mist chamber, seedlings were evaluated for anthracnose reaction based on scale of 1 to 9 (Balardin et al. 1990). Plants with disease reaction that scored of 1-3 were considered resistant, whereas plants rated from 4-9 were considered susceptible.

Results and Discussion

The inheritance studies demonstrated a 3R:1S ratio in the F_2 populations from the crosses between Jalo Vermelho/PI 207262 and Jalo Vermelho/Widusa, respectively, inoculated with race 453 and 89, revealing the presence of only one resistant gene, conferring resistance for the cultivar (Table 1).

The allelism tests in nine F_2 populations from the crosses of Jalo Vermelho and other previously characterized cultivars supported an expected 15R: 1S ratio, indicating the action of two independent dominant genes, and conferring resistance to anthracnose. The segregation ratio of 63R:1S ($p = 0.72$) was demonstrated by F_2 population from the cross Jalo Vermelho x PI 207262 supporting the hypothesis of three dominant genes in segregation. PI 207262 differential cultivar possesses two dominant genes *Co-4*^{3} and *Co-9* (Gonçalves-Vidigal et al., 1997; Geffroy et al. 1999; Alzate-Marin et al., 2002), which segregated independently from that one presented in Jalo Vermelho. In 2005, Mendez-Vigo and collaborators observed that the genes *Co-3* (present in Mexico 222) and *Co-9* (present in BAT 93) are alleles of the same gene. Thus, we are able to confirm that the gene in Jalo Vermelho is independent from the gene *Co-3* present in Mexico 222. These results support the existence of a gene in Jalo Vermelho independent from Andean and Mesoamerican resistant genes.

In conclusion, the gene present in Jalo Vermelho is independent of the prior characterized genes, which are: *Co-1, Co-1*^{2}, *Co-1*^{3}, *Co-1*^{5}, *Co-2, Co-3*, *Co-4*, *Co-5*, *Co-6*, *Co-9* and *Co-11*. Therefore, we suggest that the anthracnose resistant gene in Jalo Vermelho should be designated *Co-12*.

Table 1. Reaction of 12 F_2 populations, observed and expected ratios of resistant (R) and susceptible (S) plants to inoculation with different races of *C. lindemuthianum* in R x S and R x R crosses with Jalo Vermelho

Crosses with Jalo Vermelho	Race	Resistance Gene	Observed Ratio		Expected Ratio	χ^2	*P* value
			R	S	R:S		
Widusa (S)	89	*Co-1*[5]	62	20	3:1	0.016	0.90
PI 207262 (S)	453	*Co-4*[3], *Co-9*	108	37	3:1	0.021	0.89
Michelite	64	*Co-11*	60	4	15:1	0.0	1.00
BAT 93	64	*Co-9*	85	5	15:1	0.074	0.79
PI 207262	64	*Co-4*[3], *Co-9*	98	2	63:1	0.124	0.72
MDRK*	65		81	5	15:1	0.027	0.86
Kaboon	65	-	69	4	15:1	0.073	0.79
Perry Marrow	65	-	72	5	15:1	0.007	0.93
Widusa	65	-	58	4	15:1	0.004	0.95
Cornell 49-242	65	*Co-2*	77	5	15:1	0.003	0.96
AB 136	65		86	6	15:1	0.011	0.91
TU	453	*Co-5*	64	5	15:1	0.117	0.73

*MDRK = Michigan Dark Red Kidney.

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CHARACTERIZATION OF THE ANTHRACNOSE RESISTANCE GENES IN ANDEAN COMMON BEAN JALO LISTRAS PRETAS CULTIVAR

Pedro Soares Vidigal Filho[1], Maria Celeste Gonçalves-Vidigal, Abrão F. Medeiros, Pedrina Gonçalves Vidigal. [1]Departamento de Agronomia, Universidade Estadual de Maringá, Brazil. E-mail: psvfilho@uem.br

Introduction

Anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. & Magn.) Scrib., is one of the most widespread and economically important fungal diseases of common bean (*Phaseolus vulgaris* L.). The resistance is considered the most important factor in integrated control strategy, which includes cultural practices, use of mixed cultivars, quarantines and fungicides (Chaves, 1980). In previous studies, genotypes collected from different parts of Paraná state were screened with several races of *C. lindemuthianum* for anthracnose resistance; and among them Jalo Listras Pretas demonstrated to be resistant to races 9, 31, 65, 69, 73, 81, 89, and 95. On the other hand this cultivar revealed a compatible reaction to pathotypes 7, 19 and 55 (Andean) and to the Mesoamerican pathotype 453 (Vidigal Filho et al., 2004). Many anthracnose resistance loci have been characterized in common bean, being identified as of Mesoamerican origin. Only one anthracnose resistance gene, *Co-1*, has been characterized from the Andean gene pool; therefore, new Andean resistant genes are constantly being sought (Kelly and Vallejo, 2004). The results obtained by Vidigal Filho et al. (2004) showed that the Jalo Listras Pretas cultivar has different Michigan Dark Red Kidney (MDRK) resistance spectrum and carry a different gene from *Co-1* locus. At the moment, there is no information about inheritance and independence of the gene present in Andean cultivar Jalo Listras Pretas in relation to genes previously characterized. The objective of this work was to investigate the inheritance of anthracnose resistance and characterize the independence of the gene present in Jalo Listras Pretas.

Material and Methods

The Andean common bean cultivar Jalo Listras Pretas was crossed with Michigan Dark Red Kidney (resistant to race 73), Kaboon, Perry Marrow, Widusa, AB 136 (all resistant genotypes to race 65), Ouro Negro and MSU (resistant to race 64), also with Mexico 222 and Cornell 49-242 (susceptible to race 64 and 73, respectively). Parents, F_1 and F_2 of each cross, were spray-inoculated with standardized spore concentration (1.2×10^6 spores mL^{-1}) of each race of *C. lindemuthianum* (Table 1), using a De Vilbiss number 15 atomizer powered by an electric compressor. After inoculation, plants were maintained at high relative humidity (>95%) for 48 h at 21-23 ^oC. Plants were allowed to dry and were transferred to the greenhouse where they were kept for 5 days. Seedlings were evaluated for their disease reaction using a scale of 1 to 9 (Balardin et al., 1990). Plants with no visible disease symptoms or with only a few and very small lesions mostly on primary leaf veins were recorded as resistant (scale 1 to 3), whereas plants with numerous small or enlarged lesions, or with sunken cankers on both sides of leaves and on the seedling stem were recorded as susceptible (scale 4 to 9). The allelism tests were conducted in nine F_2 populations.

Results and Discussion

The inheritance studies demonstrated a 3R:1S ratio in the F_2 populations from the crosses Jalo Listras Pretas x Cornell 49-242, and Jalo Listras Pretas x Mexico 222, when they were inoculated with races 73 and 64, respectively. This fact indicates the presence of only one resistant gene in Andean cultivar Jalo Listras Pretas. Allelism tests in the crosses involving Jalo Listras Pretas with Michigan Dark Red Kidney, Kaboon, Perry Marrow, Widusa, AB 136, MSU 7, BAT 93 and Ouro Negro cultivars fitted a 15R:1S ratio, indicating that each of the cultivars

carries an independent dominant resistance gene. The cross involving PI 207262 showed a 63:1 ratio in F_2 population, supporting the hypothesis of three dominant genes, two of them in PI 207262 and one in Jalo Listras Pretas. These results support the independence of the gene in Jalo Listras Pretas from other Andean and Middle American resistance genes.

Conclusion

The results indicated the segregation of one dominant resistant gene in Jalo Listras Pretas. The allelism tests confirmed that the dominant gene present in Jalo Listras Pretas is independent from the *Co-1*, *Co-1*^{2}, *Co-1*^{3},*Co-1*^{5}, *Co-2, Co*-3, *Co-4*, *Co-6*, *Co*-7, *Co-9*, and *Co-10* genes. The authors propose the symbol *Co-13* to name the referred gene present in Jalo Listras Pretas cultivar.

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

Crosses	Race	Resistance Gene	Observed Ratio		Observed Ratio	χ^2	*P* value
			R	S	R:S		
JLP* x Cornell 49-242	73	*Co-2*	174	55	3:1	0.117	0.73
JLP x Mexico 222	64	*Co-3*	38	12	3:1	0.026	0.87
JLP x MDRK**	73	*Co-1*	155	12	15:1	0.249	0.62
JLP x Kaboon	65	*Co-1*^{2}	84	5	15:1	0.060	0.81
JLP x Perry Marrow	65	*Co-1*^{3}	93	8	15:1	0.481	0.48
JLP x Widusa	65	*Co-1*^{5}	167	12	15:1	0.062	0.80
JLP x AB 136	65	*Co-6*	126	10	15:1	0.282	0.59
JLP x MSU 7	64	*Co-7*	143	9	15:1	0.028	0.86
JLP x BAT 93	73	*Co-9*	111	6	15:1	0.251	0.62
JLP x Ouro Negro	64	*Co-10*	97	6	15:1	0.031	0.85
JLP x PI 207262	73	*Co-4*^{3}, *Co-9*	229	4	63:1	0.036	0.85

* JLP= Jalo Listras Pretas; ** MDRK= Michigan Dark Red Kidney

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INHERITANCE OF RESISTANCE TO LIMA BEAN DOWNY MILDEW (*PHYTOPHTHORA PHASEOLI*) AND PRELIMINARY LIMA IMPROVEMENT EFFORTS

E.G. Ernest[1], W.E. Kee[1], L. Santamaria[2], T.A. Evans[2]
[1]University of Delaware Research and Education Center, Georgetown, DE
[2]University of Delaware, Newark, DE

Introduction

Lima beans (*Phaseolus lunatus*), grown for consumption as a succulent shelled bean, are an economically important vegetable crop in Delaware. Green baby limas and Jackson Wonder-type, speckled limas are grown for freezing. Fresh market growers produce Fordhook types, and large seeded pole limas which bring premium prices. There is also interest in production of Fordhook limas for freezing. However, the lack of reliable cultivars prevents large scale production of Fordhook types. From 2000 to 2003, an average of 16,400 acres of green baby lima beans for processing were planted each year in Delaware. This accounts for 32% of U.S. processing lima bean production area. However, lima yields in Delaware are lower than in other US production areas – 0.98 tons/acre compared to the national average of 1.26 tons/acre.

The predominant constraints to lima production in Delaware are heat and diseases. The major diseases affecting limas are downy mildew (*Phytophthora phaseoli*), anthracnose (*Colletotrichum lindemuthianum* and *C. truncatum*), and white mold (*Sclerotinia sclerotiorum*). We have initiated some lima bean improvement work with the goals of improving disease resistance, particularly to downy mildew and anthracnose, increasing heat tolerance, improving plant habit with respect to suitability for mechanical harvest, increasing yields, and maintaining qualities important to processors. In the fall and winter of 2004 – 2005 green baby lima cultivars were intercrossed, as well as, crossed with downy mildew resistant and heat tolerant lines. Selections from the F_2 populations generated from these crosses were made in the field in summer '05. We also evaluated over 200 lines from the USDA germplasm collection for various traits, including flowering time, vigor, plant habit, and anthracnose resistance.

Determining the Inheritance of Resistance to Downy Mildew Races E and F

Materials and Methods

To facilitate breeding for resistance to downy mildew, we conducted a study of the inheritance of resistance to races E and F of the disease. The cultivars used in the study were 'Cypress', 'Dover Tucker', 'Maffei 15', 'B2C' and 'Jackson Wonder'. Cypress and Dover Tucker are resistant to race E but susceptible to race F while Maffei 15 and B2C are susceptible to race E and resistant to race F. Jackson Wonder is susceptible to both races E and F. Crosses were made between Jackson Wonder and the resistant parents in the greenhouse. The F_1 plants from these crosses were grown out in the greenhouse and the seed from each F_1 plant was harvested and maintained separately. The F_2 plants were inoculated by pouring on a *Phytophthora phaseoli* spore suspension when the cotyledons had just emerged from the soil. Inoculum was grown on 'Concentrated Fordhook' seedlings. After inoculation the F_2 plants were transferred to a humid chamber and maintained there until disease symptoms were evaluated five to seven days later.

Results and Conclusions

The F_2 populations from the crosses between Jackson Wonder and the race E resistant cultivars segregated 3 resistant : 1 susceptible (Table 1), indicating that in both cultivars, a single dominant gene confers resistance to race E. All of the F_2 plants from the cross between Dover Tucker and Cypress were resistant to race E, suggesting that these two cultivars carry the same resistance gene.

Table 1. Inheritance of resistance to downy mildew race E in the cultivars 'Cypress' and 'Dover Tucker'

Cross	Cross Type	Test Ratio	Resistant	Susceptible	Total	X^2 ρ-value
Jackson Wonder/Cypress	S x R	3 : 1	163	51	214	0.693
Jackson Wonder/Dover Tucker	S x R	3 : 1	227	88	315	0.229
Dover Tucker/Cypress	R x R	all resistant	148	0	148	

When inoculated, the F_2 populations from the crosses between Jackson Wonder and the race F resistant cultivars yielded plants in three different resistance categories. Some plants were completely resistant, some were very susceptible, exhibited obvious fungal growth and died, while others were obviously stunted, and had some tissue death but did not die outright. *Phytophthora phaseoli* fungal mycelium with spores was detectable on these plants using a microscope. In Table 2 the plants are categorized as either resistant or susceptible – plants exhibiting moderate susceptibility were considered susceptible in this case. The segregation ratio of resistant to susceptible plants is 3 : 1 indicating that both Maffei 15 and B2C carry a single dominant gene which confers complete resistance to down mildew race F.

Table 2. Inheritance of resistance to downy mildew race F in the cultivars 'M-15' and 'B2C' with plants categorized as resistant or susceptible

Cross	Cross Type	Test Ratio	Resistant	Susceptible	Total	X^2 ρ-value
Jackson Wonder/M-15	S x R	3 : 1	159	50	209	0.719
Jackson Wonder/B2C	S x R	3 : 1	212	59	271	0.220

In Table 3 the plants are categorized as resistant, moderately susceptible, or very susceptible. The segregation ratio of 48 resistant : 7 moderately susceptible : 9 very susceptible suggests that Maffei 15 and B2C carry 2 recessive genes, either of which will confer partial resistance to race F. It is likely that Maffei 15 and B2C carry the same genes since Maffei 15 was selected from B2C.

Table 3. Inheritance of resistance to downy mildew race F in the cultivars 'M-15' and 'B2C' with plants categorized as resistant, moderately susceptible, or very susceptible.

Cross	Test Ratio	Resistant	Moderately Susceptible	Very Susceptible	Total	X^2 ρ-value
Jackson Wonder/M-15	48 : 7 : 9	159	21	29	209	0.910
Jackson Wonder/B2C	48 : 7 : 9	212	28	31	271	0.408

SELECTION OF BACKCROSS *PHASEOLUS* GERMPLASM LINES DERIVED THROUGH INTERSPECIFIC HYBRIDIZATION OF COMMON BEAN AND TEPARY BEAN

Richard C. Pratt[1], Joseph C. Scheerens[1], Soon J. Park[2]

[1]Dept. of Horticulture and Crop Science, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster OH 44691; [2]Agriculture and Agri-Food Canada, Greenhouse and Processing Crops Centre, 2585 County Road 20 Harrow, Ontario N0R 1G0

Introduction

Many biotic and abiotic stresses can cause serious economic losses in dry bean (*P. vulgaris* L) production. The genetic base within most market classes of dry bean is narrow. Tepary bean (*Phaseolus acutifolius* A. Gray var. *latifolius* Freeman), a member of the tertiary gene pool of common bean, contains desirable genetic variation for several biotic and abiotic stresses. Interspecific hybrids from crosses between the respective species are infertile, but the production of partially fertile backcross populations and desirable breeding lines has been demonstrated. In a previous study (Pratt and Gordon, 1994) we reported that BC_1 and BC_2 populations could be produced by crossing interpsecific hybrids (derived from crosses between heterogeneous and heterozygous parents) with diverse *P. vulgaris* parents. The backcross populations displayed a range of fertility–but did not show a strong association between infertility and the presence of several tepary markers. The objective of this project was to determine if high yielding germplasm could be selected from eight BC_2 populations and one BC_3 population. An additional objective was to determine if negative traits associated with tepary bean would hinder selection in these populations.

Materials and Methods

Unreplicated, space-planted nurseries of segregating BC progeny lines were planted at OARDC experimental farms near Wooster, OH during the period 1992-1993. Early-generation selections were made within, and across, lines using the pedigree method. Selections were based on acceptable maturity, upright or short vine habit, and production of pods and seeds that remained sound until a delayed harvest was conducted in late October or early November. In 1995, replicated performance evaluations of 20 selected lines were conducted to enable selection for yield and the criteria mentioned previously. Selected lines were sorted based on seed characteristics and several color variant sister-lines of S_4 BC_2 and BC_3 lines were formed by bulking similar phenotypes. Additional replicated performance evaluations in Wayne County, OH were conducted at one site in 1997, and at two sites in 2000. In 2002 and 2003 evaluations of the responses to infection by *Xanthomonas campestris* pv. *phaseoli*, causal agent of common bacterial blight, and agronomic characteristics, of the advanced BC lines were determined in Ontario, Canada. In 2003, agronomic characteristics of the advanced BC lines also were determined in an unreplicated field plot in Michigan through the courtesy of Dr. James Kelly.

Results and Discussion

It was determined that inbred backcross breeding lines displaying both desirable tepary traits (e.g. common bacterial blight resistance) and economically important common bean characteristics, could be selected (Pratt et al., 2006). The yield of the top seven BC lines, designated Ohio *Phaseolus* germplasm (OPG) lines, was not significantly different from that of the check cultivar ‘Jamapa’. The lines displayed considerable variation in architecture, from upright bush (Type I) to prostrate vine (Type III) and this variation also was observed within some of the lines. The maturity of three lines was suitable for the Great Lakes region, whereas the others were too late-maturing (over 100 days).

Most OPG lines displayed only average lodging resistance and will likely find their greatest utility when used in breeding with elite, locally adapted cultivars with good lodging resistance.

The seven OPG lines display highly diverse seed-coat colors and patterns–ranging from white, light to dark brown, pinto-like variegation, pink and red, to black and mottled. It has not been possible to fix the seed coat color of one line (OPG-3). Seed weights of the released lines are comparable to those of the small-seeded to medium weight common beans (Pratt et al., 2006), although some very small-seeded lines were observed among the unselected progenies. The tendency for tepary pods to sometimes shatter prematurely was not observed among the selected progenies. The OPG lines were tested for water imbibition and cookability, and all were found to be satisfactory.

Two OPG lines were derived from a BC_2 population and five were obtained from the BC_3 population. Each of the lines has a high percentage of the cultivar 'Jamapa' in the pedigree. A release notice for the seven OPG lines will be published in HortScience (Pratt et al, 2006).

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Acknowledgements

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APPLICATION OF DIFFERENT *IN VIVO* POLLINATION TECHNIQUES TO IMPROVE THE FERTILIZATION EFFICIENCY OF INTERSPECIES CROSSES IN THE GENUS *PHASEOLUS*

Valarmathi Gurusamy*, Bert Vandenberg and Kirstin Bett

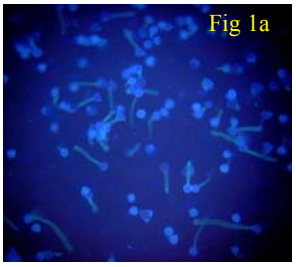
Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK S7N 5A8, Canada. e-mail: guv382@mail.usask.ca

Introduction

Observations of fertilization in the F_1 of *P. vulgaris* cv ICA Pijao (*Pv*) x *P. angustissimus* PI 535272 (*Pa*) upon backcrossing with *Pv* indicated that fertilization occurs 30 or more hours after pollination compared to 8-10 hours in *Pv* x *Pv* or *Pa* x *Pa.* Abscission of the pods in these backcrosses usually occurs within 3 or 4 days after pollination and embryo develops only to at most a four-cell stage in this time frame (Balasubramanian et al. 2004). A range of pollination techniques including cut-style, grafting and placental pollination have been applied to overcome pre-fertilization barriers in many ornamental crops. The effect of similar alternative *in vivo* pollination techniques on reducing the time to fertilization in *Phaseolus* interspecies hybrids was studied.

Materials and Methods

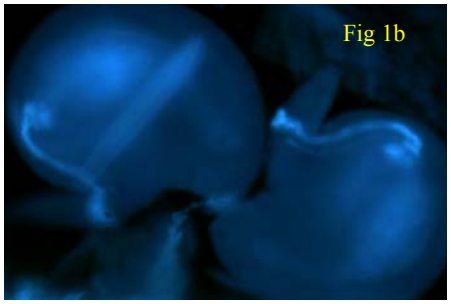
Three types of pollen *viz*., pre-germinated pollen (PGP), non-germinated pollen (NGP) and fresh pollen (FP) were used. PGP was produced *in vitro* and germinated pollen grains (~65%) with growing pollen tubes were used for pollination (Fig 1a). NGP consisted of fresh pollen grains mixed immediately in the same medium as was used to produce PGP. FP was collected from freshly opened flowers and used directly. Two types of pollination technique *viz*., cut-style (3/4 of the stylar region was removed) and stigmatic pollination, were used. Both the PGP and NGP were applied using a micropipettor. In total, four cross combinations were studied under controlled growth conditions: the two parents were manually self-pollinated; *Pa* as a female parent was crossed with *Pv* as male parent; and the hybrid (*Pv/Pa*) was backcrossed with *Pv*.



Standardization of *in vitro* pollen germination medium

Four different mineral solutions consisted of varying levels of boric acid, calcium nitrate, potassium nitrate and magnesium chloride and two levels of sucrose (30% and 40%) with a pH 8.5-8.8 were tested. A total of 30 µl of mineral solution and 30 µl of sucrose at the appropriate concentration was placed on a glass cavity slide and mixed well as per the treatment combinations. Fresh pollen from freshly opened flowers of *Pv* was mixed in the PGM. Prepared slides were placed in a Petri plate (150x15 mm) containing moist filter paper and were kept in a cold room (6-9°C) for germination. Presence of germinated pollen was determined by fluorescent microscopy using 0.1% Aniline Blue prepared in 0.5 M KH_2PO_4. Each experiment was replicated three times. Data were analyzed using Statistix (Analytical Software, Version 2) and Tukey's (HSD) comparison of means was used to determine significant differences.

Assessment of fertilization in vivo



Assessment of *in vivo* fertilization was carried out at 2, 6, 10 and 24 hours post pollination, as described by Gurusamy et al (2004). An ovule was considered fertilized when a pollen tube penetrated the micropyle (Fig.1b). Analyses were carried out using PROC GENMOD, Type III analysis, (SAS system 8.2, SAS Institute Inc., NC, USA, 2001), using unbalanced data, to study the interaction effects of the altered pollination techniques on percent fertilization.

Results and Discussion

Pollen germination under in vitro *condition*

An effective technique was developed for *in vitro* germination of *Pv* and *Pa* pollen. Maximum germination occurred between 16-18 hours after culturing. The highest level of germination (74.9% for *Pv* and 70% for *Pa*) was observed in the treatment combination of H_3BO_3 (400 mg/l) + $CaNO_3$ (600 mg/l) + $MgSO_4$ (400 mg/l) + KNO_3 (400 mg/l) + 40% sucrose. Low temperatures (6-9°C) were found to be necessary for *Phaseolus* pollen germination.

Fertilization under different pollination techniques

Alternative pollination techniques to reduce the long time required between pollination and fertilization in interspecies crosses of *Pv* and *Pa* were identified. Among the pollen types studied, pollinations with PGP using either of the pollination techniques resulted in rapid fertilization in all the genotypes studied. Among the pollination techniques studied, cut-style pollination coupled with any pollen type was superior in reducing time to fertilization. A dramatic increase in the rate of fertilization in the (*Pv* / *Pa*) interspecific hybrid upon backcrossing with *Pv* was noticed with the altered techniques. Pollination using a cut-style technique coupled with PGP reduced the time for fertilization in the backcrosses of interspecies hybrids by approximately 28 hours. Pollination with PGP on cut-styles resulted in significantly higher levels of fertilization than the other treatments, even at 2 hours after pollination (69 %). Using the cut style technique in combination with pre-germinated pollen (*in vitro*) improved the success rate in wide crosses of *Phaseolus* designed to introgress genes from wild relatives. However these techniques showed detrimental effect on further pod growth and development leading to drying out of the young pods (data not shown). This could be due to the high osmotic level of the PGM on the pistil affecting the growth. This technique is therefore only recommended for wide crosses where very early embryo rescue is planned. It is anticipated that this will lead to greater success in common bean breeding where wide crosses are used to introgress genes from wild relatives.

Acknowledgements

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REPEATABILITY OF MORPHOLOGICAL & PHENOLOGICAL TRAITS USED BY THE CROPGRO-DRY BEAN CROP MODEL

Marcos Saliceti, Elvin Román-Paoli and James S. Beaver

Dept. of Agronomy and Soils, University of Puerto Rico, Mayaguez, Puerto Rico 00681

CROPGRO-dry bean is a process-orientated model that simulates vegetative growth, reproductive development and seed yield of common bean (Hoogenboon et al., 1994). The model uses data on daily weather, soil characteristics and crop management practices to predict crop growth, development and seed yield. The model uses cultivar-specific coefficients to represent different cultivars. Use of simulation models by plant breeders, however, has been limited due to the restricted capability of models to represent genetic differences (Hoogenboom et al., 2004; Hoogenboom and White, 2002). GENEGRO integrated the action of seven genes into the common bean model (White et al., 1996). The GENEGRO model explained >80% of observed variation in days to flowering and maturity but only 31% of variation in seed yield from 14 trials conducted in Colombia, Guatemala, Mexico and Florida. Growth habit (*Fin* gene) had a large effect on seed yield. Determinate lines had low seed yields due to their short life cycles (Hoogenboom et al., 1997). Most of the determinate genotypes in the trials, however, were early-maturity, large-seeded Andean bean lines. The objectives of this research were to 1) estimate repeatability of morphological and phenological traits used in the CROPGRO-dry bean model in a population derived from a cross between parents having different growth habits and 2) determine whether growth habit should be major consideration when using the CROPGRO-dry bean model. Eighty two indeterminate and 36 determinate recombinant inbred lines (RIL) from the cross “ICA Pijao x Montcalm’ were evaluated at Isabela, Puerto Rico during 2004 and 2005. Both parents are adapted to Puerto Rico with similar yield potential and days to maturity. ICA Pijao has an indeterminate growth habit and a 19 g hundred seed weight (HSW) whereas Montcalm has a determinate growth habit and a 55 g HSW. Experiments were arranged in four randomized complete blocks each year. The experimental units were single 2 m rows. Genetic coefficients used the CROPGRO model and seed yield components were measured for each RIL. Repeatabilities were calculated to estimate the ratio of genetic variation to phenotypic variation for genetic coefficients and seed yield components. Mean seed yields of determinate and indeterminate RILs were similar in 2004 and 2005 (Table 1). Lower mean seed yields during 2004 were attributed to drought stress caused by higher temperatures, strong trade winds and low rainfall during the growing season. There were significant line x year interactions for seed yield for the determinate and indeterminate lines. Mean photothermal days (PTD) from emergence to first flower (EM-FL) of the determinate lines was 3.7 PTD earlier than the indeterminate lines in 2004 and 6.2 PTD earlier in 2005. The indeterminate lines had lower repeatabilities for EM-FL. The mean period from first flower to the beginning of seed fill (FL-SD) of the determinate RILs was 2.2 PTD shorter than the indeterminate RILs in 2004 but 8.8 days longer than the indeterminate RILs in 2005. The mean period from the beginning of seed fill to physiological maturity (SD-PM) of the indeterminate RILs was 21.2 PTD shorter than the determinate RILs in 2004 but 0.6 PTD longer than the determinate RILs in 2005. Repeatabilities of FL-SD and SD-PM were, with one exception, intermediate in magnitude. The unfavorable growing conditions in 2004 resulted in a reduction of the mean number of pods per plant and mean number of seed per pod of both the determinate and indeterminate RILs. The determinate RILs averaged 0.3 fewer seed per pod than the indeterminate RILs in 2004 and 0.4 fewer seed per pod than the indeterminate lines in 2005. The determinate lines averaged 2.5 more pods per plant than the indeterminate RILs in 2004 but averaged 0.2 fewer pods per plant than the indeterminate RILs in 2005. Repeatabilities of seed per pod and number of pods per plant were generally intermediate in magnitude. Mean individual seed weights of the determinate and indeterminate RILs differed by only 0.02 g both in 2004 and 2005. Although repeatabilities of individual seed weight were large for both growth habits only a few of the determinate RILs had seed weights similar to Montcalm. In this population, individual seed weight and growth habit were not associated with differences in seed yield. However, sensitivity of determinate lines to abiotic stress may make the prediction of seed yield of this growth habit more difficult. Number of pods per plant was the only trait in the study that had large and positive correlations with seed yield for both growth habits during 2004 and 2005 (Table 2).

Table 1. Mean, minimum, maximum and repeatability of genetic coefficients of the CROPGRO–Dry bean model of determinate and indeterminate lines from the cross 'ICA Pijao x Montcalm' tested at Isabela Puerto Rico.

Genetic coefficient	Growth habit	Mean (Minimum, Maximum)		Repeatability estimate	
		2004	2005	2004	2005
EM-FL[1]	Determinate	45.2 (42.4-50.0)	45.3 (36.7-51.7)	0.59	0.83
	Indeterminate	48.9 (44.6-61.3)	51.5 (46.0-59.3)	0.19	0.16
FL-SD[1]	Determinate	15.6 (13.2-22.2)	30.9 (19.5-36.2)	0.24	0.63
	Indeterminate	17.8 (12.6-36.8)	22.1 (17.4-26.9)	0.07	0.63
SD-PM[1]	Determinate	35.7 (18.7-45.4)	34.8 (26.6-48.8)	0.51	0.77
	Indeterminate	14.5 (7.4-25.2)	35.4 (26.6-44.6)	0.35	0.47
Pods per plant	Determinate	21.7 (13.1-34.7)	16.4 (8.3-27.2)	0.58	0.55
	Indeterminate	19.2 (9.8-32.9)	16.6 (7.0-24.0)	0.48	0.60
Seeds per pod	Determinate	3.7 (2.8-5.1)	2.7 (1.5-4.0)	0.91	0.54
	Indeterminate	4.0 (2.5-5.2)	3.1 (1.7-5.0)	0.62	0.22
Individual seed weight (g)	Determinate	0.28 (0.18-0.55)	0.27 (0.20-0.48)	0.91	0.82
	Indeterminate	0.26 (0.16-0.41)	0.25 (0.17-0.39)	0.93	0.82
Seed yield (kg/ha)	Determinate	2174 (1325-3280)	1123 (640-1785)	0.43	0.56
	Indeterminate	1903 (880-3395)	1256 (440-2390)	0.48	0.76

[1] Photothermal days

Table 2. Phenotypic correlations of seed yield components with seed of determinate and indeterminate lines from the cross 'ICA Pijao x Montcalm' tested at Isabela Puerto Rico.

	Indeterminate lines Seed yield		Determinate lines Seed yield	
	2004	2005	2004	2005
Pods per plant	0.85	0.73	0.68	0.73
Seed per pod	0.31	0.35	NS	0.47
Seed weight	NS	NS	0.35	NS

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SYSTEMATICS AND MOLECULAR VARIABILITY OF BEAN RUSTS

M.C. Aime

USDA-Agricultural Research Service, Systematic Botany and Mycology Lab, 10300 Baltimore Ave., Beltsville, MD. cathie@nt.ars-grin.gov

The systematics and genetic variability of rusts on Fabaceae (legume family) are examined within a molecular phylogenetic context. Analysis of nuclear ribosomal large subunit DNA (LSU) sequences suggests that legume rusts may have as few as three independent origins within the Uredinales. Rusts pathogenic on subfamily Papilionoideae are confined to two lineages, one within the Pucciniaceae, and the other within the Phakopsoraceae p.p. Rusts occurring on *Phaseolus* spp. form part of the crown clade in both lineages. Analysis of nuclear ribosomal internal transcribed spacer region DNA (ITS) resolves three species of *Uromyces* (Pucciniaceae lineage) found on *P. vulgaris*, but these do not correspond to prior morphologically based reports. Within the species *U. appendiculatus,* a protein gene encoding the elongation factor 1-alpha (EF1-α) was found to be hypervariable. Analyses of EF1-α haplotype data show two well-defined gene pools for this rust that correspond to the Andean and Mesoamerican gene pools of its host. Dikaryotic data, however, show the presence of a third, intermediate, genotype that may be the result of hybridization events between the two parental gene pools. There is no evidence for sexual outcrossing within the Andean gene pool nor between the Andean and Mesoamerican. A rapid diagnostic test has been developed to quickly genotype isolates of *U. appendiculatus* into one of these three main genotypes.

INTRODUCTION

The pulses (Papilionoideae: Fabaceae) contain important sources of protein for humans and domestic animals. Hundreds of species of rust fungi (Uredinales) from 33 teleomorphic genera spanning eight families are known pathogens of legumes (1, 2). Phylogenetic study of the rust flora associated with legumes can help to identify centers of origin for rust cohorts and identify and predict patterns of co-speciation and evolution. One of the major constraints to dry bean (*Phaseolus vulgaris*) production is the globally distributed rust, *Uromyces appendiculatus*. In order to select the best sources of resistance for pyramiding rust-resistance genes and to develop effective host breeding programs it is necessary to know the genetic variability and geographic and host range of the pathogen.

MATERIALS AND METHODS

DNA was obtained from fresh field collections, dried herbarium specimens (BPI and PUR), or as frozen urediniospores (*U. appendiculatus* isolates) from the collection maintained by Dr. Marcial Pastor-Corrales (USDA-ARS-VL, Beltsville, MD). Rusts were extracted and LSU and ITS were amplified and sequenced using rust-specific protocols and primers (Aime unpubl.); primers for EF1-α were modified from (3). EF1-α haplotype sequences were obtained from urediniospore preps using the cloning protocol in (4). Standard conditions for sequence analysis and phylogenetic inference are provided in (4). A detailed protocol for the EF1-α restriction enzyme test is available from the author upon request.

RESULTS AND DISCUSSION

Rusts on legumes—Sampling and LSU analysis of exemplars from 15 genera of legume rusts was conducted within a dataset containing nearly 50% of the generic-level diversity of the Uredinales. Results reveal that leguminous rusts have undergone evolutionary host tracking, or possibly co-evolution, with their hosts, and may have originated from as few as three unique colonization events. The majority of legume rust genera have specialized on Mimosoideae, and those sampled have a single evolutionary origin. Rusts on Papilionoideae are confined to two families: Pucciniaceae and Phakopsoraceae p.p.

Rusts on *Phaseolus vulgaris*—Phylogenetic analysis of LSU for 13 species of *Uromyces* on pulse crops and related legumes show that *Uromyces* spp. on Papilionoideae probably derive from heteroecious ancestors that had their aecial stages on members of the Euphorbiaceae. Three species of *Uromyces* can be found on *P. vulgaris*: *U. azukicola*, *U. vignae*, and *U. appendiculatus* (5). A similar host-association pattern is evident in the Phakopsoraceae p.p., where only two known species are capable of infecting *P. vulgaris*: *Phakopsora meibomiae* and *P. pachyrhizi*.

Uromyces appendiculatus—Comparison of EF1α data derived from two different sources, urediniospores (presumed dikaryotic) and haplotypes cloned from urediniospore isolates show: 1) *U. appendiculatus* isolates can be divided into two main gene pools corresponding to the Andean and Mesoamerican gene pools of the host plant; 2) an intermediate genotype exists that consists of urediniospores containing one haplotype from each gene pool that is potentially derived from hybrid races; 3) the Mesoamerican gene pool contains numerous alleles and a high degree of genetic diversity and probably represents isolates with a sexually outcrossing strategy; 4) the Andean gene pool (although sampling of this gene pool is limited) is homogenous at the EF1-α locus and has a single fixed polymorphism in the urediniospore stage that is suggestive of an asexual strategy. Because knowledge of the pathogen races present in different geographic regions will aid in deployment of resistant host cultivars, a restriction enzyme digest test has been developed and tested on numerous *U. appendiculatus* isolates to quickly differentiate between the three main genotypes.

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MOLECULAR ADVANCES WITH COMMON BEAN RUST RESISTANCE

Phillip N. Miklas, USDA-ARS, Prosser, WA (pmiklas@pars.ars.usda.gov)

Bean rust [caused by *Uromyces appendiculatus* (Pers.) Unger var. *appendiculatus*] is a serious fungal disease that limits common bean (*Phaseolus vulgaris* L.) production worldwide. The disease can be adequately controlled by genetic resistance in the host. For bean rust, as with most host/hypervariable pathogen interactions, co-evolution of numerous resistance genes and pathotypes has transpired. Nine named *Ur-3, Ur-4, Ur-5, Ur-6, Ur-7, Ur-9, Ur-11, Ur-12*, and *Ur-13*, and four undefined rust resistance genes: one from BAC 6; one from Ouro Negro; and two from Dorado, have all been tagged with RAPD or SCAR markers (Miklas, 2006) and placed on the genetic linkage map (see reviews by Miklas et al., 2002; 2006; and Kelly et al., 2003). Note that *Ur-12* conditions adult plant resistance.

Twelve of the R genes map to five linkage groups (Miklas et al., 2006). *Ur-9* is located on B1 near a cluster of R genes of Andean origin for anthracnose resistance. *Ur-5* and *Ur*-Dorado are linked and independent or loosely linked with *Ur*-Ouro Negro on linkage group B4 (Alzate-Marin et al., 2002), and all are associated with anthracnose resistance genes. *Ur-4* is located on B6. *Ur-13* is located on B8 near a gene for angular leaf spot resistance. *Ur-3*, *Ur-11*, and *Ur*-Dorado are linked and map near the *Co-2* anthracnose resistance gene and are independent of *Ur-6*, *Ur-7* and *Ur*-BAC 6, all on linkage group B11.

Although numerous markers are available for indirect selection and to facilitate development of resistance gene pyramids, few actual applications of MAS in breeding for rust resistance have been published, perhaps due to relative ease of direct screening with the pathogen. The occurrence of false positives and negatives (Table 1), where the coupling (*cis*) marker is present but the gene is absent, or vice versa, has also limited implementation of MAS. Currently, breeding programs in Brazil are using MAS for rust resistance with success (Souza et al., 2003; Ragagnin et al., 2003); however, the full potential for MAS for rust resistance in common bean has not yet been realized. The comprehensive linkage map of the rust resistance genes could be used to direct allelism tests for characterization and naming of new rust resistance genes.

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Table 1. Survey of genotypes with known rust resistance genes for presence/absence of linked RAPD or SCAR markers.

	Genetic Markers							
Line	Known Rust Resistance Genes	SK14 SCAR (*Ur-3*)	A14 RAPD (*Ur-4*)	SI19 SCAR (*Ur-5*)	SF10 SCAR (*Ur*-ON)	A04 RAPD (*Ur-9*)	AC20 RAPD (*Ur-11*)	GT02 SCAR (*Ur-11*)
		B11	B6	B4	B4	B1	B11	B11
OAC 88-1	*Ur-3*	+	-	-	-	-	+	-
Kodiak	*Ur-3*	+	-	-	-	-	-	+
Early Gallatin	*Ur-4*	-	+	-	-	+	-	-
Mexico 309	*Ur-5*	+	-	+	+	-	+	-
Montrose	*Ur-5*	-	-	+	+	-	+	-
BelNeb-RR-1	*Ur-5*	-	-	+	+	-	+	-
Ouro Negro	*Ur*-Ouro Negro	+	-	-	+	-	+	-
Olathe	*Ur-6*	-	-	-	-	-	+	-
PC 50	*Ur-9*	-	+	+	+	+	-	-
BD-RGMR-4	*Ur-3*$^{+}$*,Ur-4*	-	+	-	-	-	+	-
BARC RR4,13,16	*Ur-4,Ur-5*	-	+	+	+	+	-	-
BARC RR25	*Ur-4,Ur-5*	-	+	+	-	+	-	-
BG-RMR-1,2,3	*Ur-4,Ur-11*	-	+	-	-	+	+	-
BDM 14	*Ur-3,Ur-6,Ur-11*	+	-	-	-	-	+	-
BMN RMR 7	*Ur-3,Ur-4,Ur-11*	+	+	-	-	-	+	-
BDM 18	*Ur-3,Ur-4, Ur-6,Ur-11*	+	+	-	-	-	+	-

Shaded boxes (columns 1 and 2) indicate lines of Andean background.
Shaded boxes (columns 3 to 9) with + or – are false positives and negatives, respectively.

THE OCCURRENCE OF ASIAN SOYBEAN RUST (CAUSED BY *PHAKOPSORA PACHYRHIZI*) ON COMMON BEAN IN SOUTH AFRICA

MM Liebenberg[1*], AJ Liebenberg[1] and ZA Pretorius[2]
[1]ARC-Grain Crops Institute, Private Bag X 1251, Potchefstroom 2520; [2]Department of Plant Sciences, University of the Free State, P.O. Box 339, Bloemfontein 9300, South Africa
[*]LiebenbergM@arc.agric.za

INTRODUCTION

Phakopsora pachyrhizi was first reported on soybeans (*Glycine max*) in South Africa in 2001 (Pretorius *et al*., 2001). However, none was found on *Phaseolus vulgaris* until April 2004, when it occurred on a small patch of *Ph. vulgaris* (surrounded on three sides by heavily infected soybeans) at Cedara Agricultural Research Station near Pietermaritzburg, South Africa (Du Preez *et al*., 2004). At this time, no *P. pachyrhizi* could be found on common bean in trials ± 1 km down wind of the soybeans. A trial was therefore planted to obtain information on the possible threat of Asian soybean rust to the common bean, to determine differences in susceptibility between *Ph. vulgaris* genotypes, and to identify potential sources of resistance.

METHODS

Field Trial The 91 entry, single row trial, included a wide spectrum of *Ph. vulgaris* germplasm containing all known (RR) genes conveying resistance to bean rust (caused by *Uromyces appendiculatus*) as well as breeding lines with multiple RR genes and the South African National Dry Bean Cultivar Trial (30 entries). Important additional USA genotypes were contributed by Drs. JR Steadman (University of Nebraska) and MA Pastor-Corrales (USDA, Beltsville). It was planted at Cedara Research Station (latitude: 29.5 south; longitude: 30.3 east; altitude: 1076 m above sea level) on 17 January 2005. Each *Ph. vulgaris* row was flanked by a row consisting of a mixture of short and long season soybeans (Stork, LS444, LS677), planted on 9 December 2004, and all highly susceptible to *P. pachyrhizi*. No fungicides were applied.

Greenhouse inoculations were done at the University of the Free State (UFS), Bloemfontein, and at the ARC-Grain Crops Institute (ARC-GCI), Potchefstroom, South Africa. At UFS, an isolate collected from and increased on soybeans was used. Fresh spores (in water + Tween 20) were applied to both leaf surfaces of seedling plants using an atomizer. Plants were incubated for 16 h incubation at 21 ºC, >95 % relative humidity (RH), then removed to a greenhouse at 16/25 ºC night/day. Consistent results, but generally with low severity, were achieved. Better results were achieved with Soltrol 130 as spore carrier, but this caused leaf burn. In addition, inoculation of *Ph. vulgaris* with dry spores of *P. pachyrhizi* in a settling tower gave satisfactory results. Exposing primary leaves of the common bean cultivar Teebus to 16 mg spores for a 3 min, followed by a high-humidity period as described above, provided sufficient rust lesions for rating purposes.

At ARC-GCI, an unpurified sample collected from the common bean, but increased on soybeans, was used. Only isolated pustules were obtained on *G. max* after transfer of the rust from common bean leaves, and only faint necrotic flecking with no pustules was obtained on common bean. Two increases on soybean were necessary to obtain sufficient spores for inoculation. Results using spray inoculations were unsatisfactory. The best method was application of a high concentration fresh dry spores using a No.1 artist's paint brush on mature leaves wetted with

0.01% Tween 20 in tap water. Plants were incubated for 24 h at >95 % humidity at ±19 °C and then removed to a greenhouse at ±20/24 °C night/day. Well-developed sporulating pustules were observed 18 d after inoculation. At both the UFS and ARC-GCI, pustules appeared gradually and considerably later than on soybeans.

RESULTS AND CONCLUSIONS

Field ratings (summarized in Pastor-Corrales *et al*., this edition) ranged between 0 for the upper canopy to 8 for the lower canopy, lines with a deteminate growth being the most seriously affected. Serious defoliation of *Ph. vulgaris* can take place as a result of infection by *P. pachyrhizi*. This is accompanied by both chlorosis and necrosis. However, serious infection of *Ph. vulgaris* by *P. pachyrhizi* is dependent on the close proximity of heavily infected soybeans, the relative timing of the rust epidemic and planting date, and the presence of ideal conditions, in particular high humidity. Leaf maturity may also play an important role in the field. *Ph. vulgaris* may not be able to maintain the epidemic in the absence of *G. max* as a source of inoculum, and spore production of *P. pachyrhizi* on *Ph. vulgaris* appears to be lower than on *G. max*. There were also fewer pustules per unit area, and *P. pachyrhizi* appeared to have a less adverse effect on yield of *Ph. vulgaris*. The viability of *P. pachyrhizi* may be adversely affected by *Ph. vulgaris*. At this stage, *P. pachyrhizi* is probably not a serious threat to *Ph. vulgaris* under normal farming conditions, unless unusually high levels of inoculum are present, ideal conditions for soybean rust prevail, *Ph. vulgaris* is planted adjacent to heavily infected soybeans, and *P. pachyrhizi* is not controlled on the soybeans. Differences in the reaction of *Ph. vulgaris* entries appeared to be a function of maturity at the time of infection rather than differences in genetic resistance to *P. pachyrhizi*, and no resistance appeared to come from "*Ur*"-genes. However, cooler temperatures and lower rainfall (but consistent nightly high humidity) that may have affected levels of *P. pachyrhizi*, were experienced after defoliation of the soybeans. Due to the high variability of *P. pachyrhizi*, the danger does exist that the pathogen may adapt to *Ph. vulgaris* and become a more serious threat, especially in countries such as Brazil, the USA and South Africa, where *G max* and *Ph. vulgaris* are cultivated in the same area, and where *P. pachyrhizi* can overwinter.

Future plans

A simplified, replicated version of the *Ph. vulgaris* germplasm trial is planned for 2005/6, using only a few selected entries. Two planting dates for soybeans will be used in order to provide inoculum pressure over the full growth period of common beans. Miniature trials may be included to test a) the ability of common bean to maintain the epidemic and b) the effect of a single fungicide application. Monitoring of *Ph. vulgaris* breeding trials will be continued for signs of damage due to *P. pachyrhizi*. Greenhouse inoculations will be continued and optimized for screening of common bean genotypes. Other possible areas of study include latent period, pustule forming, sporulation, viability, and the effect of *P. pachyrhizi* on yield of *Ph. vulgaris*.

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DIVERSITY OF THE RUST PATHOGEN AND COMMON BEAN GUIDES GENE DEPLOYMENT FOR DEVELOPMENT OF BEAN CULTIVARS WITH DURABLE RUST RESISTANCE

M. A. Pastor-Corrales
Vegetable Laboratory, USDA-ARS, Beltsville, MD, 20705-2350, USA

The high virulence diversity of *Uromyces appendiculatus,* the rust pathogen of the common bean (*Phaseolus vulgaris*), greatly complicates the development of cultivars with durable disease resistance. The virulence diversity of this pathogen varies in time and space, thus bean varieties that are resistant in one year or location may be susceptible in another. Understanding the virulence and genetic diversity and the evolution of the rust pathogen, as well as the diversity of its common bean host is essential to the development of bean cultivars with durable resistance.

Diversity of the Common Bean

Two major groups of beans, known as the Middle American and Andean gene pools, are recognized in cultivated common bean. Many distinguishing characteristics such as morphology (5), allozymes (6), seed proteins (3), DNA polymorphisms (1), and chloroplast data (2) separate these two very distinct groups of beans. These gene pools reflect multiple and independent domestications events within distinct wild populations in Middle America (Central America and Mexico) and the Andes region of South America.

Diversity of the Bean Rust Pathogen

The rust pathogen is autoecious, biotrophic and macrocyclic (8). It readily produces uredionospores and teliospores but the basidiospores, pycniospores and aeciospores are found infrequently. The five spore stages of *U. appendiculatus* are evidence of the genetic recombination capacity of this fungus and its plausible ability to produce new virulent strains (4). Recent virulence diversity studies of the rust pathogen using Andean and Middle American bean differential cultivars, and genetic diversity studies using molecular markers show that *U. appendiculatus* has two distinct groups of isolates mirroring the diversity of its common bean host (5). One group identified as Andean, is made of isolates that have narrow and specific host range; they are compatible only with or mostly with Andean cultivars. These isolates occur in Mozambique and Ecuador where Andean beans predominate. Another group, called Middle American is comprised of isolates having a broad and nonspecific host range; they are compatible with Andean and Middle American beans (5). These isolates are often found in Central America, Mexico and other countries where Middle American beans predominate. A phylogenetic analysis of sequence data of approximately 2.2 KB of the elongation factor 1-alpha gene (EF-1a) used to explore genetic diversity of the same isolates, resolved two distinct groups of isolates that corresponded to the Andean and Middle American virulence groups (5). The notable correspondence of the two groups of isolates of the rust pathogen with those of its bean host suggests coevolution between these organisms.

Development of bean cultivars with durable rust resistance

The diversity and coevolution studies described above have shown that rust resistance genes from beans of Andean origin tend to be susceptible to Andean races of rust pathogen; however, these genes are often very effective against many important Middle American races. Conversely, the rust resistance genes from Middle American beans often have broad resistance and but are

susceptible to many Middle American races and particularly resistant to most Andean races. For example, *Ur-11* a rust resistance gene of Middle American origin is resistant to all known races of the rust pathogen except race 108 which is of Middle American origin. On the other hand, the Andean *Ur-4* rust resistance gene is susceptible to most Andean races of the rust pathogen, but *Ur-4* is resistant to race 108 and to many other Middle American races. The *Ur-3* and *Ur-5* rust resistance genes of Middle American origin are susceptible to several Middle American races of the rust fungus but they provide resistance to most Andean races. Therefore, a practical consequence of the bean-rust diversity and coevolution studies is the realization that combining rust resistance genes (gene pyramiding) from Andean and Middle American gene pools could result in bean cultivars with effective and durable rust resistance throughout the world. In collaboration with scientists from Michigan and North Dakota state universities and the university of Nebraska, we have developed six great northern (known as BelMiNeb-RMR-8, -9, -10, -11, -12, and -13) and five pinto (known as BelDakMi-RMR-19, -20, -21, -22, and -23) bean germplasm lines that are unique in the world for the genes they combine. They contain two Middle American (*Ur-3* and *Ur-11*) and two Andean (*Ur-4* and *Ur-6)* genes for resistance to all known races of *Uromyces appendiculatus* and two genes (*I* and *bc-3*) for resistance to all known strains of the also variable bean common mosaic and bean common mosaic necrosis potyviruses. So far, these beans have been evaluated as resistant under greenhouse conditions to all 90 strains of the rust pathogen maintained at Beltsville, MD and under field conditions the US, South Africa and Honduras. In summary, understanding the diversity of the rust pathogen and of its common bean host has provided a rational basis for gene pyramiding of Andean and Middle American disease resistance genes and the possibility of having durable resistance in bean cultivars to highly variable pathogens.

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PROCEEDINGS FROM THE RUST WORKSHOP HELD AT THE 2005 BIC MEETING

Challenges to and Priorities for Management of Rusts of Common Bean

Compiled by H. F. Schwartz – Colorado State University, J. R. Steadman – University of Nebraska, and M. A. Pastor-Corrales – USDA/ARS at Beltsville

The 18th Biennial Meeting of the Bean Improvement Cooperative was hosted by Dr. W. E. Kee at the University of Delaware – Newark Campus on October 31 to November 2, 2005. A special workshop (4th International Rust Workshop) was convened by Greg Varner (USDBC representative) on November 2 to provide an overview on common bean (dry, snap, lima) vulnerability to and advances in management of Common Rust (caused by *Uromyces appendiculatus*) and Asian Soybean Rust (caused by *Phakopsora pachyrhizi*). Invited speakers from the USDA/ARS (Drs. Morris Bonde, Catherine Aime, Phil Miklas, Marcial Pastor Corrales), University of Nebraska (Dr. James R. Steadman), and ARC-Grain Crops Institute of South Africa (Dr. Merion Liebenberg) reviewed research progress and provided worldwide perspectives on Asian Soybean Rust, molecular variability within *U. appendiculatus* and compared to other plant rust pathogens, diversity of Common Rust in cultivated and wild beans in the Americas and Africa, and Common Rust resistance gene deployment strategies. Additional information is available in the proceedings of these individual papers elsewhere in this annual report, as well as at the BIC web site: http://www.css.msu.edu/bic/

During the course of these common bean rust presentations and discussions, a number of critical issues, needs and challenges emerged that have been compiled in this paper for use in strategic planning for and prioritization by the research community and the bean industry. Please feel free to cite and expand upon the following points that were generated by the collective participants at this biennial BIC meeting and workshop.

General Issues for Rusts of Common Bean:

- Develop universal protocols for the collection, preservation, inoculation and description of pathogenic responses (disease incidence and severity) and expression of genetic variability (race typing, virulence patterns);
- Expand the knowledge of pathogen biology, epidemiology, and especially the role of the sexual stage;
- Incorporate disease forecasting and the critical timing of effective fungicides in an integrated pest management context;
- Expand the use of molecular markers for pathogen resistance in germplasm improvement and varietal release efforts;
- Determine linkage of rust resistance markers to other disease resistance or plant trait markers;
- Continue to support the African Bean Rust Network.

Specific Issues for Asian Soybean Rust of Common Bean:

- Identify and characterize genetic resistance from cultivated and wild plant sources, with emphasis upon varied mechanisms including major gene, slow rusting, induced resistance;
- Expand the scope of germplasm rust resistance evaluation of *Phaseolus vulgaris*, other *Phaseolus* and legume species, and interspecific hybrids;
- Study the effects of mixed legume cropping systems upon endemic survival and epidemic development of the pathogen in tropical and temperate ecosystems;
- Investigate potential host range and vulnerability of cultivated and wild plant species under laboratory and field conditions;
- Preserve and expand a working collection of pathogen isolates at a USDA/ARS facility such as Beltsville or St. Paul;
- Identify and expand funding resources to emphasize research at the national and international level with this pathogen and its hosts;
- Search for resistance genes in *P. vulgaris* for transfer to *Glycine max*;
- Use *P. vulgaris* differential resistance reactions to phenotype *P. pachyrhizi* races.

Specific Issues for Common Rust of Common Bean:

- Develop molecular markers to facilitate elucidation of coevolution of the common rust pathogen and its bean host and expand knowledge base on the common bean – common rust model; identify specific races or pathotypes in support of diagnostic and epidemiological objectives; measure contributions from sexual and asexual stages in Andean and Middle American populations and ecosystems;
- Develop and implement breeding strategies for deployment of resistance genes (stacking, pyramiding, rotation, use of defeated genes) in varied cropping systems and regions;
- Preserve and expand the historical and working collection of pathogen isolates at the USDA/ARS Beltsville facility, and duplicate other collections as projects terminate and active rust personnel retire (e.g., Univ. of Nebraska);
- Improve the utility of race or pathotype differentials by replacing the widely susceptible Andean material Montcalm with red mottled JeMa (Ecuador) in 2006 and the Middle American material GN 1140 with a candidate like PI 310762 in the future;

Identify and expand funding resources to emphasize research at the national and international levels with this pathogen and its hosts.

INITIAL AFLP TAGGING OF THE GENE (CL) FOR CIRCUMLINEATED PATTERN

L. Aranda[1], T.G. Porch[2], M.J. Bassett[3]
[1]Agronomy and Soils Dept., University of Puerto Rico, Mayaguez, PR; [2]USDA/ARS/TARS, Mayaguez, PR; [3]Horticultural Sciences Dept., University of Florida, Gainesville, FL

Introduction

The *circumlineatus* gene (*Cl*) was discovered by R. Prakken (1972). *Circumlineatus* is recessive and is expressed in partly colored seed coats (*t*) with restricted patterns, such as virgarcus. *Cl* was discovered in a cross between 'White J' (*T z cl Rk*) x 'Soldaat K' (*t z Cl rk*^{d}) and is denoted by a "precipitation line" (Prakken, 1972) demarcating the boundary between the white and colored zones of the seed coat. Bassett (2004) further clarified the anatomy of the demarcation, by describing the precipitation line as a physical groove in the seed surface between seed coat colors in various genetic backgrounds, including mineral brown (*v* BC_3- 5-593), yellow brown (*b v* 5-593), and shamois (*g b v* BC_3 5-593). He found that only the black (- *B V*) F_2 seed from each backcross did not present the *cl* phenotype, but that non-black virgarcus types were true breeding.

The genetic location of *Cl* has not been confirmed, however molecular markers promise to resolve its location given that they have served as a useful tool in the study of seed coat color genetics. Early evidence for the genetic location of *Cl* indicated a weak linkage to *T* (partly colored seed coats) (Prakken, 1972). The linkage data from Prakken's work indicated a distance of approximately 36 cM between the loci, while *T* is located on linkage group B9 (McClean et al., 2002). Thus, the proposed location of *Cl* on linkage group 9 needs to be confirmed especially considering that it remains one of the few seed color loci that has not been tagged or mapped. The goal of this study was to tag *cl* using AFLP to identify co-segregating markers with bulk segregant (BSA) analysis. The long-term goal is to verify the genetic location of *Cl*.

Materials and Methods

Population development and *Cl* Genetics

A population segregating for *cl* was created from the cross, *t z cl G b v* virgarcus BC_3 5-593 × *t* z^{sel} *Cl G b v* sellatus BC_3 5-593. A selection was made in the BC_3-F_2 for a virgarcus (*z/z*) partly colored seed coat type without the circumlineated border. The F_3 generation segregated for *Cl* in plot 2-44, but was true breeding for virgarcus. The F_3 was planted under greenhouse conditions in Mayaguez, Puerto Rico and the F_4 seed was phenotyped for *circumlineatus*. The phenotypic data was verified by progeny testing the F_4 generation in the field in Isabela, Puerto Rico.

ALFP Analysis

DNA was extracted from the F_3 lines using Qiagen (Valencia, California) DNeasy extraction kits. AFLP analysis was performed using a AFLP pre-amplification kit from Licor (Lincoln, Nebraska) and selective amplification was performed according to Vos et al. (1995) using labeled *Eco* RI primers from Licor and non-labeled *Mse* I primers from Illumina (San Diego, California). The ALFP products were separated on a Licor 4300 sequencer. Bulk segregant analysis was performed on bulks of four *cl/cl* or *Cl/Cl* genotypes. Two bulks for each genotype were generated. Two *Eco* RI primers, one labeled with IR700 and the other with IR800, were amplified with a non-labeled *Mse* I primer. Both sets of primers contained three selective nucleotides. Initial bulk segregant analysis was performed using 96 primer-pair combinations

(Table 1) and primer combinations generating polymorphisms were tested on the whole population. Co-segregating markers were identified and the genetic distance between the AFLP marker and the locus was determined on the population derived from the 2-44 plot.

Results and Discussion

***Cl* Genetics**

Circumlineatus was found to be suppressed by *V* in the black seed coat color background in both virgarcus and sellatus patterns, but was expressed in other seed coat colors as a recessive gene (Bassett, 2004). In the virgarcus pattern with yellow brown seed coat color used in this study, we found a 1:2:1 ratio confirming a single recessive gene (Table 1).

Table 1. *Cl* segregation ratios in the F_3 based on progeny testing in the F_4 of plot 2-44

	Cl/Cl	*Cl/cl*	*cl/cl*		
Plot number	Normal virgarcus	Normal virgarcus	Circumlineated	χ^2 (1:2:1)	P
2-44	10	28	11	1.04	0.59

AFLP survey

Because of the high level of genetic similarity between the two parents of the population, *t z cl G b v* virgarcus BC_3 5-593 and *t* z^{sel} *Cl G b v* sellatus BC_3 5-593, a large survey of AFLP primers was conducted in order to identify linked markers. In total, 8 *Eco* RI and 32 *Mse* I primers combinations were surveyed, for a total of 256 AFLP primer combinations. One primer combination was found to yield unambiguous polymorphisms between the bulks of *cl/cl* and *Cl/Cl* genotypes: *Eco* RI GACTGCGTACCAATTC**ACC** + *Mse* I GATGAGTCCTGAGTAA**CTC.** The *E*-**ACC**, *M*-**CTC** primer pair amplified two dominant markers, one in coupling phase and at 12 cM from *Cl* (~90 bp) and the other in coupling phase and at 7 cM from *cl* (~120 bp).

The tagging of *Cl* will continue with additional populations and future plans include the mapping of the genetic location of *Cl* in common bean.

Literature Review

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EVALUATION OF COMMON BEAN FOR RESISTANCE TO *CLOVER YELLOW VEIN VIRUS*

Richard C. Larsen and Phillip N. Miklas
USDA-ARS, 24106 N. Bunn Rd., Prosser, WA 99350
rlarsen@pars.ars.usda.gov

A virus disease causing severe mosaic, top necrosis, stunting, and pod necrosis ("chocolate pod") on infected plants has been plaguing bean production in the Great Lakes region since 2000. Disease incidence and severity corresponds with influx of the soybean aphid (*Aphis glycines*) in the region. *Clover yellow vein virus* (ClYVV) was identified as a major component of the disease initially and as the cause of pod necrosis in 2003 (2). ClYVV and the pod necrosis disease also occurs sparingly some years on snap beans in the Willamette Valley of Oregon (3). Resistance to certain ClYVV strains exists in certain genotypes (4) but overall resistance to chocolate pod-inducing strains is not well understood. The objective of this study was to identify specific genes conferring resistance to ClYVV that could be successfully integrated into breeding programs.

Materials and Methods

In preliminary virus screening experiments to identify resistance to ClYVV, the host differentials for *Bean common mosaic virus* (BCMV) and *Bean common mosaic necrosis virus* (BCMNV) (1) were mechanically inoculated with a necrosis-inducing strain of ClYVV. Host response to ClYVV indicated that UI-31 and lines with *I* + *bc-3* genes may be resistant to the virus. Based on these results, recombinant inbred populations R31 and H31 developed previously for tagging other traits were evaluated for ClYVV resistance. The R31 population consisted of 103 $F_{5:7}$ RILs derived from Raven (*I* + *bc-3*) and I9365-31 (*I*). H31 consisted of 25 $F_{7:8}$ RILs derived from Harold ($bc\text{-}1^2$) and UI-31 ($bc\text{-}1^2$, $bc\text{-}2^2$). Separate sets of susceptible (Sutter Pink) checks, parents, and RILs for each population were mechanically inoculated at the primary leaf stage with an isolate of ClYVV from Wisconsin and Oregon, respectively. Symptoms expressed on primary leaves were recorded 12 days post-inoculation (dpi) and symptoms on secondary leaves were recorded 28 dpi. A random sampling within each RIL population was collected and evaluated for the virus by RT-PCR using primers designed specifically to detect ClYVV (2).

Results

Susceptible lines expressed typical symptoms resulting from infection with ClYVV All BCMV host differentials screened were susceptible to the Wisconsin and Oregon strains except UI-31 and lines TARS-VR-1s and TARS-VR-7s with *I* + *bc-3*. Some lines including 92US-1006, UI-114-8 Pinto, and UI-59 initially appeared to be resistant but subsequently showed characteristic virus symptoms. Reactions to inoculations with each strain of ClYVV were consistent across resistant and susceptible lines, although symptoms were more severe with the strain from Wisconsin than with the Oregon strain. ClYVV was not detected by RT-PCR in symptomless lines and thus, were considered resistant (Table 1). R31 parental line Raven was resistant and I9365-31 was susceptible. Response to ClYVV in the R31 RILs resulted in 52 resistant and 51 susceptible individuals and fit the 1:1 segregation ratio expected for a single resistance gene in a RIL population. All 52 RILs with *bc-3*//*bc-3* resistance to the NL-3 strain of BCMNV also were resistant to both strains of ClYVV, and conversely, 50 of 51 RILs lacking *bc-3* resistance were susceptible to the ClYVV. One RIL lacking *bc-3* was resistant to ClYVV. Other dry bean lines USLK-1, USLK-2, USLK-3, USDK-4 USDK-5, USWK-6, USCR-7, USCR-8, and USCR-9 with *I* + *bc-3* or *bc-3* alone remained symptomless after 50 days, further implicating *bc-3* gene in resistance to ClYVV. In the H31 population, UI-31 was resistant to the virus and Harold was susceptible. Nine individuals within the population were resistant and 16 were susceptible, which does not fit a 1:1 ratio suggesting that more than one gene maybe required in UI-31 to condition a resistance response.

Discussion

There was nearly complete co-segregation between *bc-3* and resistance to ClYVV among the 103 RILs from the R31 population. These results clearly demonstrate that a gene tightly linked with *bc-3,* or the *bc-3* gene itself, confers resistance to ClYVV. The resistant reaction for UI-31 which lacks *bc-3* suggests that additional genes are involved in resistance to ClYVV. Work of Tachel et al (4) identified two resistance genes conferring resistance to ClYVV that fit a recessive gene hypothesis. It is conceivable that the two resistance genes are actually the *bc-1*^{2}*, bc-2*^{2} combination found in UI-31. However, susceptibility of other BCMV and BCMNV host differentials with *bc-1*^{2} or *bc-2*^{2} indicate that resistance to BCMV or BCMNV in the case of UI-31 is not associated with resistance to ClYVV, as also supported by the literature. Additional experiments to identify the gene(s) for resistance to ClYVV in UI-31 are in progress.

Table 1. Reaction of genotypes within the BCMV and BCMNV host groups to ClYVV.

Host Group	Host differential	Resistance genes	Primary symptoms 11 dpi	Secondary symptoms 21 dpi	Secondary symptoms 50 dpi
0	Sutter Pink	None	VN	TN, D	---
1	Stringless Green Refugee	*bc-u*	VN	D	---
2	Redlands Greenleaf C	*bc-1*	CLL	sst, sM,->D	---
3a	Redlands Greenleaf B	*bc-1*^{2}	CLL	sst, sM,->D	sM
3b	UI-59	*bc- 1*^{2}	NS	NS	M
4	Sanilac	*bc-2*	VN	D	---
5	UI-114-8 Pinto	*bc-1, bc-2*	NS	NS	M
6a	UI-31	*bc-1*^{2}*, bc-2*^{2}	NS	NS	NS
6b	Othello	*bc-2*^{2}	VN	TN	TN, ->D
7	IVT 7214	*bc-2, bc-3*	NLL	sM, st	sM, st, TN
8	Black Turtle I	*I*	NLL, VN	D	---
9	Jubila	*I, bc-1*	NLL	M->D	---
10	Red Kloud	*I, bc-1*^{2}	CLL, Vchl	sst, sM, Bl	sst, sM, Bl
11	92US-1006	*I, bc-u, bc-2*^{2}	NS	NS	TN, pod lesions
12	TARS-VR-1s	*I, bc-3*	NS	NS	NS

CLL = chlorotic local lesions; Bl = blistering; M = mosaic; mM = mild mosaic; NLL = necrotic local lesions; NS = no symptoms; D = plant death; sM = severe mosaic; st = stunted growth; sst = severe stunting; TN = top (systemic) necrosis; Vchl = vein chlorosis; VN = vein necrosis.

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TILLAGE, PESTICIDE AND RESISTANCE MANAGEMENT OF WHITE MOLD IN DRY BEAN

Howard F. Schwartz, Mark A. Brick, J. Barry Ogg, Kris L. Otto and Mark S. McMillan, Dept. of Bioagri. Sci. & Pest Management, Dept. of Soil & Crop Sci., Colorado State University, Fort Collins, CO 80523

This project with the National Sclerotinia Initiative investigated the roles of cultural practices (plant growth habit, plant spacing) and timely application of chemicals in reducing damage from *Sclerotinia sclerotiorum* to *Phaseolus vulgaris*. Work was conducted in 2004 and 2005 to investigate the roles of plant growth habit (pinto Type III vine - 'Montrose' vs Type II upright - 'Vision'), plant spacing (1 vs 2 lines per 75 cm wide bed), and timely application of chemicals (none, thiophanate methyl = Topsin, and boscalid = Endura) within an Integrated Pest Management context. Unfortunately, the regional drought effects of 2004 and 2005 did not support appreciable disease development in this nursery in spite of our inoculations with white mold sclerotia prior to planting as well as mycelial inoculation of flowers. The field data were analyzed as a factorial, and plant spacing was the only significant main effect for yield (kg/hectare). There was a significant interaction between cultivar and spacing, as one would expect when comparing vine (Montrose) and upright (Vision) growth habits in different plant spacings. Both cultivars yielded more with the 2-line spacing that provided more uniform distribution and utilization of resources (light, moisture, nutrients). Yield of the vine cultivar was increased by 10% and 0%, while the yield of the upright cultivar was increased by 50% and 9% in 2004 and 2005, respectively. Additional testing of released cultivars and future germplasm releases with different growth habits is warranted under varying environmental conditions and disease pressure in the dry bean producing regions with a history of white mold.

Table 1. Dry bean yield responses of 'Montrose' and 'Vision' dry bean cultivars to varying plant density during 2004 and 2005 at the CSU ARDEC research farm.

Treatment		**2004 Trial***		**2005 Trial**	
Cultivar	**Spacing**	**Yield (kg/Ha)**	**100 Seed Wt (g)**	**Yield (kg/Ha)**	**100 Seed Wt (g)**
Vision	1 line	2274.05	73.45	2002.60	66.74
	2 lines	3411.87	72.24	2188.93	64.26
Montrose	1 line	2662.00	76.66	2066.04	68.53
	2 lines	2936.15	74.75	2054.56	65.30

During 2004 and 2005, a set of laboratory and greenhouse experiments systematically evaluated the efficacy of a standard (Topsin) and new (Endura) fungicides applied to foliage of susceptible cultivars 'Montrose' and 'Vision' before inoculation of leaf disks with the white mold pathogen. The rate of leaf colonization by the fungus was recorded over time (2 to 5 days post-inoculation and incubation at 23^{o}C). This series of replicated experiments was run 4 times, and statistical analyses allowed us to combine data over cultivars since there were no differences in responses between the susceptible cultivars (Montrose vs Vision) as expected. The conventional fungicide,

Topsin, provided very good control (80 to 84%) of white mold, even after 5 days of incubation. The newer fungicide Endura provided even greater control (97 to 99%), and offers a lot of potential for enhanced fungicide management for future IPM programs on dry bean and other crops that are affected by *Sclerotinia sclerotiorum*.

A second set of experiments systematically evaluated the response (efficacy) of these fungicides applied in varying gallonage to foliage of susceptible cultivar 'Montrose' before inoculation of leaf disks with the white mold pathogen. This series of replicated experiments was run 2 times, and statistical analyses allowed us to combine data over runs since there were no differences in responses between the two runs. The conventional fungicide, Topsin applied in 46 to 2337 liters of water per hectare (5 to 250 gallons of water per acre), provided very good control (84 to 96%) of white mold, even after 5 days of incubation. The newer fungicide Endura provided less control (39 to 93%) in this series of experiments with different gallonages. Both fungicides were more efficacious when applied in 234 or more liters of water per hectare (25 or more gallons or water per acre). These rates are typically associated with ground rig or low volume chemigation equipment; while rates less than 234 liters per hectare are typically associated with aerial equipment.

A final series of experiments demonstrated that the conventional fungicide, Topsin, and the newer fungicide, Endura, applied in 46 to 2337 liters of water per hectare (5 to 250 gallons of water per acre), provided very good control of white mold, even after a simulated rain event and 5 days of incubation. Topsin provide 93 to 95% control and Endura provided 87 to 95% with different gallonages and the post-treatment rain event. Both fungicides provided 90 to 98% control when there was no simulated rain event post-treatment.

Integrated Pest Management approaches such as selection of a resistant or at least less susceptible cultivar, use of certified seed, moderate plant population and fertility, crop rotation of 3 years or longer out of white mold susceptible hosts, tillage to promote healthy roots and more efficient use of moisture later in the season, aggressive scouting, weather monitoring and disease forecasting, and the timely application of appropriate fungicides can be successfully used to manage white mold of beans under varying cropping systems.

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CONSTRUCTION AND CHARACTERIZATION OF A COMMON BEAN BAC LIBRARY

Kangfu Yu, Margaret Haffner, and Soon J. Park
Greenhouse and Processing Crops Research Centre, Agriculture and Agri-Food Canada,
2585 County Road 20, Harrow, Ontario, N0R 1G0

Abstract
A bacterial artificial chromosome (BAC) genomic DNA library of the HR45 common bean line (*Phaseolus vulgaris* L.), which is highly resistant to common bacterial blight of common beans incited by *Xanthomonas axonopodis* pv. *phasoli* (Xap), was constructed and preliminary characterization was done. The library consists of 33024 clones arrayed in 86 384-well microtiter plates. Based on a genome size of 588MBP for *P. vulgaris* L, the library would cover 5 fold of the bean genome. The library is stable, all clones analyzed to date have inserts and the average size of the insert is 100kb.

Methods
Leaf nuclei were isolated by differential centrifugation and embedded in plugs of low melting temperature agarose (TAMU, 2002). This high molecular weight nuclear DNA was partially digested with Hind III (Folkertsma et al., 1999), size selected twice by pulsed-field gel electrophoresis, electroeluted from the gel (Strong et al. 1997) and ligated into the vector pIndigoBAC -5 (Epicentre). The ligation mixture was electroporated into TransforMax EC100 Electrocompetent *E.coli* cells (Epicentre). 33,024 individual clones were picked and grown in 384 well (capacity 120µl/well) microtiter plates in 90µl of modified LB medium containing 4.4 % glycerol. A total of three copies of this library were stored at -80^{o} C.

Results and Discussion
Not I digestion of 100 randomly picked clones indicated that all of the clones analyzed have an insert and the insert size ranges from 30 kb to 200 kb (Fig. 1) with an average insert size of 100 kb (Fig 2.). Because leaf nuclei were used as the source for high-molecular-weight DNA, contamination of the library with organelle sequences should be low. Stability analysis of clones with larger inserts from cultures of generation one and 100 with restriction enzymes has shown that the large insert library is stable in the *E coli* host. In addition, the use of Hind III for this library would complement the previously reported BAC libraries for generating overlapping clones.

This library will facilitate analysis of the physical organization of the bean genome and positional cloning of genes and QTLs associated with various traits such as disease resistance. Detailed analysis of this BAC library is still underway.

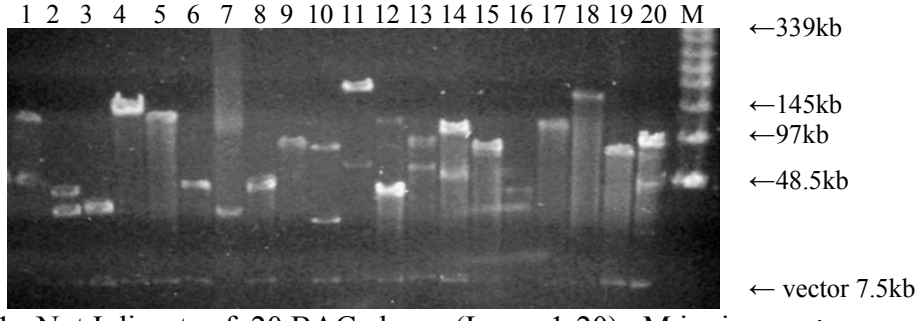

Figure 1: Not I digests of 20 BAC clones (Lanes 1-20). M is size marker.

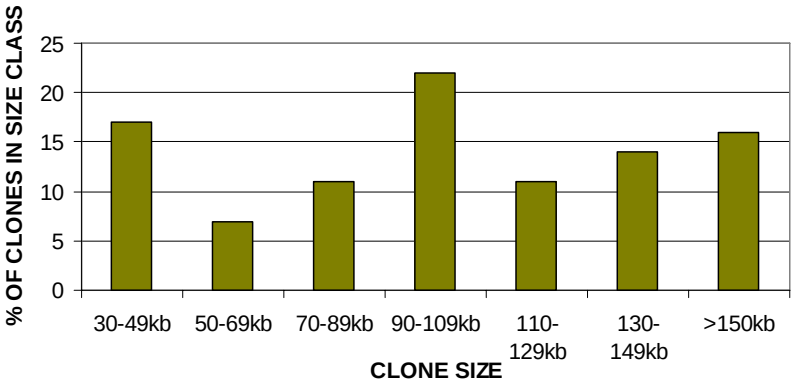

Figure 2: Size distribution of inserts

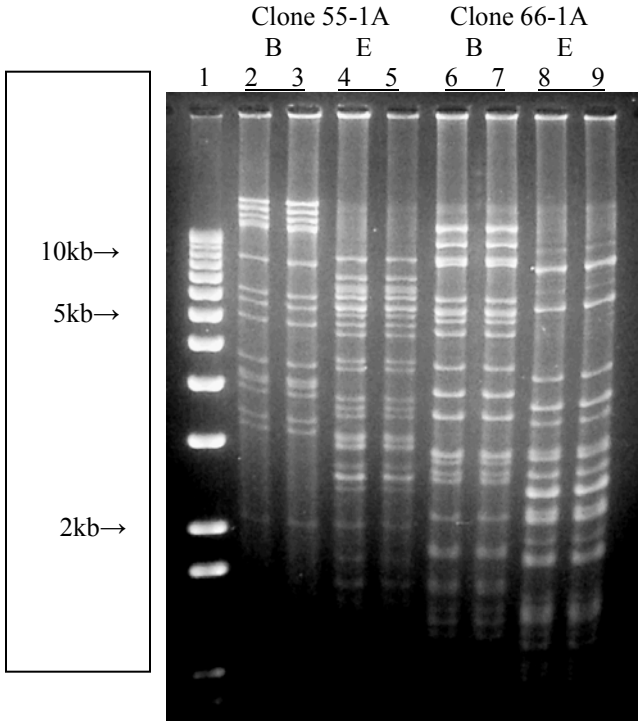

Figure 3. Stability Test. Lane 1 is a size marker. Lanes 2-5 are clone 55-1A. Lanes 6-9 are clone 66-1A. Lanes 2,3,6,7 were digested with Bam HI and 4,5,8,9 were digested with Eco RI. The first of each pair is generation one and the second is generation 100.

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QUANTITATIVE TRAIT LOCI FOR RESISTANCE TO WHITE MOLD IN COMMON BEAN.

Judd Maxwell, Mark Brick, Patrick Byrne, Howard Schwartz, Xueyan Shan, James B. Ogg, and Robert Henson. Colorado State Univ., Fort Collins, CO and North Dakota State Univ., Carrington Research and Extension Center, Carrington, ND.

White mold disease of common bean (*Phaseolus vulgaris* L.), caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, is a serious disease and causes annual average yield loss of 20 to 30% worldwide. Development of cultivars with physiological resistance combined with avoidance mechanisms, such as upright plant architecture, is the current strategy to minimize yield losses due to white mold. This research was initiated to verify the effect of a major QTL for resistance found in the Andean bean line G 122 (Miklas et al., 2001), and search for additional resistant QTL. A recombinant inbred line (RIL) population was developed from a cross between adapted pinto line CO72548 and G122 at Colorado State University (CSU RIL). The RIL population was screened in the greenhouse using the straw test (Petzoldt and Dickson, 1996) and a subset of the population was screened in an artificially induced white mold nursery at the Carrington Research and Extension Center, Carrington ND. Field reaction was based on % of plant tissue with mycelial growth. Polymerase chain reactions were conducted on the CSU RIL population to find QTL linked to resistance according to procedures by Kami et al. (1995). Amplified DNA was separated by electrophoresis on either 4% agarose gels or 6% denaturing polyacrylamide gels. All RAPD primers considered for this study were present on the core map developed by Freyre et al. (1998). SSR reactions were performed as described by Blair et al. (2003). AFLP reactions were performed as described by Inventrogen Life Technologies (AFLP analysis system II).

Two RIL had higher resistance than G 122 based on the average of three evaluations using the straw test. CSU RIL lines 31 and 67 had DSI of 3.2 and 3.4, respectively, compared to the resistant parent G 122 with 4.5 (Table 1). Both lines also showed lower levels of disease infection in the field however, the difference was not significant. One hundred twenty four molecular markers were used to map the CSU RIL population based on AFLP, SSR, RAPD and SCAR markers. A significant relationship ($P < 0.01$) was found between the B7 QTL (Miklas et al., 2001) and white mold reaction in both the greenhouse straw test and field. In total, four markers were found to be significantly associated with white mold resistance in the CSU RIL Population using single factor analysis and composite interval mapping (CIM). Based on CIM strong evidence (LOD >2.9) indicated that three QTL influenced physiological resistance to white mold (Table 2). The QTL were linked with marker loci a5p4195, ataca300, and *Phs* on linkage groups B2, B6a, and B7, respectively. The ataca300 region of B6a had the largest effect and accounted for 19.3% of the phenotypic variation for white mold reaction in the straw test. The a5p4195 region of the B2 linkage group accounted for 17.6%, and the *Phs* region of the B7 linkage group accounted for 16.3% of the phenotypic variation. A fourth QTL was significant at a genome-wide empirical threshold of LOD = 2.8 at the BM184 region of the B9 linkage group (Table 2). All QTL loci for resistance were contributed from parent G122 in the RIL population.

Table 1. Entry, phaseolin type and mean straw test DSI among check cultivars and the two most resistant recombinant inbred lines.

Entry	Phaseolin Type†	Mean DSI
31	T	3.2 a‡
67	T	3.4 a
G 122	T	4.5 bc
CO72548	S	5.8 c
PC-50	T	5.9 c
Montrose	S	8.0 d

† Denotes the seed storage protein type; where T = Tendergreen and S = Sanlic.
‡ Scores followed by the same letter are not significantly different ($p < 0.05$).

Table 2. Most important QTLs for white mold resistance; location, position, LOD score, estimated effect, nearest marker locus, and source of QTLs for resistance to white mold based on the mean straw test ASI. Results were obtained by composite interval mapping, with genome-wise empirical threshold significance levels.

Location of QTL	Position	LOD score	R^2	Nearest Marker	Source	Additive
	--- cM ---	--Score--	----%-----	Name		
B2	18	3.63	17.6	a5p4195†	G122	-0.5786
B6a	131	3.83	19.3	ataca300	G122	-0.5530
B7	42	3.59	16.3	*Phs*	G122	0.5127
B9	28	2.86	9.4	BM184	G122	-0.4015

† a5p4195 and ataca300 are AFLP markers, *Phs* is a SCAR marker, and BM184 in a microsatellite marker

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GENETIC DIVERGENCE IN LANDRACE BEAN (*PHASEOLUS VULGARIS* L.) GERMPLASM IN THE STATE OF PARANÁ, BRAZIL

L. P. Bonett**;** M. C. Gonçalves-Vidigal; A. R. Schuelter[2]; P. S. Vidigal Filho; Klasmann[2], J. P. [1/]
[1/] University State of Maringá; [2/] University Paranaense.

Introduction

In Paraná state (Brazil), several common bean (*Phaseolus vulgaris* L.) cultivars are commercially produced, mainly those representing the more common commercial groups, such as "Carioca", "Preto", "Mulatinho", "Roxo", "Rosinha" and "Amarelo". According to Voysest (2000), few cultivars of the groups "Carioca", "Jamapa" and "Synthetic Porrilo" have been used as parents, which results in the reduction of the additional genetic gain, due to use of a restricted gene pool.

The goal set for this study was to evaluate landrace bean germplasm collected in the State of Paraná and five additional controls, using uni- and multivariate techniques based on morpho-agrononomic traits as well as to make them available to bean genetic improvement programs.

Materials and Methods

Sample collection of the material used in this study was based on the spatial distribution of bean plantations in the state of Paraná carried out by Yokoyama et al. (2001). The treatments consisted of 58 landrace cultivars and five additional controls ("Pérola", "Aporé", "Diamante Negro", "FT Nobre" and IAPAR 81). The experimental design used was completely randomized blocks, with three repetitions, and plot size was of 2 rows x 5,0 m with 0.50 m between rows, and 5,0 m^2 useful area, plant density of 15 seeds/m.

The following traits were assessed: plant height (AP), flowering date (NDF), number of pod per plant (NVP), number of seeds per plant (NSP); number of seeds per pod (NSV), mean weight of 100 seeds (M100), grain yield per plant (PGP), total grain yield (PG), insertion height of the first pod (AI) and number of days to maturity (NDAC).

Initially, an analysis of variance was carried out for each trait and the landrace bean germplasm. The multivariate analysis evaluated the genetic divergence among accessions by the UPGMA and Toche's algorithm, based on the Mahalanobis' generalized distance. The relative contribution of each trait to genetic diversity was determined by the Singh's method (Singh, 1981). All analyses were performed with the Genes Software (Cruz, 2001).

Results and Discussion

There were significant differences ($P < 0,05$) between the landrace cultivars for all the traits, indicating the existence of genetic variability.

The method proposed by Singh (1981) showed that (PG), (CI) and (PGP) gave the greatest relative contribution, representing 71.35% of the total variation.

The Tocher's method grouped the landrace cultivars and the controls in nine clusters, being group I divided in seven subgroups (Ia to Ig) with 36 landrace cultivars and the five controls. A possible explanation for the similarity among the large number of "Carioca" cultivars is the prevalence of hybridizations involving a small number of ancestry belonging to the Mesoamerica race, among them, the "Carioca" cultivars "Jamapa", "Synthetic Porrillo" and "Turrialba" (Voysest, 2000). Nine groups were formed by the UPGMA method. Group I with 18 landrace cultivars of the "Carioca" group, four cultivars of the "Preto" group and the five controls.

The UPGMA method grouped in the clusters VIII and IX all landrace cultivars of the type Jalo, being group VIII consisted of cultivars 7, 20, 8 and 18; the same grouping was obtained by the Tocher's method. The dissimilarity dendrogram showed that the most divergent cultivars were "Carioca Pitoco" and "Jalo Vermelho", whereas the most similar cultivars were "Carioca Pitoco" and "Carioca".

Conclusions

The most divergent cultivars were "Carioca Pitoco" (1) and "Jalo Vermelho" (43), whereas "Carioca" (41) and "Carioca Pitoco" (1) were found as the most similar. Cultivars "Carnaval" (33), "Carioca Pitoco" (16), "Pérola" (14) and "Carnaval" (27) are recommended for obtaining superior genotypes in interpopulation improvement programs. The traits (PG), (CI) and (PGP) gave the greatest relative contribution, representing 71.35% of the total variation.

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POTENTIAL MARKER-ASSISTED SELECTION FOR RESISTANCE TO SCLEROTINIA WHITE MOLD IN PINTO AND GREAT NORTHERN BEAN

Phillip N. Miklas

USDA-ARS, Prosser, WA, 99350 (pmiklas@pars.ars.usda.gov)

Resistance to white mold (*Sclerotinia sclerotiorum*) in dry bean (*Phaseolus vulgaris*) is quantitatively inherited with low heritability. The identification of QTL conferring resistance may enable MAS to combine resistance sources and expedite development of resistant cultivars. Our objective was to backcross white mold resistance linked QTL markers into pinto and great northern bean.

A QTL on linkage group B7 conferring white mold resistance linked with *Phs* SCAR marker from G 122 source (Miklas et al., 2001) was introgressed by marker-assisted backcrossing (MAS-BC) into 'Winchester' pinto bean, and another QTL on B8 linked with RAPD and SCAR markers SS18, AW9, and C5 from NY6020-4 source (Miklas et al., 2003) was similarly transferred by MAS-BC into 'Maverick' pinto and 'Mayflower' great northern bean.

Resulting $BC_nF_{4:6}$ lines from five populations were screened for the markers and for reaction to white mold in the greenhouse straw test and in replicated field trials.

Table 1. Segregation and phenotypic variation explained by the QTL linked markers for disease reaction in the MAS BC-lines.

Population	Observed Lines#	Expected marker	Variation explained ratio	straw test	field
MAS for B7 QTL				R2	
Winchester*2/CO8112034//G122/Winchester ($BC_3F_{4:6}$)					
PS02-011A	50	32+ / 18-	1:1	42%	17%
PS02-011D	38	18+ / 20-	1:1	64%	12%
MAS for B8 QTL					
Maverick*3/NY6020-4 ($BC_2F_{4:6}$)					
PS02-005C	52	24+ / 28-	1:1	47%	NT
Maverick/3/OT9630-17-25*2/NY6020-4 ($BC_2F_{4:6}$)					
PS02-006D	33	14+ / 19-	1:1	35%	17%
Matterhorn*3/NY6020-4 ($BC_2F_{4:6}$)					
PS02-029C	41	15+ / 26-	1:1	NT	27%

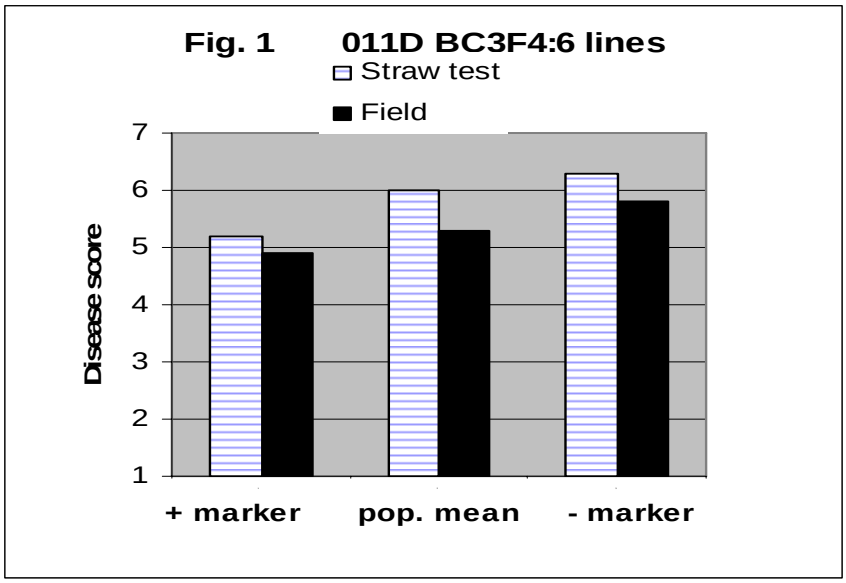
NT = not tested

The markers effectively transferred the resistance QTL into the recurrent parents (Table 1). Presence/absence of the marker equated to a difference of 1 unit of disease score (Figure 1). No linkage drag due to presence of the markers was observed (Table 2), but overall yield was depressed in the MAS BC-lines compared to the recurrent parents.

Overall results indicate that MAS-BC of QTL has potential to compliment phenotypic selection in breeding pinto and great northern bean for improved white mold resistance.

Table 2. Effect of presence/absence of QTL linked markers on performance of MAS-BC lines

Trait	+ marker	RP mean	- marker
PS02-11A (B7 QTL)		Winchester	
Yield (lbs/A)	2702	3496	2820
Harvest maturity (DAP)	88	88	87
PS02-011D (B7 QTL)		Winchester	
Yield (lbs/A)	2156	3004	2383
Seed size (g 100-1 seed)	40	41	37
Harvest maturity (DAP)	90	88	87
PS02-006C (B8 QTL)		Maverick	
Yield (lbs/A)	3390	3158	2925
Seed size (g 100-1 seed)	41	43	42
Harvest maturity (DAP)	110	92	112
PS02-029C (B8 QTL)		Matterhorn	
Yield (lbs/A)	2144	2469	2052
Harvest maturity (DAP)	90	93	89



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HALO BLIGHT RESISTANCE IN HOST DIFFERENTIAL CULTIVAR ZAA 12 IS CONDITIONED BY THREE MAJOR GENE LOCI

Phillip Miklas[1] and Deidré Fourie[2]
[1]USDA-ARS, Prosser, WA, pmiklas@pars.ars.usda.gov;
[2]ARC-Grain Crops Institute, Potchefstroom, South Africa, FourieD@arc.agric.za

Halo bacterial blight caused by *Pseudomonas syringae pv. phaseolica* (*Psp*) is a serious seed-borne disease limiting common bean production worldwide. Genetic resistance provides the most effective means for combating this disease. ZAA 12 (A43) is the most resistant host differential cultivar (Table 1) conditioning resistance to 7 of 9 differential races of the pathogen (Taylor et al., 1996). Our objective was to study the inheritance of halo blight resistance in ZAA 12.

A recombinant inbred population consisting of 79 $F_{6:7}$ RILS was developed from the cross ZAA 12/Canadian Wonder. ZAA 12 is resistant to *Psp* Races 2, 3, 4, 5, 7, 8, and 9. Canadian Wonder is a universal susceptible. Separate greenhouse inoculations of the RILs and parents were performed for each race.

ZAA 12 has three major gene loci: *Pse-2*, *Pse-3*, and *Pse-4*. There were 36 Resistant / 35 Susceptible / 8 Segregating RILs with complete co-segregation for reaction to Races 2, 7, 8, and 9. There was 51% segregation for resistance among the RILs indicating a single gene (gene cluster) conditions resistance to Races 2, 7, 8, and 9.
We suggest that *Pse-2* confers resistance to Races 2, 7, 8, and 9 (Table 2); whereas, Teverson (1991) postulated that resistance to these races was governed by a dominant gene *Pse-2* and recessive gene *pse-5*. *Pse-2* also conditions resistance to Races 3, 4, and 5 (see details below)

There were 66 R / 9 S / 4 segregating RILs for reaction to Races 3 and 4 with complete co-segregation for resistance to both races. Thus, 88% of the RILs exhibited hypersensitive resistance to Races 3 and 4, indicating at least two genes affect resistance as also observed by Teverson (1991). We suspect that *Pse-3* which conditions hypersensitive resistance to Races 3 and 4, and completely cosegregates with the *I* gene that conditions resistance to *Bean common mosaic virus* (BCMV), is one of the genes present. The other dominant gene *Pse-2* was proposed by Teverson (1991). The resistance affect of *Pse-2* against Races 3 and 4 is supported by our results because all RILs susceptible to Races 3 and 4 lacked this gene (Table 2).

There were 59 R / 19 S / 1 segregating RILs for reaction to Race 5. Thus, 76% of the RILs were resistant to Race 5, indicating presence of two genes affecting resistance.
Pse-4 conditions resistance to Race 5, and is present in ZAA 12 based on allelism tests conducted by Teverson (1991). Our results also support presence of a second gene *Pse-2* as reported by Teverson (1991) because all RILs susceptible to Race 5 also lacked *Pse-2* gene (Table 2).

Preliminary results for the inheritance of halo blight resistance in ZAA 12 (A43) support the findings of Teverson (1991) that: i) two independent dominant genes *Pse-3* and *Pse-2* condition resistance to Races 3 and 4, and ii) two independent dominant genes *Pse-4* and *Pse-2* condition resistance to Race 5. A new finding from this study is that a single gene *Pse-2* (or gene cluster) conditions resistance to Races 2, 7, 8, and 9. The recessive gene *pse-5* described by Teverson (1991) has not been observed in tests conducted thus far. If *pse-5* is present in ZAA 12 then it must be tightly linked with *Pse-2* gene. Available (*I* gene and SW13 SCAR, and SB10 SCAR for *Pse-3*) and newly developed markers linked with the genes will be generated and used

to further study and confirm the inheritance of halo blight resistance in ZAA 12 and other sources.

Table 1. Host/pathogen differential set for reaction to *Psp* (Taylor et al., 1996; Teverson, 1991).

		Race								
Differential	**R-genes**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Canadian Wonder	-	+	+	+	+	+	+	+	+	+
ZAA 54 (A52)	4	+	+	+	+	-	+	+	+	+
Tendergreen	3	+	+	-	-	+	+	+	+	+
Red Mexican UI 3	1,4	-	+	+	+	-	+	-	+	-
P.acutifolius (1072)	2	+	-	+	-	-	+	-	+	+
ZAA 55 (A53)	3,4	+	+	-	-	-	+	+	+	+
ZAA 12 (A43)	2,3,4,5	+	-	-	-	-	+	-	-	-
Guatemala 196-B	3,4	-	+	-	-	-	+	-	+	-

Table 2. Reaction of RILs (ZAA 12/Canadian Wonder) with putative halo blight resistance genes to 7 differential races of the pathogen (*Psp*).

RILs	Race 2	Race 3	Race 4	Race 5	Race 7	Race 8	Race 9
Resistant (*Pse-2*)	R	R	R	R	R	R	R
Susceptible (*pse-2*)	S	R or S	R or S	R or S	S	S	S
Resistant (*Pse-3*)	R or S	R	R	R or S	R or S	R or S	R or S
Susceptible (*pse-3*)	S	S	S	R or S	S	S	S
Resistant (*Pse-4*)	R or S	R or S	R or S	R	R or S	R or S	R or S
Susceptible (*pse-4*)	S	R or S	R or S	S	S	S	S

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BIOLOGICAL CONTROL OF SCLEROTINIA DISEASES (*SCLEROTINIA SCLEROTIORUM*) OF BEAN AND CANOLA BY *CONIOTHYRIUM MINITANS*.

McLaren, D.L. [1]*, Huang, H.-C. [2], Conner, R.L.[3], McAndrew, D.W.[3], Irvine, R.B.[1]
[1] Agriculture and Agri-Food Canada, Brandon, Manitoba R7A 5Y3, Canada;[2] Agriculture and Agri-Food Canada, Lethbridge, Alberta T1J 4B1, Canada; [3] Agriculture and Agri-Food Canada, Morden, Manitoba R6M 1Y5, Canada.*(dmclaren@agr.gc.ca)

Introduction

White mould of bean (*Phaseolus vulgaris*) and sclerotinia stem rot of canola (*Brassica napus*), caused by *Sclerotinia sclerotiorum*, are destructive diseases of these crops and have caused significant economic losses in the United States and Canada. Spraying chemical fungicides to protect canola and bean blossoms can be effective in controlling sclerotinia disease but proper timing and effective coverage of crop canopy are necessary to obtain adequate disease control. These conditions can be difficult to achieve and application of chemical fungicides does not reduce the numbers of ascospores released from soil-borne sclerotia. There is increasing concern that pesticide residues in water and soil may be harmful to the environment and therefore it is important to explore alternative methods to chemical control of sclerotinia diseases. The fungus *Coniothyrium minitans* is a mycoparasite capable of attacking *S. sclerotiorum* under natural conditions. The objective of these studies was to evaluate the potential of application type (foliar and/or soil) and application timing of *C. minitans* for the control of white mould of dry bean and sclerotinia stem rot of canola under Canadian prairie conditions.

Materials and Methods

Biocontrol of sclerotinia diseases was evaluated in two provinces, Manitoba and Alberta during 2001-04 (bean) and 2004 (canola). The treatments at each site in each year included applications of fungicide (vinclozolin or boscalid), biological control agent (BCA) and an untreated control. Two seeding rates and two cultivars (Envoy and NW63) were incorporated into the 2004 bean trials at two locations. Canola tests involved one cultivar only. The fungicide and biocontrol were applied at the recommended crop development stages and in 2004, biocontrol treatments included a spring soil treatment and a foliar spray at 30-50% bloom. Severity and incidence of sclerotinia were assessed at maturity and plot yields were determined.

Results and Discussion

The incidence of white mould of bean was reduced with the application of *C. minitans* and the fungicide in most trials. In 2002 at Brandon, *C. minitans* reduced the proportion of plants infected by an average of 32%. The BCA applied as a single or split application resulted in greater yields and fewer sclerotia per kg of seed than in the untreated control . White mould incidence and severity for all of the *C. minitans* treatments and the fungicide treatments were lower than the control of *S. sclerotiorum* at Lethbridge in 2004 (Figure 1). This trend was evident with both seeding rates of NW63 and Envoy at Brandon. The combined treatment of *C. minitans* foliar spray plus soil application resulted in the highest seed yields at Lethbridge (cv. NW63). Seed yields for NW63 (low seeding rate) and Envoy at Morden were highest with the *C. minitans* combination treatment when compared to all BCA treatments, but were not as high as those obtained with the fungicide spray applications. The *C. minitans* foliar spray(s) produced the highest yields of all treatments at Brandon. *C. minitans* significantly reduced the number of sclerotia of *S. sclerotiorum* in harvested seed and was consistently recovered from sclerotia

produced on diseased bean plants. A numerical trend towards reduction of white mould severity with the low compared to the high seeding rate was evident at Morden and Brandon in untreated control plots but these differences were not significant. In canola, application of the BCA as a spore suspension to the soil and later to the foliage at the early flowering stage was as effective as fungicide spray in reducing sclerotinia stem rot at the Lethbridge site. These two treatments resulted in the highest seed yields and lowest recovery of sclerotia from seed. At Morden, application of the BCA as a foliar spray and application of the fungicide spray were the most effective treatments in reducing incidence of stem rot compared to the untreated control and resulted in the highest average yields and lowest recovery of sclerotia from seed. A similar trend was observed at Brandon (Figure 2). These studies suggest that *C. minitans* is a promising agent for the management of *S. sclerotiorum* in bean and other susceptible crops such as canola.

Acknowledgements
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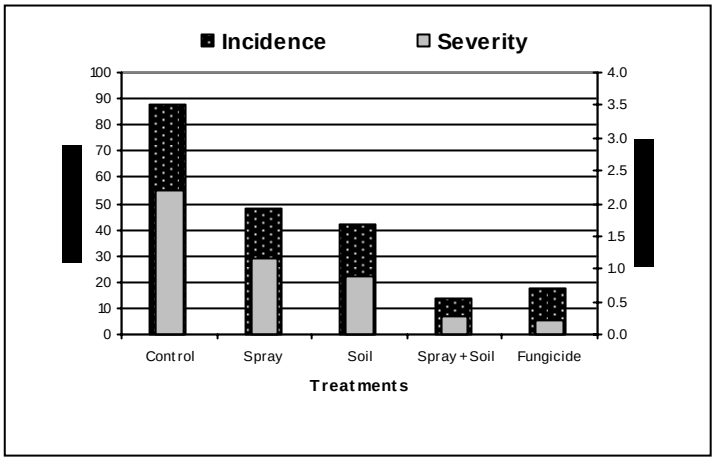

Figure 1. Impact of *C. minitans* and fungicide on incidence and severity

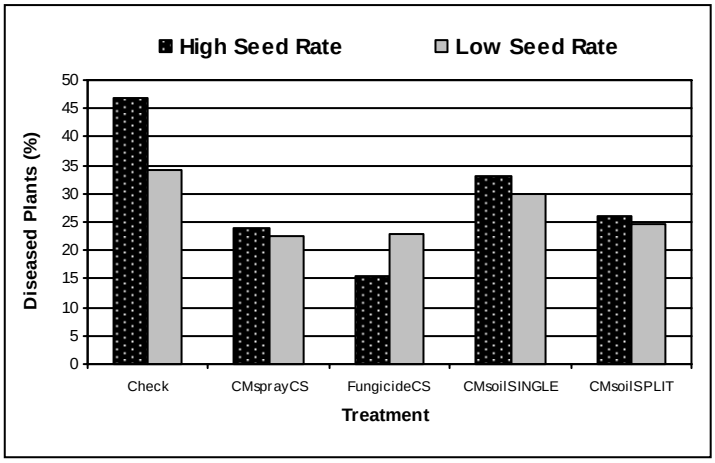

Figure 2. Impact of *C. minitans* and fungicide on sclerotinia stem rot of canola

A MOLECULAR MARKER LINKAGE MAP OF SNAP BEAN (*PHASEOLUS VULGARIS*)

J.W. Davis[1], D. Kean[1], B. Yorgey[2], D. Fourie[3], P.N. Miklas[4], & J.R. Myers[1]. [1]Dept. Horticulture & [2]Dept. Food Sci. & Tech., Oregon State Univ., Corvallis, OR USA; [3]ARC-Grain Crops Inst., Potchefstroom, S. Africa, [4]USDA-ARS, and IAREC, Prosser, WA USA.

Bush Blue Lake (BBL) snap beans are grown in the Willamette Valley of Oregon for canning and freezing. While they have desirable processing and production traits compared to other snap beans, plant architecture, seed production traits, and white mold resistance need improvement (Myers, et al, 2004a). Previous breeding work at OSU demonstrated that it is very difficult to recombine Oregon BBL materials with other snap beans (Myers and Baggett, 1999). Using molecular markers Myers and Davis (2002) showed that Oregon BBL snap beans are of the Mesoamerican center of origin whereas most other North American snap beans are of the Andean center of origin, which could explain the lack of useful recombinants between these types. A molecular marker linkage map should allow us to monitor the introgression of new traits while maintaining the BBL traits in future germplasm and cultivar development.

A recombinant inbred (RI) population of 80 lines from the cross 'Minuette' × 'OSU 5630' was grown in an observation trial as described in Myers, et al (2004a). Pods harvested at processing maturity were graded into sieve sizes 1-6, and samples were evaluated for pod length, cross section (height × width), straightness, smoothness, color, ace (shininess), and for the presence of suture strings The framework map consisted of 200 marker loci (173 RAPD's, 24 SSR's, 1 EST, and 2 phenotypes) in 11 linkage groups for a total of 470 cM (Figure 1).

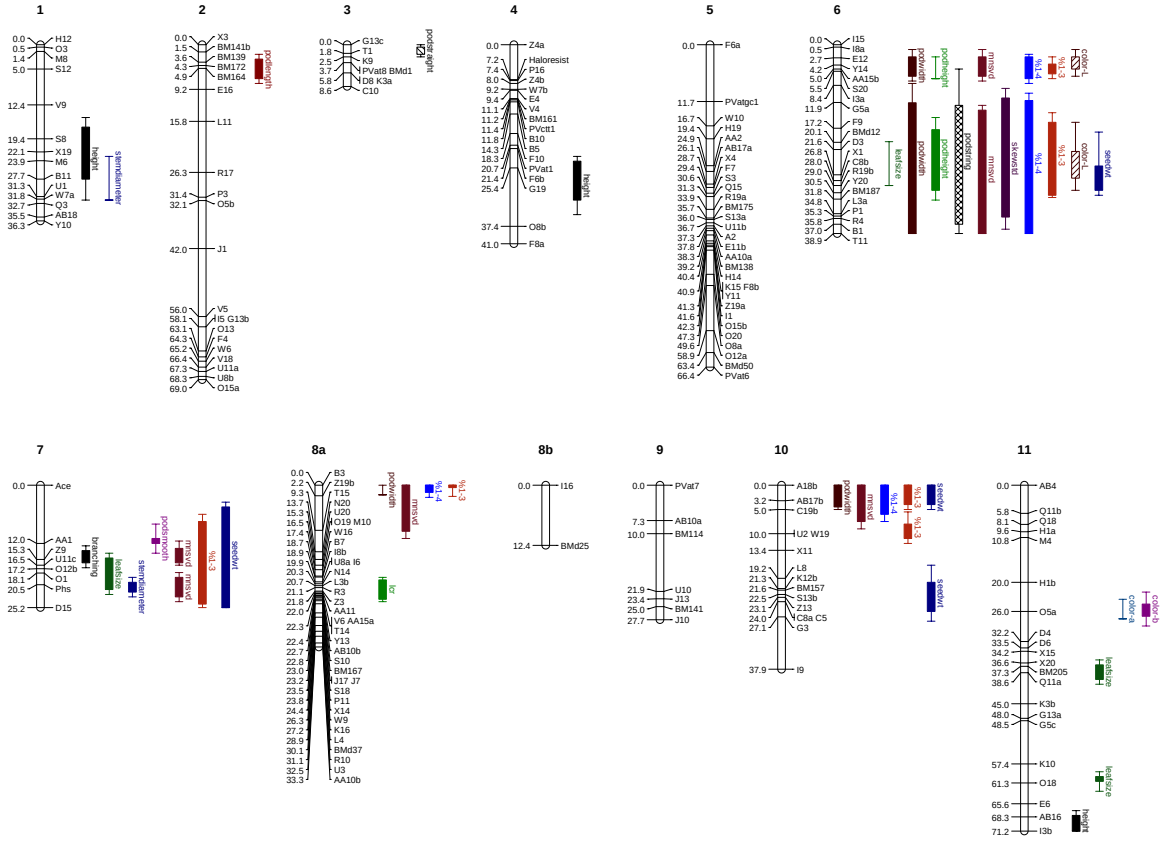

Figure 1. Linkage map for 'Minuette' × 'OSU 5630' RI population. QTL's are indicated with lower LOD of 2.0 and upper LOD (box) at the p = 0.01 significance level for each trait.

Table 1. QTL's for pod and architecture traits.

TRAIT	Group	Marker Loci	*LOD*	% Expl. Var.
Plant height (cm)	1	M6	3.9	22.5
	4	G19	3.4	19.1
	11	I3b	2.7	15.4
Branching [1]	7	Z9	3.0	17.0
Stem diameter	1	U1	2.3	13.0
(cm)	7	Phs	2.7	15.3
Leaf size[1]	6	D3	2.4	13.5
	7	Phs	2.9	16.1
	11	Q11a, O18	3.0, 2.7	16.6, 15.4
Leaf crinkle (lcr)	8a	Z3	2.7	15.1
Pod length (mm)	2	BM164	3.3	19.1
Pod straightness[1]	3	T1	2.5	14.4
Pod width (mm)	6	X1, AA15b	9.1, 3.8	43.6, 20.8
	8a	B3	2.6	15.0
	10	AB17b	3.1	17.7
Pod height (mm)	6	BMd12, S20	4.9, 2.3	26.5, 13.3
Pod smoothness[1]	7	AA1	2.7	15.5
Pod string[1]	6	X1	4.8	25.8
Seed weight	6	R19b	3.7	21.6
(g/100)	7	Phs	5.3	28.8
	10	BM157	3.3	19.6
%1-4 sieve[2]	6	X1, S20	13.5,	57.7, 21.8
	8a	B3	3.9	16.1
	10	AB17b	2.8	23.6
			4.3	
%1-3 sieve[2]	6	X1, S20	3.7, 2.5	14.4, 21.1
	7	Phs	4.5	24.8
	8a	B3	3.0	17.4
	10	A18b	2.7	15.8
Mean sieve	6	X1, S20	8.8, 3.3	43.0, 18.7
distribution[3]	7	Phs, Z9	3.2, 2.8	18.3, 16.1
	8a	B3	3.8	21.1
	10	AB17b	3.8	21.4
Skewness (std) [3]	6	X1	8.4	42.2
Color L[4]	6	X1, E12	3.0, 2.9	19.4, 17.9
Color a[4]	11	O5a	2.3	14.6
Color b[4]	11	O5a	2.9	17.6

[1]Scale of 1 to 9 where 1 = smallest/least and 9 = largest/most. [2]Proportion of pods in 1 – 4 or 1 – 3 sieve size class out of six classes. [3]Distribution of pods among six sieve size categories. [4]L*a*b colors for processed pods.

Table 1 lists the QTL's with their LOD and % explanation of variance values. The trait plant height mapped to three locations, and a small cluster of architecture traits was observed on LG7.*lcr*, which represents hybrid incompatibility between the two centers of origin, did not fit any known segregation ratio and was therefore mapped as a quantitative trait. Many of the pod traits were found in clusters of various combinations. The largest of these clusters, LG6, also had the highest % explanation of variance values for the traits pod width, pod height, %1-4 sieve, mean sieve distribution, and standardized skewness. Also located on LG6 is the QTL for pod string. *st*, a gene controlling pod suture string, was previously mapped to LG2 on the consensus map (Koinange et al., 1996), therefore our QTL probably represents a different gene for stringy pods. While both OSU 5630 and Minuette have round pod section (width × height) and are stringless, transgressive segregation was observed in the RI lines, suggesting that different genes control these traits in the two parents (Myers, et al, 2004a).

The amount of phenotypic variation and transgressive segregation observed in these RIL lines suggests that OSU 5630 has a different complex of genes for the snap bean phenotype compared to Minuette, which may relate to the difference in centers of origin of the two parents (Myers, et al, 2004b). The utility of a molecular marker map may facilitate the introgression of these and other traits of interest through marker-assisted selection methods and by developing a better understanding of the origins and contributions of desired traits from parental material.

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ARCELIN–LIKE AND α-AMYLASE–LIKE INHIBITOR DNA SEQUENCES COSEGREGATE WITH A NOVEL SEED STORAGE PROTEIN IN *PHASEOLUS VULGARIS* X *P. ACUTIFOLIUS* HYBRIDS

Paul M. Kusolwa and James R. Myers
Department of Horticulture, Oregon State University, Corvallis, OR, USA.

Wild tepary beans (*P. acutifolius* A. Gray) are a potentially valuable source of desirable traits, including drought tolerance, disease and insect resistance. So far, only common bacterial blight resistance has been transferred from tepary beans (Singh and Munoz, 1999; Munoz et al., 2004). Several accessions of wild tepary beans confer strong resistance to the two major bruchid pests of common bean. The main objective of this project is to develop common bean cultivars resistant to the bean bruchid *Acanthoscelides obtectus* through interspecific transfer of resistance from tepary bean.

The resistance mechanism in tepary beans is not well understood. Various types of lectin family proteins (arcelin, α-amylase inhibitor and phytohemagglutinin) may play a role in bruchids resistance (Kornegay & Cardona, 1991; Mirkov et al., 1994; Finard-Filho, 1996; Yamada et al., 2005). In this work, the wild tepary bean accession G40199 identified by CIAT as resistant to *A. obtectus* was hybridized to 'ICA Pijao' and F_1 plants were obtained via embryo rescue after 22 to 28 days. Seeds from the first backcross were used to screen for the presence of novel protein profiles corresponding to those found in the *P. acutifolius* accession G40199. A small portion of the seed were scratched on sand paper to obtain a fine powder for total protein extraction and electrophoresis of proteins fragments on SDS – PAGE followed by standard staining with coomasie blue in 10% acetate and 40% methanol. A 33 – 35 kDa seed storage protein (similar in size to arcelins) was stably integrated after two backcrosses to 'ICA Pijao'. The protein has also been successfully introgressed into 'Rojo', an improved Tanzanian cultivar, via bridge interspecific hybrid lines. More than one seed storage protein may be responsible for conditioning resistance to bruchids. The unique arcelin-like isoform and α-amylase inhibitor-like proteins appear to be tightly linked. Other storage proteins with potential insecticidal properties are also likely to be co-transferred into *P. vulgaris*.

BLAST search and screening of related storage protein gene sequences for phytohemaglutinins, α-amylase inhibitors and arcelins isoforms was conducted. DNA sequence alignment and primer designing for different genes was performed manually. These primers were used for screening for DNA amplifications from leaf tissues of tepary G40199, cultivated brown tepary bean and the interspecific hybrid lines. Only sets of primers that generated polymorphic DNA fragment with stable and repeatable size after optimization of PCR conditions were used to screen for the presence of the introgressed novel protein among interspecific hybrids. The DNA fragment generated by primers for the co segregating proteins was sequenced and their DNA sequence homology aligned and compared by the BLASTP alignment. Two DNA markers associated with arcelin-like and α-amylase inhibitor-like proteins, respectively, co-segregate with seed

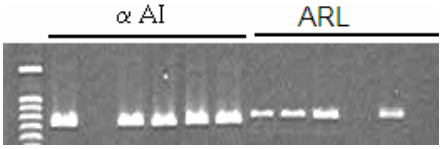

Figure 1 DNA PCR fragments amplifications for α-amylase-like (α-AI) and arcelin-like(ARL) proteins from leaf tissues of *P. acutifolius* and *P. vulgaris* interspecific hybrids. Lane 1: 100 bp ladder; Lanes 2 & 12: G40199; Lanes 3 & 11: ICA Pijao; Lane 4: Cultivated brown tepary; Lanes 5-9: Interspecific backcross lines.

storage proteins. An arcelin like (ARL) DNA fragment with molecular size of ~800bp was only detectable in genomic DNA from G40199 and among interspecific backcross lines that inherited the equivalent size of the storage protein (**fig. 1**). An additional DNA fragment corresponding to α-amylase inhibitor-like (α-AIL) protein is found in both tepary accessions (G40199 and cultivated brown tepary), but not in *P. vulgaris* cultivar ICA Pijao (**fig. 1**). This fragment of ~750bp also co-segregated with the novel storage protein inherited among interspecific hybrids.

The ARL and α-AIL genomic DNA sequence alignment in tepary bean shows a 93% and 80% homology to ARL-2 arcelin-like and α-AI-2 pa, respectively.(**fig. 2**). The two proteins ARL and α-AIL shares a high percent sequence homology and they may be tightly linked into a single super gene family.

We are in the process of associating the presence of one or more of the isoforms of seed storage proteins and resistance for bruchids in our backcross lines. Determining the actual sequence of the open reading frame for these storage proteins in our materials will be necessary so as to target a full sequence homology of the proteins to those in the data base and consequently cloning of the genes.

Acknowledgements
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ARL G: 669 TDTYFNFDFFKQNDADTNRLILQRDATISSGGRLRLTGVGSNEDPWVDSMGRAFYSDPIQ 490
           TDTYFNFDFFKQNDADTNRLILQRDATISSGGRLRLTGVGSNEDPWVDSMGRAFYSDPIQ
ARL 2: 23  TDTYFNFDFFKQNDADTNRLILQRDATISSGGRLRLTGVGSNEDPWVDSMGRAFYSDPIQ 82

ARL G: 489 IRDSTGNLASFHTNFTFIIRANNAGHSAYGLAFSLVPVGSQPKRKREYLGLFPDAHTVAV 310
           IRDSTGNLASFHTNFTFIIRANNAGHSAYGLAF+L PVGSQPKRKRE LGLFPDAHTVAV
Sbjct: 83  IRDSTGNLASFHTNFTFIIRANNAGHSAYGLAFALFPVGSQPKRKRENLGLFPDAHTVAV 142

ARL G: 309 AFNTLNNSIDIDVNSNSPSHTGFCDFNKHNGEKTDVQITYESPKKNLRVVLHFTKSNVQY 130
            FNT++N +         +  GF     HNGE TDVQITYESPKKNL++VL  T SNVQ
ARL 2: 143 -FNTVSNVMKSTSTPTRLAQRGFAISTNHNGETTDVQITYESPKKNLKIVLPSTNSNVQ- 200

ARL G: 129 EYDFNAPLYLENDVDRSVKRWVGFSATSGLKEETTETHDILS 4
            YDFNAPLYLEN+VDR+V   VGFSATSGL EETTETHDILS
ARL 2: 201 -YDFNAPLYLENEVDRNVS--VGFSATSGLTEETTETHDILS 239



AMYLG: 641 THANSASDT-FNFHSFNETNLILQGDATVSSNGNLQLHTMDSMCSAFYSAPIQIRDSTTG 465
           THANSA DT FNFHSFNETNLILQG ATVSSNG L+L+T DSMCSAFYSAPIQIRDSTTG
AILpa: 20  THANSACDTSFNFHSFNETNLILQGQATVSSNGKLKLNTYDSMCSAFYSAPIQIRDSTTG 79

AMYLG: 464 NVASFHTNFTMNITTYRKANSAVGLDFALVPVQPKSKGRLLGLFKTPDYDRNAGIVTVEF 285
            VASF TNFTMNITT    NSA+GLDFALVPVQPKSKG     FKTPDYDRNAG VTVEF
AILpa: 80  KVASFDTNFTMNITTNYYKNSAIGLDFALVPVQPKSKGH----FKTPDYDRNAGTVTVEF 135

AMYLG: 284 DTLRRRISIDGNYNDIESVPWNVDDYDGQKAEVRITYNSSTKVLAVSLLNPSTGKSNNVS 105
           DT R+ ISID N+ND+ SVPWNV DYD Q  EVRITYNSSTKVLAVSLLNP TGKSN VS
AILpa: 136 DTFRKCISIDSNHNDLNSVPWNVLDYDRQNTEVRITYNSSTKVLAVSLLNPITGKSNKVS 195

AMYLG: 104 ARMELEKKLDDWVSVGFIGTSGVHEYSLKRE---TWS 3
           ARMELEK LDDWVSVGF  TSG +++  +     +WS
AILpa: 196 ARMELEKILDDWVSVGFSATSGAYQWGFETNEVLSWS 232
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Figure 2. BLASTP -alignment of protein sequences (translated DNA sequences) of two storage proteins from *P. acutifolius*: arcelin-like and α-amylase inhibitor-like from G40199 (ARL G & AMYLG) demonstrate homology with ARL 2 (373HAAF71744) and AILpa (374HBAB72259) , respectively, from the NCBI Enterz database (375Hhttp://www.ncbi.nlm.nih.gov/).

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NEW SOURCES OF RESISTANCE TO BEAN RUST AND IMPLICATIONS FOR HOST-PATHOGEN COEVOLUTION

Acevedo, M*. [1], Steadman, J.R.[1], Rosas, J.C.[2], J. Venegas[2], [1]University of Nebraska-Lincoln, Plant Pathology Department, [2]Universidad Agrícola Panamericana, Honduras.
*maceved1@bigred.unl.edu

Introduction

Bean rust is one of the limiting factors for bean production in tropic and subtropic regions. The bean rust pathogen*, Uromyces appendiculatus* is characterized by high diversity of virulence phenotypes in both natural and cropping systems. High virulence diversity of this pathogen in Honduras supports the hypothesis that this area is a center of diversity for this fungal pathogen. While disease resistance is the most economical management option, pathogen variability presents a formidable challenge to resistance gene deployment. Although rust is commonly found in Honduran wild, landrace and commercial beans some wild beans were free of rust. The objective of this study was to evaluate "wild" beans collected in the highlands of Honduras for rust resistance and effectiveness of the resistance to a broad array of pathotypes.

Methods

Thirty *Phaseolus* accessions including wild *P. vulgaris* (similar to *P.v. aborigineus*)*, P. lunatus, P. agusti* (species not confirmed) and *P. coccineus* were grown from seeds collected from plants without rust symptoms in the highlands of Honduras. Samples areas were selected based on the CIAT GIS map for wild bean probability (Beebe., *et al.,* 1997). Each accession was inoculated with five *U. appendiculatus* isolates that represented the most common pathotypes identified in Honduras (Acevedo *et al*., 2005). Six-day-old primary leaves of the 30 accessions were sprayed with a suspension of 22-25000 urediniospores per ml and 40µg/l Tween-20.

For a second resistance screening test, a group of 18 wild *P. vulgaris* accessions collected in Honduras with rust symptoms was inoculated with urediniospores of *U. appendiculatus* isolates from four different locations within Honduras. In both tests, plants were placed overnight in a mist chamber after inoculation then incubated in a greenhouse. Disease reaction was determined 14 days after inoculation based on uredinium size (J.R. Stavely *et al.,* 1983). An accession was considered resistant to a pathotype if no symptoms, necrotic spots, or pustules smaller than 300nm were present.

Results and discussion

Ten of the 30 accessions preliminarily showed high levels of resistance. These accessions included one wild *P.vulgaris,* three *P. lunatus*, five *P. coccineus* and one landrace (Table 1). From the second screening test, only 2 of the 18 wild *P. vulgaris* accessions susceptible in the wild, showed a high level of resistance to most of the isolates. The low number of resistant wild *P. vulgaris* accessions (3 out of 30) and the considerably higher number of *P. coccineus* (5 out of 13) and *P. lunatus* (2 out of 3) accessions with rust resistance, may have some implications for the host-pathogen coevolution especially in the case of *P. coccineus* since this species is commonly found in the same areas were wild *P. vulgaris* grows in Honduras. Moreover, *P. coccineus* plants without symptoms have been observed in the vicinity of rust infected wild *P. vulgaris*. This finding suggests that selection pressure on the pathogen may be at different levels in each *Phaseolus* host population based on species composition. In this case, a metapopulation structure needs to be considered for evaluating coevolution and for implications in resistance gene deployment strategies. Variability in rust resistance within and between areas of collections was observed in the second test (Table 2). Highly resistant individual plants were present in some of the populations. The diversity of resistance

structure captured in this small sample of individuals and populations reflects the complexity of the host-pathogen system under study and the importance of evaluating natural data from wild populations to improve resistance gene deployment.

Table 1. Examples of disease reaction of wild beans to the most common *U. appendiculatus* from Honduras .

Collection ID	*Phaseolus* species	Disease reaction of wild beans to Honduras' pathotypes				
		HON03-2-1	HON03-3-5	HON03-12-3	HON03-13-7A	HON03-7-7A
01-004-1	*Wild P. vulgaris*	R	*	R	R	R
01-006-2	*Wild P. vulgaris*	S	S	S	S	S
01-006-3a	*Wild P. vulgaris*	S	S	S	S	S
02-018-1	*Wild P. vulgaris*	S	S	S	R	S
02-018-2	*Wild P. vulgaris*	S	R	S	R	S
02-008-2c	*P. lunatus*	R	R	R	R	S
01008-7b	*P. lunatus*	R	S	R	R	S
01-008-1	*P. lunatus*	R	S	R	R	R
01-006-3b	*P. coccineus*	R	R	R	R	R
01-006-4	*P. coccineus*	R	R	R	R	R
01-006-5	*P. coccineus*	S	R	R	S	S
01-006-6b	*P. coccineus*	R	R	R	R	R
01-006-7b	*P. coccineus*	R	R	R	S	R
01-017-4	*P. coccineus*	R	R	R	R	R
01-004-2	*P. augusti*	R	S	*	R	*
01-004-3	*P. vulgaris* landrace	R	R	R	S	R

S=Susceptible, R=Resistant. * No data.

Table 2. Disease reaction of wild *P. vulgaris* to *U. appendiculatus* pathotypes from different locations in Honduras.

LOCATION	TATUMBLA			SANTA LUCIA			MARCALA		LA ESPERANZA
ISOLATE Collection ID	HON03-17-2	HON03-17-1	HON03-17-4	HON03-19-3B	HON03-19-4B	HON03-19-07	HON03-7-7A	HON03-3-2	HON03-19-3B
01-010-1	S	S	S	S	S	S	S	S	S
01-015-1	*	S	S	S	S	S	S	S	S
01-012-1	S	S	S	S	*	S	S	S	S
01-011-1a	S	R	R	R	R	*	R	R	R
01-001-3	S	S	S	S	S	S	S	S	R
01-002-4	S	S	S	S	S	S	*	S	S
01-001-1a	S	S	S	S	S	S	S	S	S
02-017-2b	S	R	R	R	R	R	R	R	R
01-006-1	S	S	S	S	S	S	S	R	R
01-006-7a	S	S	S	S	S	S	S	S	S

S=Susceptible, R=Resistant. * No data.

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TIMING OF ANTHOCYANIN DEPOSITION IN BLACK BEAN

Adams Frimpong, Perumal Vijayan and Kirstin Bett
Department of Plant Sciences, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK, Canada S7N 5A8.

Introduction

Common bean (*Phaseolus vulgaris* L.) is an important pulse crop of increasing acreage in Canada. The market value of beans is largely determined by the colour of the seed, making it a very important quality characteristic. The seed coat colour of dry beans is known to be determined by the amounts of flavonol glycosides, anthocyanins and condensed tannins (proanthocyanidins) in the seed coat.

Inheritance of seed coat colour is well studied but the actual developmental control of color deposition in black beans is not well understood. In some genotypes, the yellowing of the pods does not coincide with the completion of seed coat color deposition. Thus under some conditions, incomplete colour deposition at harvest can cause undesirable purple seed colouration under some conditions. By studying the deposition of anthocyanin through time in selected black bean varieties, we hope to gain an insight into the genetics underlying the initiation, rate and completion of colour deposition

Materials and Methods

Five bean varieties (AC Black Diamond, Expresso, 5-593, Nighthawk and T39) were grown in 6 inch pots in the greenhouse in the summer of 2005 in Saskatoon, SK. Flowers were individually tagged when they opened and pods were harvested daily, beginning at 15 days after flowering (DAF). Four pods were sampled from each genotype each day after flowering.

Four seeds from each pod were imaged using an imaging system designed by the College of Engineering, University of Saskatchewan. The imaging system measured the total seed surface area and pigmented area. Total seed weight and seed coat weight was determined for two seeds from the same pod.

Seed coat pigments were extracted to completion, from two seed coats from the same pod (2 reps per pod) in 1.5ml to 10ml in methanol containing 0.1% HCl. Absorbance spectra of seed coat extracts were recorded using a diode array spectrophotometer (Ocean Optics Inc., Dunedin, FL, USA). Absorbance values were normalized and the total anthocyanin content calculated based on a per seed coat weight.

Results and Discussion

At 20 DAF, all genotypes contained significant amounts of chlorophylls (absorption peaks at 657nm and 420nm) and another pigment, probably carotenoids (absorption peak at 475nm) in the seed coat acidified methanol extracts. All the genotypes accumulated anthocyanins (combined absorbance maximum at 540 nm) in the seed coat acidified methanol extracts. These results agree with previous reports on the presence of anthocyanins in black beans (Strack and Wray, 1994; Takeoka *et al*., 1997).

The normalized (based on volume of extract) absorption spectra can be used to quantify the anthocyanin deposition in the seed coat. Pigmented spots were visually observed as early as 15DAF in Expresso and AC Black Diamond. Appearance of pigmented spots was delayed by 2-3 days in Nighthawk, 5-593 and T39. Colour began to spread around the hilum as a ring and bled towards the dorsal side as the seed enlarged. Correspondingly, imaging experiments revealed expansion of pigmented area on the seed surface as a discontinuous episodic process. Anthocyanins could initially be detected in the seed coat extracts of all genotypes (excluding 5-593) between 20 and 25DAF. Anthocyanin deposition could be detected the earliest in AC Black Diamond and it also had the fastest rate of deposition.

100% pigment coverage occurred at 27DAF in AC Black Diamond, T39 and Nighthawk; 29DAF in Expresso and 30DAF in 5-593. In all the genotypes, except AC Black Diamond, anthocyanin deposition continued after reaching 100% pigment coverage as the seed turned from purple to black. Maximal pigment deposition was attained in AC Black Diamond at 30DAF and the seed coat became black and started desiccating. In 5-593, very little anthocyanin was detected until 34 DAF; thereafter it increased rapidly until it reached a maximum at 38DAF.

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EFFECT OF RATE OF SEED INFECTION ON ANTHRACNOSE SEVERITY AND YIELD LOSS IN DRY BEANS.

Conner, R.L.*, McAndrew, D.W., Balasubramanian, P. and Kiehn, F.A.
Agriculture and Agri-Food Canada Research Station, Morden, MB *(conner@agr.gc.ca).

Introduction:

Bean anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus.) Lams.-Scrib., is one of the most serious diseases of dry beans (*Phaseolus vulgaris* L.) in Manitoba and Ontario as well as other parts of the world. Anthracnose has been shown to strongly reduce seed yield and quality. Reductions in seed quality are directly related to the extent of the brown to black anthracnose lesions on the seed coat. Severe early anthracnose outbreaks in dry bean crops are often associated with planting badly infected seed. The use of disease-free seed is widely recommended for anthracnose control. Currently, there are no tolerance standards for anthracnose levels in certified seed. However, seed producers still must maintain very high standards of disease control during seed production in order to maximize yield and provide bean producers with high quality, disease-free seed. A two-year field study was established to examine the effect of different rates of anthracnose seed-borne infection on subsequent disease development and seed yield and quality.

Materials and Methods:

The study was conducted at field sites near Morden and Portage La Prairie, Manitoba, Canada in 2003 and 2004 to compare anthracnose development in the upright, indeterminate navy bean cultivar Navigator and the upright, indeterminate pinto bean cultivar AC Ole at six different seed-borne infection rates, (i.e., 0, 1, 2, 5, 10 to 20%). Disease-free seed of each cultivar was obtained commercially. The plot dimensions were 1 x 5 m with 30 cm row spacings. The bean plots were separated by plots of soybeans (*Glycine max* L.). The treatments were organized in a randomized complete block design with four replications at each field site. Anthracnose severity was assessed in late July and mid-August. Just prior to harvest, the severity of pod infection was determined. The plots were harvested with a small-plot combine and seed yield and the incidence of seed discoloration were determined. An analysis of variance was carried out on all the disease and agronomic data from each year and each field site. Fisher's Protected LSD test ($P < 0.05$) was used to compare treatment means.

Results and Discussion:

Each year, frequent rainfalls occurred throughout the growing season providing favorable conditions for anthracnose development. The results from the Portage La Prairie site in 2004 are summarized in Table 1. On all the rating dates, significant differences in anthracnose severity in the crop canopy were apparent among the different seed-borne infection treatments. Disease severity in the crop canopy increased with higher rates of seed-borne infection. Early in the growing season, differences in the rate of seed-borne infection on anthracnose development in the crop canopy were most apparent in AC Ole, but they also became evident in Navigator by the end of the growing season. These differences in disease severity resulted in different rates of pod infection by the end of the growing season. The rate of seed-borne infection increased the incidence of seed discoloration in both cultivars. Yields in the 0% infection treatment of both cultivars were higher than those of the 20% seed-borne infection rate treatment, but these differences were not significant. Yields in the

other seed-borne infection rate treatments usually were not significantly different from those of the 0% treatment.

Conclusions:

There was a linear relationship between initial seed-borne infection and subsequent disease development in the crop canopy and pods. Low incidences of seed-borne infection did not affect yield, but would have led to downgrading losses due to high incidences of seed discoloration. These results demonstrated there should be zero tolerance for anthracnose infection in bean seed. High levels of seed-borne infection resulted in more uniform anthracnose development within the crop than low levels of seed-borne infection. Anthracnose ratings in the upright navy bean cultivar Navigator were consistently lower than those of the upright, indeterminate pinto bean cultivar AC Ole. This difference in disease development between these two cultivars might have been at least partially due to higher emergence rates of infected seed in AC Ole.

Table 1. Effect of rate of seed-borne infection on anthracnose severity, yield and seed discoloration in AC Ole and Navigator beans.

Treatment	Plant % infection[1]	Plant % infection[2]	% Pod infection[3]	Yield (kg/ha)	% Seed discoloration
AC Ole-0%	0.05 d	0.8 de	0.5 e	1664 ab	1.2 d
AC Ole-1%	0.09 d	0.9 cde	4.1 de	1470 ab	1.6 cd
AC Ole-2%	0.21 cd	1.3 cde	6.0 cde	1352 ab	2.2 bcd
AC Ole-5%	0.87 a	2.8 bcd	13.2 bc	1336 ab	3.3 bc
AC Ole-10%	0.47 b	2.6 bcde	19.4 ab	1520 ab	7.8 a
AC Ole-20%	1.08 a	5.3 a	19.2 ab	1271 b	7.1 a
Navigator-0%	0.04 d	0.7 e	0.5 e	1730 a	1.6 cd
Navigator-1%	0.16 d	1.0 cde	1.3 e	1255 b	2.2 bcd
Navigator-2%	0.11 d	2.3 bcde	11.8 bcd	1314 ab	2.6 bcd
Navigator-5%	0.24 bcd	2.0 bcde	14.8 bc	1505 ab	2.1 bcd
Navigator-10%	0.25 bcd	2.9 bc	17.6 ab	1294 ab	4.0 b
Navigator-20%	0.42 bc	4.0 ab	26.0 a	1438 ab	3.6 b
LSD (0.05)	0.243	2.05	8.82	444.9	2.0

Growth stages: [1]full bloom, [2]pod filling stage, and [3]maturity.

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BEAN ROOT ROT EVALUATION PROTOCOLS CURRENTLY USED IN NEW YORK

George S. Abawi, John W. Ludwig, and Beth K. Gugino
Dept. Plant Pathology, NYSAES, Cornell University, Geneva, NY 14456

Root rot diseases are widespread and often considered a major constraint to bean production, reducing both yield and profitability worldwide. Depending on the pathogen(s) involved, general root rot symptoms might include any combination of the following: poor seedling establishment, damping-off, uneven growth, chlorosis, premature defoliation, death of severely infected plants, and lower yield. The roots of infected plants are reduced in size, discolored, and exhibit various stages of decay. Several soilborne pathogens are known to cause root rot diseases on beans, but their prevalence and damage varies from one production region to another. In New York State, the major soilborne pathogens found causing damage to beans are *Fusarium solani* f. sp. *phaseoli*, *Pythium ultimum*, *Rhizoctonia solani*, *Thielaviopsis basicola* and *Pratylenchus penetrans* (lesion nematode).

The involvement of multiple soilborne pathogens with different mechanisms of pathogenicity has made it difficult to develop a simple and effective disease management program. Currently, the management of root rot diseases is possible only through the use of a combination of control options (cultural, chemical and biological) which utilize the concept of Integrated Pest Management (SOIL-IPM). Therefore, a thorough knowledge of the fields' cropping and management history, including the identity of causal pathogen(s), is critical for the formulation of practical and effective IPM program. However, the single most effective and practical management strategy is the use of bean cultivars that are resistant to the most common soilborne pathogen(s) in a production region. Bean germplasm lines with resistance to a single or multiple pathogens have been reported and in few cases, fully characterized. Unfortunately, commercial bean varieties currently grown in New York State still do not exhibit a high level of tolerance to the prevailing root pathogens and similar needs exist in the majority of the bean producing regions of the world. The following is a field and greenhouse protocols which can be used to screen germplasm for resistance to soilborne pathogens. The techniques can be adapted based on the predominant pathogens in the region of interest.

Field Evaluation Protocol

To effectively screen germplasm for resistance to the predominate soilborne bean pathogens in New York, a root rot nursery (~ 1 ha) was established twelve years ago at the Vegetable Research Farm, NYSAES, Geneva, NY. To build-up pathogen inoculum, the field was double cropped to a susceptible bean variety during each of the first two years of establishment. In the first year, emerging seedlings in each planting were inoculated with *F. solani*. f. sp. *phaseoli* (Fsp), *T. basicola* (Tb), *P. ultimum* (Pu) and *R. solani* (Rs). Inocula of Fsp and Tb consisted of conidial suspensions that were applied to the base of the seedling stems. Inoculum of Pu consisted of a suspension of sporangia or colonized rye or wheat seeds, whereas the inoculum of Rs was a suspension of hyphal fragments or colonized rye or wheat seeds. All seedlings were hilled immediately after inoculation and irrigated. After two years (4 bean crops), root incidence and severity became uniform and severe enough that additional infestations have not been required during the subsequent 10 years of continuous bean germplasm plantings.

Seeds of all the bean germplasm to be evaluated are first treated with two fungicides (Apron + Captan) and an insecticide (Lorsban) at recommended commercial rates. The latter seed treatments make it possible to evaluate bean germplasm for the root rot phase and not the seed-decay and damping-off stages which are effectively controlled by chemical or biological products. Seeds of each germplasm are then planted in two 4-m-long-rows, 76-cm apart and replicated a minimum of 4 times and arranged in a completely randomized block design. The plots are irrigated as needed and all additional maintenance practices are performed according to recommended commercial guidelines. Seedling emergence and stand establishment are recorded at 3 and 6 weeks after planting, respectively. The number of productive plants

and seed or pod weight are recorded at harvest. Root rot severity is assessed at the full-bloom stage (usually 6 weeks after planting) on 20 or more plants dug from one of the two rows. The washed roots are rated on a scale of 1 (normal root/healthy) to 9 (75% of root and stem tissues affected and decaying). Generally, germplasm lines with an average root rot severity ratings of 1-3, >3-6, and >6-9 are described as resistant, intermediate, and susceptible, respectively. Known susceptible and known or reported resistant germplasm (if available) are included for comparison and for determining fluctations in root rot severity between growing seasons and monitoring which pathogen(s) are present.

Greenhouse Evaluation Protocol

Bean germplasm lines that exhibit field resistance to root rot pathogen(s), are adapted to local conditions, and/or exhibit other desirable traits are candidates for greenhouse root rot screening. Their reaction to a single pathogen, specific race/strain of a pathogen or combination of pathogens can be tested under greenhouse conditions. Seeds or seedlings of bean germplasm are planted in clay pots (≥10 cm in diameter) containing pasteurized soil (30 min at 60ºC). Depending on the target pathogen, the soil can be infested with the inoculum prior to planting, at planting time or at a specific seedling stage. However, several suggested methods for inocula preparation and inoculation techniques for the various root rot pathogens are available (Abawi and Pastor-Corrales, 1990). For example, soil-potato inoculum is effective for screening for resistance to Rs. Bean seeds are planted in pasteurized soil mixed thoroughly with this inoculum preparation at a rate of 1-5% (vol. to vol.) or the infested soil is placed around the stem of emerging seedlings in a plastic or paper collar placed on top of the pots. Seed of grain crops or beet seed colonized by Rs can also be used as an inoculum source and mixed with pasteurized soil or placed directly next to the seedling stems near the soil surface. For screening for resistance to the Fusarium Yellows pathogen, it is best to first plant seeds in sterile sand or light soil. After one week, seedlings are removed; 1-cm segments are cut from the root tips, seedlings are dipped in a spore suspension of Fop (10^6 conidia/ml) and then transplanted into pots filled with pasteurized soil. Greenhouse evaluations have resulted in uniform, high infection rates and are generally simple, cost-effective and rapid. Greenhouse evaluations are ideal for characterization of resistance gene(s) to specific pathogens and assist in the development of molecular markers for such factors. However, greenhouse results are dependable in the development of resistant cultivars only if they correlate closely to the reaction of bean germplasm under field conditions. In contrast, field screening under naturally fluctuating conditions accurately measure the reaction of bean germplasm to root rot pathogens and to assess the impact on the quantity and quality of marketable yield (seeds or pods). Field screening also permits the selection for local adaptation, reaction to other disease pathogens and pests, and to tolerance abiotic stresses.

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DRY BEAN TRANSFORMATION TO ENHANCE WHITE MOLD RESISTANCE

Ann Roselle O. Armenia, Richard F. Allison*, and James D. Kelly
Departments of Crop and Soil Sciences, and Plant Biology*, Michigan State University, East Lansing, MI 48823

Introduction:

White mold, caused by the aggressive fungal pathogen *Sclerotinia sclerotiorum,* is among the most destructive diseases to limit dry bean productivity. Previous studies have identified oxalic acid as the major pathogenic factor during white mold infection (Godoy, et al. 1990). The wheat germin gene *gf-2.8* (Lane, 2002), which encodes an oxalate oxidase, has previously conferred enhanced white mold resistance in soybean, and sunflower (Donaldson, et al 2001; Hu et al, 2003). Our goal was to enhance dry bean resistance to *S. sclerotiorum* through the introduction and expression of the germin gene in *Phaseolus vulgaris*.

Materials and Methods:

The plant transformation plasmid, pBKS*bar/gf-2.8* was constructed by excising the 1.7kb fragment of *gf-2.8* driven by enh*35S* promoter and *nos* terminator from the pRD400-germin and the 3.1kb fragment of *bar* driven by *35S* promoter and *nos* terminator from pCAMBIA3300. These fragments were cloned into the SspI and HindIII sites of pBluescriptKSII(-).

Using *bar/gf-2.8* fragment DNA from pBKS*bar/gf-2.8*, eighteen different apical meristem treatments (Table 1) were tested on the two bean cultivars Olathe and Matterhorn using the electrotransformation approach developed by Richard Allison (MSU, Plant Biology). The protocol involved inserting the apical meristem of 5-7 day old bean seedlings into a pipette tip with approximately 1 µl of DNA (10 ng/µl) and 49 µl of 2% TAE agarose. The pipette and the transformation tube containing the seedling was filled with the 1X TAE transformation buffer and connected to a power source which is allowed to run at 125 V and 0.15 mA for 15 min of direct current and 30 sec alternating current. Pretreatments that consisted of injecting various levels of hormone (identity preserved), lipofectin and ascorbic acid on the apical meristems of bean seedlings were performed prior to transformation.

Results and Discussion:

A total of 1,150 dry bean seedlings were transformed with the linearized SspI/HindIII fragment of pBKS*bar/gf-2.8* containing both the bar and germin gene. About 92% seedling survival was noted on both Matterhorn and Olathe plants. Using a subset of the T_1 seeds from each transformed seedling (T_0), 4,250 Matterhorn and 2,888 Olathe plants were screened for herbicide resistance by spraying with glufosinate ammonium at a level predetermined from a kill curve. Herbicide screening resulted in 161 herbicide resistant plants.

Polymerase chain reaction (PCR) with T_1 genomic DNA from the herbicide screen surviving plants using *gf-2.8* specific primers showed integration of the germin gene in a total of 18 Matterhorn and 11 Olathe T_1 plants (Fig. 1a). About 88% of the PCR positive plants from the cultivar Matterhorn and 91% of Olathe were produced from plants that were pretreated with various levels of Hormone (identity preserved) prior to transformation. Meanwhile, PCR analysis of 208 T2 plants from 23 PCR+ T1 lines has confirmed integration of *gf-2.8* in 3 Matterhorn and 1 Olathe T2 plants (Fig 1b).

Southern hybridizations to verify gene integration in T1 and T2 plants; and oxalic acid and fungal bioassays to evaluate white mold resistance T2 plants is currently underway.

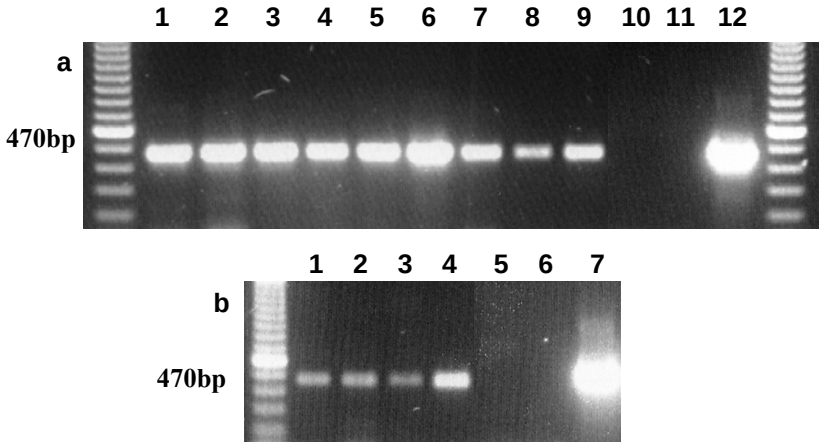

Figure 1. PCR results showing integration of the *gf-2.8* gene a) T1 plants: 1=D2M-1, 2=E13M-1, 3=D7M-3, 4=D7M-4, 5=H35M, 6=E19M, 7=G22O-1, 8=G21O-1, 9=G4O-2; 10=Matterhorn, 11= Olathe, 12=pBKS*bar/gf-2.8*; b) T2 plants: 1=D23O-5, 2=E13M2-1, 3=E13M1-3, 4=D2M1-5, 5=Matterhorn, 6=Olathe, 7=pBKS*bar/gf-2.8*

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DNA SEQUENCING AND ANALYSIS: A TOOL FOR IMPROVING WEB BLIGHT MANAGEMENT AND RESISTANCE BREEDING.

Godoy-Lutz G [1], Kuninaga S[2], Steadman JR[3]*, Beaver JS[4], Rosas JC[5].
[1]IDIAF, Dominican Republic. [2]University of Hokaido, Japan. [3]University of Nebraska, Lincoln, NE.
[4]University of Puerto Rico, U.S. [5]EAP, Honduras.

Web blight of dry edible beans is caused by aerially dispersed isolates of *Rhizoctonia solani* Kuhn [teleomorph *Thanatephorus cucumeris* (Frank) Donk] and is endemic in the bean growing areas of the Americas and some areas of East Africa. Fungicides are a costly disease management option, not always effective and have negative environmental effects. Resistance is the most economical management strategy, but no commercial varieties have web blight resistance. Progress in breeding for web blight resistance has been limited due to the lack of high levels of resistance in *P. vulgaris* and a poor understanding of pathogen variation. Identification of the pathogen subgroups of local isolates will assist breeding for resistance and implementing other disease management practices.

Genetic variation of 117 isolates of *Rhizoctonia solani* from 11 countries in the Latin American and Caribbean region was evaluated. These isolates previously had been classified as belonging to AG-1 and AG-2-2. The sequences coding for the 5.8S nuclear rDNA gene and the internal transcribed spacers (ITS 1 and ITS2) were amplified by PCR and sequenced for 68 *R. solani* isolates and compared by sequence alignment to other *R. solani* isolates sequences from the Genbank.

The ITS region varied in both length and composition but the 5.8S nuclear rDNA sequence was similar among the web blight isolate sequences and other *R .solani* isolates. Phylogenetic analysis of the sequence data sets by neighbor joining and parsimony methods clearly separated most of the web blight isolates into three new subgroups AG-1,IE, AG-1,IF and AG-2-2 WB from the other known subgroups in AG-1 and AG-2-2 (Fig. 1). Within the new subgroups many isolates displayed polymorphism at the ITS region while others did not. Specific primers sets WB-A, WB-B and 2-2 WB were designed on the basis of sequence differences and used to examine another 49 web blight isolates. Based on sequence analysis and specific primer amplification of the ITS rDNA region we determined that web blight of common beans is caused by isolates in subgroups AG-1,IA, AG-1,IB,AG-1,IE,AG-1,IF,AG-2-2 IIIB and AG-2-2 WB. Primers were designed to separate web blight isolates from rootrot isolates (Fig. 2). The results demonstrate that there is ample genetic variation of the web blight pathogen which is composed of clonal and outbreeding populations.

Disease management including breeding for web blight resistance in common beans has been a challenge to plant pathologist as well as breeders. The variable composition of the pathogen may have affected the dynamics of the host-pathogen relationships since its genetic flexibility allows for adaptation to changing environmental conditions, including the introduction of new interspecific populations or genotypes of host *Phaseolus* species. Screening for web blight resistance should include isolates representing the genetic variation occurring in bean fields of the region The availability of molecular tools such as specific primer sets should facilitate seed health monitoring and identification of the subgroups associated with the disease in areas with high risk for web blight epidemics.

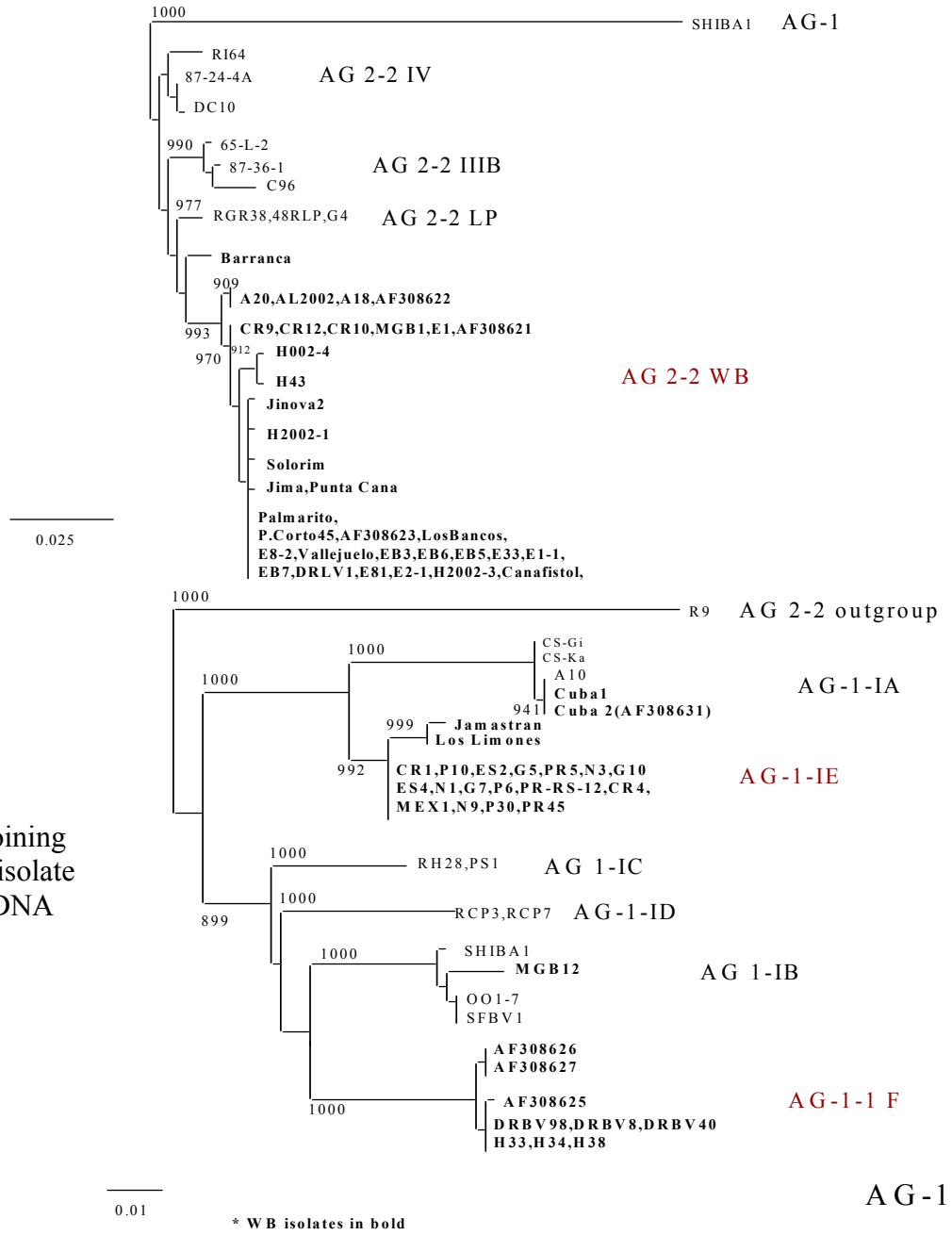

Fig. 1. Neighbor-joining trees of web blight isolate sequences of ITS rDNA

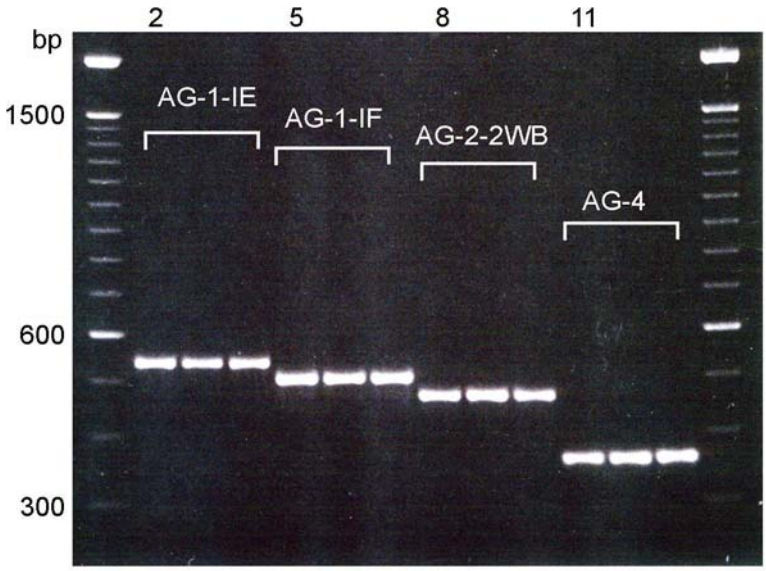

Fig. 2. Amplification with primers WB-A (2-4), WB-B (5-7), 2-2 WB (8-10) and AG4 (11-13).
R. solani causing web blight (2-10) or stem and root rot (11-13)

DEVELOPMENT OF DROUGHT-RESISTANT BEAN GENOTYPES FOR ECUADOR

Esteban Falconí[1,2], Jose Pinzón[2], and James D. Kelly[1].

[1]Crop and Soil Sciences, Michigan State University, East Lansing, MI. [2]Instituto Nacional de Investigaciones Agropecuarias, Quito, Ecuador.

Introduction:

In Ecuador, the major bean production areas are subject to intermittent drought. The rainfall pattern throughout the year is bimodal, allowing farmers to produce two crops. However, total precipitation and the irrigation systems do not always provide the minimum water requirements for the crop. Consequently, bean genotypes with improved water use efficiency should be considered as the more suitable and practical strategy to help stabilize production. The objective of this research was to identify drought tolerant genotypes with commercial seed characteristics from a cranberry bean population of inbred backcross lines (IBLs) developed using the method described by Bliss (1993).

Materials and Methods:

A population of 26 IBLs, developed in Michigan State University (Roman-Aviles and Kelly, 2005) was studied in Tumbaco, Ecuador (0o 12' S; 78o 24' W; 2330 masl). The IBLs were grown under two water regimes to identify the potential new sources of drought resistance in large seeded Andean beans. The IBL population was derived from the cross of lines C97407*2/Negro San Luis (NSL). The recurrent parent (C97407) had a cranberry seed type, whereas the donor parent (NSL) was a medium-sized black-seeded genotype identified as drought resistant genotype. Both treatments were irrigated prior to flowering and, subsequently, the irrigation was suspended in one treatment to create water stress conditions. The irrigated treatment received a total of 359 mm of water, while the treatment under water stress received 309 mm of water. Analyses of variance (ANOVA) of the yield under the two water regimes, geometric mean (GM), and seed quality were utilized to identify superior genotypes under Ecuadorian growing conditions.

Results:

The ANOVA showed statistical differences among the IBLs for both water treatments. The top yielding genotype was the NSL parent in both water treatments (2648 kg/ha and 4259 kg/ha and a GM = 3358 kg/ha), followed by the local cultivar Mil Uno. Based on GM, six IBLs surpassed 2000 kg/ha, which were superior to the recurrent parent C97407 (Table 1). Three IBLs showed good seed quality with a score of 3, whereas the recurrent parent C97407 had seed quality score of 5. The drought intensity index (DII) for the experiment was moderate at 0.36.

Table 1. Geometric means, yield under non-stress, yield under stress conditions, seed quality, and 100 seeds weight of the IBL population evaluated in Tumbaco, Ecuador. 2005.

	Genotypes	GM	Water Regimes		Seed quality (1-9)[2]	100 seeds weight
			Stress	Non-stress		
	IBLs	----------	**Kg/ha**	----------		**(g)**
*[1]	**C03131**	2235	1746	2860	5	48
	C03122	2142	1637	2803	5	53
*	**C03102**	2078	1722	2508	4	52
	C03110	2055	1698	2488	6	55
	C03160	2032	1485	2780	4	49
*	**C03121**	2028	1606	2560	3	56
	C03163	1985	1672	2358	4	54
	C03127	1797	1544	2091	6	47
*	**C03155**	1792	1530	2098	3	52
*	**C03151**	1746	1297	2349	3	45
	C03149	1558	1306	1859	6	41
	C03157	1523	1225	1894	4	53
	C03161	1466	1295	1659	5	45
	Parents					
	NSL	3358	2649	4259	NA[4]	41
	C97407	1861	1414	2448	5	49
	Checks					
	Mil Uno	2395	1866	3073	NA	62
	L88-63[3]	2017	1435	2835	NA	19
	MEAN	**1893**	**1521**	**2364**		**48**
	LSD (0.05)		**346**	**574**		**4**
	CV (%)		**13.9**	**14.9**		**4.6**

[1] Selected genotypes; [2] Scale from 1 to 9, where 1 is the best quality and 9 is the worst. [3] Drought resistant line (Frahm, 2004): [4] Comparisons performed only among cranberry seed types.

Conclusions:

Based on yields under irrigated and rainfed conditions and seed type, genotypes C03102, C03121, C03131, C03151 and C03155 were selected. The first three genotypes exhibited higher yields than the recurrent parent and the last two genotypes exhibited an improved seed quality. The IBL method for developing drought-resistant lines suitable for Ecuadorian conditions and markets is promising, given the difficulty of generating commercial seed types through single crosses. The drought resistant-lines selected from the IBL population need to be tested in different environments in the Ecuadorian Highlands to confirm drought resistance. The selected IBLs with high levels of drought resistance, but lacking desirable seed quality characteristics can be utilized in single crosses with local cultivars to improve the drought resistance of large red mottled types as part of overall breeding strategy to improve drought tolerance of common beans in Ecuador.

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USE OF MULTI-SITES TO IDENTIFY PARTIAL RESISTANCE TO *SCLEROTINIA SCLEROTIORUM* IN COMMON BEAN OVER MULTIPLE YEARS

L.K. Otto-Hanson and J.R. Steadman (University of Nebraska-Lincoln). Data from C. Kurowski (California), R. Mainz (Minnesota), J. Kelly (Michigan), P. Griffiths (New York), K. Grafton (North Dakota), J. Myers (Oregon), P. Miklas (Washington), H. Schwartz (Colorado), S. Singh (Idaho), K. Kmiecik (Wisconsin), R. Felix (Mexico), E. Kee (Delaware), and A. Oppelaar (France).

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 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 over multiple years.

Sclerotinia sclerotiorum poses many challenges for utilizing effective management practices. Fungicides are available to producers, but can be expensive. Other management strategies that reduce disease also reduce yield potential. The development of bean cultivars with partial physiological resistance and architectural avoidance to white mold would reduce disease losses but not yield potential and require no input costs to growers. Key issues for bean breeders and pathologists when they are improving bean lines for white mold resistance are the variability of the system, including variability of the screening method, and variability of different test sites.

Sources of partial resistance incorporated into bean breeding lines were evaluated by greenhouse/lab and field screening methods in 14 different US locations. Each collaborator used their standard protocols for screening in the greenhouse or lab. Most collaborators used the straw test. Collaborators were asked to use their normal number of replications for their lab/greenhouse tests. The disease severity means and individual readings were sent to the University of Nebraska-Lincoln for statistical analysis which compares all tests.

The following 12 lines/cultivars: G122, Beryl, Ex Rico 23 (Bunsi), Cornell 501, Cornell 601, AN-1, AN-37, AN-69, CO75944, NO2302, IO 1892-115M, and USWA-6, were the entries for field screening. These are the same lines that were tested in the greenhouse/lab tests. The plot design was a randomized complete block with three replications of two row plots for each entry, 15 feet (~ 4.5 meters) long. Each plot consisted of three rows--- two entry rows and a third row of a local susceptible cultivar. The disease severity rating, the percent of above ground plant canopy with white mold symptoms/signs (bleached and pithy, shredded stems often with sclerotia at or near plant maturity), was recorded for the center row entry of each plot.

Use of multiple sites allowed us to identify sources of resistance that were broadly effective. Despite variability between and within field and greenhouse sites, the rankings over multiple locations in 2003 and 2004 were consistent (Table 1 and 2). One of the breeding lines identified in our test has been released as a source of resistance in snap beans.

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For the 2005, 26 lines/cultivars were evaluated in the greenhouse/lab tests, an increase of 14 lines over the 2003 and 2004 entries. Unfortunately, many collaborators are still harvesting and screening, so rankings from the 2005 multi-site nursery are not available.

Table 1. Mean rankings of bean lines (1=most resistant) for white mold reaction over all greenhouse/lab and field tests in 2003.

Entry	Mean Ranking	T Grouping	Seed Class
Beryl	9.063	A	GN
CO75944	7.875	B A	PTO
AN 69	7.594	B A	PTO
USWA 6	7.533	B A	SR
N02 302	7.469	B A	NAVY
AN 1	7.063	B A C	GN
AN 37	6.719	B A C	PTO
Ex Rico	6.625	B A C	NAVY
IO1892-115M	5.781	B C	BLK
Cornell 501	**5.500**	**B E C**	**SNAP**
G122	**4.688**	**E C**	**CRAN**
Cornell 601	**4.219**	**E**	**RK**
Dwarf Bees	**3.167**	**E**	***P. coccineus***

Table 2. Mean rankings of bean lines (1=most resistant) for white mold reaction over all greenhouse/lab and field tests in 2004.

Entry	Mean Ranking	T Grouping	Seed Class
Beryl	10.292	A	GN
CO75944	9.000	B A	PTO
AN 1	8.042	B A C	GN
AN 69	7.542	B D A C	PTO
USWA 6	7.417	B D C	SR
IO1892-115M	7.167	B D C	BLK
N02 302	6.583	B D E C	NAVY
Ex Rico	6.208	F D E	NAVY
AN 37	5.083	F D E	PTO
Cornell 501	**4.917**	**F D E**	**SNAP**
G122	**4.167**	**F E**	**CRAN**
Cornell 601	**4.083**	**F E**	**RK**
Dwarf Bees	**3.600**	**F**	***P. coccineus***

References:

Individual rankings by locations for 2003 and 2004 can be found in the Annual Report of the BIC, volumes 47, 2004, pg. 281-282 (for 2003 rankings) and volume 48, 2005, pg. 124-125, (for 2004 rankings).

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EARLY GROWTH PROMOTION OF DRY BEANS (PHASEOLUS VULGARIS L.) BY GIBBERELLIC ACID

Alexander D. Pavlista, James A. Schild, Gary W. Hergert, and Carlos A. Urrea
Department of Agronomy and Horticulture, Panhandle REC, University of Nebraska - Lincoln

Abstract

The current cultural practice for harvesting dry beans (*Phaseolus vulgaris* L.) is to undercut vines at harvest, windrow and combine. One strategy for directly harvesting beans is to raise pod height to increase ground clearance for combining. Gibberellic acid (GA3) is a known plant hormone that stimulates cell elongation and plant growth. In a 2005 greenhouse study, GA3 was applied to cultivars Matterhorn (Great Northern, Type 2b growth habit, upright) and Poncho (Pinto, Type 3b growth habit, vine) as either a seed soak or as a spray solution to the unifoliate leaves at V2 stage. Both cultivars responded similarly to the seed soak showing a log response growth curve. GA3 doubled plant height at the first trifoliate for Matterhorn when 250 to 2000 ppm GA3 were applied; Poncho responded similarly when 125 to 1000 ppm GA3 were applied. However, the two cultivars responded differently when GA3 was applied to the unifoliate leaves. To double the height to the first trifoliate, Matterhorn required 256 ppm GA3 while Poncho needed only 4 ppm. Exposure to higher rates (1024 ppm for Matterhorn and 32 ppm for Poncho) inhibited emergence, caused stem breakage and prevented flowering. These results demonstrated the potential for using GA3 to raise bean pods off the ground thus allowing direct harvest and identified rates and methods for field successful field trials.

Introduction

Producers have expressed interest in growing dry beans in no-till cropping systems using direct harvest. Machinery modifications and changes in plant architecture will be required to accomplish this goal. Changes in plant architecture can be achieved by plant breeding or by growth regulators. Gibberellic acid (GA3) is a natural growth stimulant known to stimulate cell elongation (Haber and Luippold, 1960). Recently, a greenhouse bioassay using snap beans (*Phaseolus vulgaris* L.) with GA3 applied to unifoliate leaves was developed and a dose response for stem elongation on an indeterminate cultivar was reported (Knoche et al., 2000). The objectives of this study were to identify the GA3 dose response of two dry bean cultivars (upright and vine growth), and to evaluate two methods of GA3 application to elevate bean pods so they may be harvested directly.

Materials and Methods

Seeds of cv. Matterhorn and Poncho were used in a greenhouse study during spring 2005. A 4% ai formulation of GA3 (Valent, Release LC) was diluted in tap water. For the seed soak application, GA3 was diluted to the range of 62.5 to 16,000 ppm and applied in 2x increments with tap water as the control. Thirty-two seeds of each cultivar were soaked in 15 ml of each GA3 solution for five min, then allowed to air-dry for several days. Seeds were planted 2.5 cm deep in 20 cm diameter by 20 cm deep fibrous pots containing germination medium (Fafard Superfine Mix). Each pot had six seeds per soaking solution, three from each cultivar with four pots per treatment. For the unifoliate leaf spray application, four seeds were planted per pot and the cultivars were kept separate. When the unifoliate leaves expanded to V2 (CIAT, 1986), two weeks after planting, the leaves and growing tip were sprayed with 1 ml of a GA3 solution. All spray solutions including controls contained a surfactant (0.2% X77). Concentrations of GA3 ranged from 2 to 2,048 ppm for Matterhorn in 2x increments. For Poncho, 2 ppm was too high for a no-effect response and the test was repeated lowering the dose in 2x levels to 0.03125 ppm (31 ppb). Pots were distributed in a RCBD with four replicates blocked on benches. Seed soak and foliar treatments were in separate trials, and for the foliar treatment the cultivars were in separate trials. Data consisted of percent emergence, heights to different plant nodes from the ground, progression through growth stages, stem breakage, flowering, and pod set during the first five weeks after planting for both treatments. Data analyses were done using PROC ANOVA (SAS)

.

Results

The dose response for height to the first trifoliate node at stage V3 measured two weeks after planting showed a clear S-shaped curve for GA3 indicating rate ranges in sensitivity. The log phase of growth stimulation began at 250 ppm and ended at 2000 ppm for Matterhorn and started at 125 ppm and ended at 1000 ppm for Poncho (Figure 1). The active rates of GA3 also advanced stages of growth that is plants from treated seeds reached the V3 and V4 before the controls. Seed soaking with GA3 at 8,000 and 16,000 ppm decreased emergence. Unifoliate (V2) application of GA3 had similar effects on growth. Height from soil level to the first trifoliate node (V3) showed dose response curves as early as one week after treatment (3 wk after planting). The dose ranges were different for the two cultivars (Figure 2). For Matterhorn, the log phase started at 32 ppm and ended at 256 ppm while for Poncho, it started at 0.125 ppm (125 ppb) and ended at 4 ppm GA3. Advancement through growth stages was observed with 64 ppm for Matterhorn and 4 ppm with Poncho compared to controls. For Matterhorn, stem breakage was significant at 1024 ppm one week after treatment, and for Poncho, flowering was observed to be significantly reduced three weeks after treatment by 32 ppm GA3 and prevented by 128 ppm.

Discussion

Although seed soaking with GA3 was more effective, high rates eliminated emergence and weak stems were observed. Application to the unifoliate leaves was also significantly effective but plants appeared healthier. Unlike seed soaking, there was a major difference between the two cultivar in their sensitivity to GA3 doses. Knoche et al. (2000) did not identify differing sensitivities in snap beans as they did not compare cultivars. However, the amount of GA3 applied to unifoliate leaves of cv. Black Seeded Blue Lake was comparable to cv. Matterhorn. The GA3 doses used on the leaves were based on the label rates to stimulate pepper plant growth and rhubarb bud break. The seed soaking rates were based on the label rates for GA3 as a soak for rice and wheat seed. These studies helped refine GA3 rates and application methods for field trials. Previously, there has not been work done to alter dry bean architecture for direct harvest. By lengthening the lower vine internodes and thereby raising pods, GA3 can help develop cultural practices that compliment no-till production.

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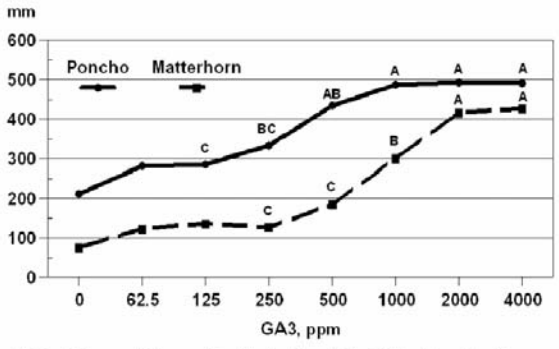
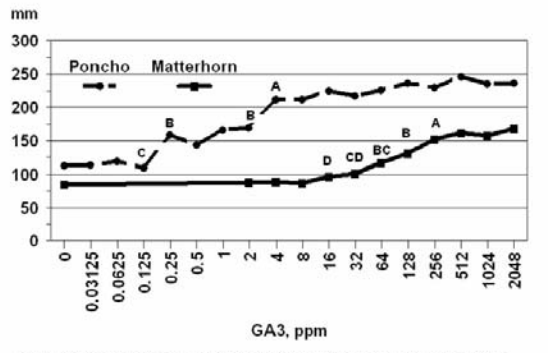
Figure 1. Height to 1st trifoliate node after GA3 seed soak, 2 WAP.



Points with the same letter are not significantly different at the 95% level for each cultivar.

Figure 2. Height to 1st trifoliate node after GA3 applied at V2, 3 WAP.



Points with the same letter are not significantly different at the 95% level for each cultivar.

PROGRESS IN BREEDING DRY BEANS WITH RESISTANCE TO FUNGAL DISEASES IN SOUTH AFRICA

MM Liebenberg*, AJ Liebenberg and CMS Mienie
ARC-Grain Crops Institute, Private Bag X 1251, Potchefstroom 2520, South Africa.
*LiebenbergM@arc.agric.za

Introduction

The most important fungal diseases in South Africa are rust and angular leaf spot (ALS). Andean-type races are the most prevalent, but Middle-American types are increasing. Many of the local cultivars, especially red speckled sugar (RSS) seed types, share a common genetic base and the rust resistance gene *Ur-13*. Progress in the past was also limited due to constrictions with regard to acceptable canning quality, adaptation and seed quality. Important seed types include RSS, or cranberry, beans, which are the most popular for both commercial and informal markets, and small white (SW) (Navy) beans (exclusively for the canning industry). There is also a limited demand for large white (Alubia) (for export and informal markets), Carioca (for small farmer home use and export), other coloured seed types (for subsistence farmers) and Painted Lady (niche market). Dark Red Kidney (DRK) beans are presently imported (canned) from overseas and there is no local production. Foreign DRK germplasm with good cooking quality (especially red colour retention) is very poorly adapted (susceptible to rust, ALS, halo blight, scab, and other diseases and generally low yielding).

Methods

Pathotype characterization was undertaken for rust and ALS. Resistance sources are now monitored in the field for changes in pathogenicity. Screening of local and imported germplasm, and backcross breeding programmes for rust & ALS (since 1995) and root rot (since 2003) has been undertaken. Screening of germplasm and breeding material is done in the greenhouse and field. Selected material is entered in check-row, preliminary, and advanced yield trials (four to seven localities each), followed by National Cultivar Trials (at ±30 localities). Collaboration with other breeders/pathologists has been an important aspect, and teamwork between the pathologist, bacteriologist, biotechnologist and breeder is essential.

Results

A functional picture of the most important rust and ALS races present in southern Africa has been obtained, and selected races are used for screening of germplasm and breeding material. Backcross breeding programmes include the following: RSS for rust, ALS and *Pythium*; SW, Carioca, LW and DRK for rust & ALS; other seed types, such as pinto and calima, for rust, ALS and *Pythium* resistance. Many lines of various seed types have been sent to other African countries (via the Southern African and East and Central African Bean Research Networks (SABRN and ECABREN respectively), one of which was widely adapted and gave the highest yield in regional trials.

Marker assisted selection (MAS) has been undertaken to a limited degree. Three markers were developed for *Ur-13* (which is hypostatic to *Ur-3, Ur-5* & *Ur-11*), and work on suitable markers for *Ur-11* is in progress. Most of the existing markers tested are not polymorphic in local germplasm, but those for *Ur-4* (from which a SCAR marker, OA14, was developed) and *Ur-5* (SCAR SI19) have been used with success.

Resistance to rust and ALS is being combined with resistance to common bacterial blight (CBB) and halo blight (HB) (from the bacterial resistance breeding programme). Two cultivars have been released, namely Teebus RR-1 in 2002 (Teebus *3/BelDak-RR-2), a small white canning bean

with improved rust (*Ur-3*+) resistance, which also retains the ALS and BCM(N)V resistance of the recurrent parent, and Teebus RCR-2 in 2005 (Teebus *4/BelNeb-RR-1//Teebus *5/XAN 159), a small white canning bean with combined resistance to rust (*Ur-5*), CBB, ALS and BCM(N)V. Application has also been made (2005) for registration of Sederberg (Jenny*2/BelMiDak-RR-9), a RSS type with combined rust (*Ur-11* + *Ur-13*) and ALS resistance. All three have the *I-gene* or recessive resistance to BCM(N)V.

Problems encountered include yield drag with *Ur-11* (with small seeded Teebus backcrosses, but not with large seeded lines), lengthy tests for canning quality at both Institute and factory levels, lodging of high yielding RSS lines, small size of improved LW lines, extreme susceptibility of improved DRK lines to halo blight, poor repeatability of ALS inoculations, theft of seed from trials, and delays in seed of new cultivars reaching farmers (commercial and small-scale). The presence of other diseases, especially anthracnose, scab and ascochyta, has somewhat retarded progress. Some field selection against these diseases is done, but as yet they have not been included in any formal resistance breeding programme.

Acknowledgements

Our sincere thanks to Drs Steve Beebe, Matthew Blair, Robin Buruchara, Roland Chirwa, Ken Grafton, Everaldo G. de Barros, Paul Kimani, Jim Kelly, Colin Leakey, George Mahuku, Phil Miklas, Jim Myers, Talo Pastor-Corrales, Shree Singh, Rennie Stavely, and Jim Steadman for seed, literature, advice and support, as well as the ARC-GCI "Bean Team" for technical support.

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BEAN QUALITY ACCESSED BY DIFFERENT SOAKING AND COOKING METHODS.

P.Z. Bassinello, M.G.C. Oliveira, L.L. Rodrigues, D.M. Soares, M.J.D. Peloso, M. Thung.
Embrapa Arroz e Feijão. Caixa. Postal 179. 75.375-000 Santo Antônio de Goiás/GO, Brazil

Common bean is the most important staple foods for Brazilian population and also an important source of protein, carbohydrate and fiber. Among the bean quality parameters grain appearance (size, color and shape) is the most decisive that leads the consumer to buy the product, followed by cooking time and taste. More than 80% of Brazilian population live in the cities and bean consumption is diminishing due to long cooking time. The bean industries try to capture bigger market share by offering canned bean, but the majority of the commercial cultivars do not have the appropriate canning quality.

The cooking time of large grains such as WAF 69, SUG 33 and DRK 18 (from CIAT), and small grains as BRS Radiante, Pérola and BRS Valente (from Embrapa Rice and Beans) were evaluated by Mattson method after soaking at different salt solutions. Two concentrations (0,5% and 1%) of NaCl, KCl, Urea, K_2SO_4 and distilled water were applied and compared to potash water (extracted from upland rice straw) in full strength and 50% diluted in water. The beans were evaluated for cooking time and the content of soluble solids in broth. Another experiment was to evaluate the cooking time of BRS Horizonte (carioca) and BRS Grafite (black), both are Embrapa Rice and Beans cultivars, with variable soaking time and water:grain ratio, in presto cooker and conventional cooking pan on common gas stove with high and low heat.

The greatest reduction of cooking time was observed when beans were soaked in potash water (Table 1). Soaking in KCl at 0,5 % did not shorten the cooking time, but reduced the cooking time by 4 minutes when soaked in 1.0% solution. At 0.5 % concentration, the SO_4^- anions were more efficient in reducing the cooking time than the Cl^-. The cooking time for beans in K_2SO_4 at 0.5 and 1.0% was almost the same, whereas urea at 0,5% showed shorter cooking time than in 1.0% solution. There was a positive correlation between cooking time and soluble solids content for beans. The grains soaked in full strength potash water gave the second highest soluble solids content and the highest tegument cracking, what lead to cooking time reduction. The high potassium content (4,3%) found in potash water might cause this cooking time reduction. Potassium derived from KCl or K_2SO_4 did not show the same efficiency as potash water in reducing cooking time, hence other chemical components in potash water may be involved in this process, which deserves further studies. Large grain size, such as SUG 33 and WAF 69, needed longer cooking time and gave lower content of soluble solids in the broth. Large seeded DRK 18 showed good canning properties with the shortest cooking time, it has an intermediary soluble solids content and higher percentage of non cracked seed coat. Medium grain size such as BRS Radiante had shorter cooking time when compared to small seeded Pérola or BRS Valente at all salt concentrations for soaking. The best grain:water ratio for cooking beans with presto cooker was 1:3 (w/v) and 1:5 (w/v) with and without previous grains soaking, respectively. For conventional pan, the satisfactory ratio was obtained at 1:4,5 (w/v) and 1:6,5 (w/v) with and without grain soaking, respectively (Table 2). Suitable appearance was obtained, when beans were presoaked or not, and cooked either by presto cooker or conventional pan with beans:water ratio of 1:7,5 (w/v) and at low heat for 20 minutes (as previously determined by Mattson method) (Table 2).

It can be concluded that the canning quality depends on bean cultivars, seed size and presoaking in salt solutions. The potash water gave overall positive effects on some grain quality parameters, hence it deserves further research to identify the factors that are related to beans cooking time reduction. When beans are cooked in presto cooker after soaking, a better appearance and less cooking time can be achieved. This method showed better correlation to Mattson cooking test.

Table 1(*). Bean quality of different cultivars of common beans submitted to different soaking solutions at variable chemical concentrations.

Identification	H_2O	0.50% NaCl	0.50% KCl	0.50% Urea	0.50% K_2SO_4	50% Potash	Average	H_2O	1.00% NaCl	1.00% KCl	1.00% Urea	1.00% K_2SO_4	100% Potash	Average
Cooking time (min.)														
WAF 69	39.5	30.5	34.0	26.0	29.5	26.5	31,0a	39.5	24.5	26.5	29.5	28.5	21.5	26,9a
SUG 33	37.0	32.0	32.0	32.0	24.5	26.0	30,6a	37.0	28.5	21.5	33.0	25.5	21.0	26,7a
DRK 18	21.0	21.5	21.0	20.5	18.5	18.5	20,2c	21.0	17.0	22.0	20.5	16.0	14.0	18,3d
BRS Radiante	19.5	18.5	17.5	17.0	20.5	16.5	18,3d	19.5	18.0	19.5	21.5	22.5	16.0	19,3c
Pérola	23.5	22.0	22.5	19.0	23.5	19.0	21,6b	23.5	19.5	21.5	23.5	24.5	16.5	21,2b
BRS Valente	22.5	21.5	23.5	17.5	19.5	16.5	20,2c	22.5	18.0	19.5	23.5	20.5	14.0	19,3c
Average	27,2a	24,3b	25,1b	22,0c	22,7c	20,5d		27,2a	20,9d	21,8d	25,3b	22,9c	17,2e	
Soluble Solids (%)														
WAF 69	7.6	5.4	6.2	7.6	6.8	7.8	6,9c	7.6	7.6	5.7	7.9	7.0	7.7	7,1e
SUG 33	6.5	4.3	5.0	7.3	7.0	7.4	6,3d	6.5	6.9	5.0	7.2	6.6	7.2	6,5f
DRK 18	7.7	5.9	7.6	8.2	7.9	8.0	7,6b	7.7	7.9	7.9	10.3	7.5	7.5	8,1c
BRS Radiante	6.3	5.9	7.1	9.3	8.5	8.6	7,6b	6.3	8.1	5.9	9.4	8.7	8.2	8,0d
Pérola	6.2	7.8	8.8	10.9	10.5	10.9	9,2a	6.2	10.2	9.1	11.1	10.1	9.6	9,9b
BRS Valente	6.3	9.0	9.3	11.0	10.5	10.5	9,4a	6.3	11.0	9.7	12.0	10.5	9.5	10,4a
Average	6,8d	6,4e	7,3c	9,1a	8,5b	8,9ab			8,6b	7,2d	9,7a	8,4bc	8,3c	
Whole Grains after Cooking (%)														
WAF 69	76	63	93	99	72	79	80d	76	93	93	94	81	86	88d
SUG 33	97	88	99	94	97	96	95bc	97	90	97	96	97	81	93bc
DRK 18	99	100	99	98	99	89	97a	99	96	98	97	99	86	96a
BRS Radiante	100	94	96	97	87	87	94c	100	96	98	98	90	81	93ab
Pérola	96	95	93	98	97	89	95c	96	93	94	98	92	87	93ab
BRS Valente	99	90	98	98	96	93	96ab	99	88	92	86	91	87	90c
Average	95b	88cd	96ab	97a	91c	89d		95a	92ab	93a	93a	92b	92c	

*Values followed by the same letters are not different at 5% probability.

Table 2. Effects of different cooking methods on common beans appearance.

Bean Identification	Cooking Method	Pan Type										
		*Presto cooker					*Conventional					
		Presoaked grains		Non presoaked grains			Presoaked grains			Non presoaked grains		
		Cooking time (min.)/ **Grain:water ratio** (w/v)										
		10	20	20	30	35	20	40	45	20	60	65
		1:3		1:7,5	1:5		1:7,5	1:4,5		1:7,5	1:6,5	
BRS Horizonte (carioca)	Low fire	S	S	S	VS	VS	B	S	VS	VB	LS	VS
	High fire	Nd	nd	LS	nd	nd	VS	Nd	nd	VB	nd	Nd
BRS Grafite (black)	Low fire	S	VS	S	S	VS	B	S	VS	B	LS	VS
	High fire	nd	nd	S	nd	nd	S	Nd	nd	B	nd	Nd

*nd: not determined; S: Satisfactory (Whole cooked grains); VS: Very Satisfactory (Whole grains very cooked); B: Bad (Whole hard grains); LS: Less Satisfactory (Cooked split grains); VB: Very Bad (Hard and split grains).

RESPONSE OF DRY BEAN CULTIVARS TO FUSARIUM ROOT ROT UNDER FIELD AND CONTROLLED CONDITIONS

V. N. Bilgi[1], C. A. Bradley[1], J. B. Rasmussen[1], S. D. Khot[1] and K. F. Grafton[2]
[1]Department of Plant Pathology, [2]Department of Plant Sciences,
North Dakota State University, Fargo

INTRODUCTION

Fusarium root rot (FRR) in dry edible beans (*Phaseolus vulgaris*) is caused by a soilborne fungal pathogen, *Fusarium solani* f.sp. *phaseoli* (Fsp), and is a major yield-limiting disease in North Dakota and Minnesota. Chemical seed treatment and cultural practice, mainly crop rotation, are currently the best methods of root rot management in dry beans. Since fungicide seed treatments render protection only against the seed and seedling blight phases of the disease, planting resistant cultivars would be a profitable strategy for disease control. The objective of this research were to 1) evaluate eleven dry bean cultivars for resistance to Fsp using three different methods under controlled conditions and in the field and 2) determine which greenhouse method best correlates with the field results.

MATERIALS AND METHODS

In all trials, the eleven dry bean cultivars Eclipse, Matterhorn, Maverick, Montcalm, Norstar, Othello, Red Hawk, Rojo Chiquito, T-39, Vax 3 and Vista were evaluated for their resistance level to Fsp. Roots were evaluated for root rot severity using a 1 to 7 scale (Abawi and Pastor-Corrales, 1990). All trials were arranged in a randomized complete block design with 3 to 4 replications. Trials conducted under controlled conditions were repeated once over time.

Controlled condition trials. The eleven cultivars were evaluated using an Fsp-infested cornmeal-sand inoculum layer, an Fsp conidial suspension method, and a direct root inoculation method. The cornmeal-sand inoculum layer method (Strausbaugh et al., 2004) was conducted in the greenhouse, and consisted of planting seed above a layer of inoculum so that the roots would growth through the inoculum layer. With the conidial suspension layer (Mondal et al, 1995), bean seedling roots were inoculated with a Fsp conidial suspension (5×10^4 conidia/ml). The direct root-inoculation method (Mitchell et al., 1968) consisted of growing bean seedlings for 10 days, removing them and inoculating the roots directly with Fsp-infested ground wheat and wrapping them with paper towels inside a sealed plastic bag, allowing them to incubate for 18 days in a growth chamber (25°C).

Field trials. Field trials were performed in the summer of 2005 at 3 locations. Trials at Perham and Park Rapids, MN relied on natural disease pressure whereas the trial at Fargo, ND was artificially inoculated with Fsp. Plots at Park Rapids and Perham were 6 m long x 4 rows wide (76 cm row spacing). Small hill plots spaced 30 cm apart (10 seeds per hill) were utilized at Fargo. The Fargo plots were infested prior to planting with a layer of Fsp–infested ground wheat inoculum below the seeds. Stand was measured at Perham and Park Rapids 21 days after planting (DAP) and 30 DAP at Fargo.

Statistical Analyses: Data were analyzed using the general linear model procedure (PROC GLM) in SAS (SAS Institute, Inc., Cary, NC). Fisher's least significant difference (LSD) test was used to compare means, where alpha = 0.05. Pearson correlations were conducted using SAS (PROC CORR) to analyze relationships between root rot data from the greenhouse methods and field trials.

RESULTS AND DISCUSSION

Controlled condition trials. Disease severity among cultivars was significantly different ($P<0.0001$) for all three methods. The cultivar Rojo Chiquito had the greatest FRR severity of all the cultivars in all 3 methods. The cultivar Vax 3 had the least FRR severity of all the cultivars in all 3 methods.

Field trial. Disease severity among cultivars was significantly different ($P<0.0001$) for all three field trials. Vax 3 had a significantly lower FRR severity rating than all other cultivars in the field trials at Perham and Fargo while T-39, Eclipse, and Vax 3 had the lowest FRR severity ratings at Park Rapids. At Fargo, the cultivars Norstar and T-39 had no emergence; therefore, no FRR ratings could be recorded on those cultivars.

Correlations. FRR severity ratings from all controlled condition methods positively correlated with FRR ratings from the field trials at Perham ($P \leq 0.001$) and Fargo ($P \leq 0.05$) (Table 1). Only FRR severity ratings from the direct root inoculation method positively correlated with FRR severity ratings from the field trial at Park Rapids ($P \leq 0.05$).

From these trials, the cultivar Vax 3 appears to have a greater level of resistance to Fsp than most other cultivars tested. All inoculation methods evaluated had good correlations to field data, suggesting that they could be used in an efficient manner to screen germplasm or cultivars for resistance to FRR.

Table 1. Pearson correlation coefficients for Fusarium root rot severity in greenhouse and field trials.

Location	Cornmeal-sand inoculum	Conidial suspension	Direct root inoculation
Perham	0.87**	0.88**	0.90**
Park Rapids	0.60	0.57	0.61*
Fargo	0.67*	0.79*	0.77*

*, ** Significant at P = 0.05 or 0.001, respectively.

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'ALMONGA', A NEW SPANISH PLANCHADA DRY BEAN.

Carmen ASENSIO[1]*, M.Carmen ASENSIO S-MANZANERA[1], Ruth LÓPEZ[1]
[1]Dep. Hortofruticultura y Protección Vegetal, ITACyL. Ctra. Burgos km. 119, 47071-Valladolid. SPAIN. *Presenter (asevegma@itacyl.es)

INTRODUCTION

Current bean breeding program at ITACyL (Instituto Tecnológico Agrario de Castilla y León) started in 1987, yielding fourteen varieties until now. The main objective of this program is to introduce bacterial and viral resistance into landraces, preserving quality characteristics of the seed. Registered cultivars belong to different Spanish market classes, prevailing large white seeded beans, although canela, cranberry and red seeded beans are also presented.

One of these varieties is 'Almonga' that is a white large dry bean (57 g 100 seed^{1}) of "planchada" market class, and was developed and released in 2002 by the ITACyL, Valladolid, in cooperation with the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Spain. Almonga is a high culinary quality cultivar with resistance to halo blight caused by *Pseudomonas syringae* pv. *phaseolicola* (Burkholder 1926) Young, Dye & Wilkie 1978], *Bean common mosaic virus* (BCMV, potyvirus), and *Bean common mosaic necrosis virus* (BCMNV, potyvirus).

DESCRIPTION

Almonga was derived from the cross ZJ-724/4J-132-1_92. Landrace ZJ-724 has large white flat seeds and indeterminate growth habit Type IIIb, high culinary quality, and moderate resistance to halo blight. Early maturing halo blight tolerant F_4 breeding line 4J-132-1_92 was derived from the cross 'Cueto'/'Jules' using the pedigree method. Cueto is a white kidney selection from a landrace. Cueto has growth habit Type I, high culinary quality, and is susceptible to BCMV, BCMNV, and halo and common bacterial blight [caused by *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye] (Asensio Vegas et al., 1990). Jules is a great northern dry bean cultivar tolerant to common and halo bacterial blights (Coyne and Schuster, 1970). Almonga possesses the recessive *bc-1*^{2} resistance allele derived from great northern Jules via breeding line 4J-132-1_92. Thus, in greenhouse tests, Almonga was resistant to BCMV (US-6 strain) and BCMNV (NL-3K strain) and was also resistant to races 1, 2, 5, 7, and 9 of *P. syringae* pv. *phaseolicola* in both leaf and pod, and moderately resistant to race 6 in pod. Almonga is susceptible to common bacterial blight.

Almonga was tested in replicated yield trials in five locations in Spain during 5 yr. Mean seed yield for Almonga was 2976 kg ha^{1} compared with 1719 kg ha^{1} for Cueto. Almonga bloomed in an average of 50 d and matured in 102 d after planting. Almonga plants have an indeterminate prostrate growth habit Type IIIb, dull green leaves, and white flowers. Almonga pods are 170 mm long and have an average of five seeds per pod. Almonga has rhombohedric, shiny white seeds, high culinary quality with very soft integument, and highly buttery albumen, tested by a trained sensory panel. These characteristics are highly desired by Spanish consumers.

Breeder and Foundation Seed of Almonga will be maintained by Instituto Tecnológico Agrario de Castilla y León (ITACyL), Subdirección de Investigación y Tecnología, Departamento de Hortofruticultura y Protección Vegetal, Ctra. Burgos Km. 119, 47071 Valladolid, Spain. Small samples for research purposes can be obtained from the corresponding author.

'ALMONGA' DESCRIPTORS*

	Trait			Mean (Standard deviation))
Market class				Planchada
Phenology	Days to first flower			50.6 (6.42)
	Days to blooming end			85.5 (8.9)
	Blooming length			35.9 (4.4)
	Days to maturity			102.3 (15.6)
Plant	Growth habit			IIIb
	Length of internodes			3.0 (1.0)
	Number of nodes to flower			5.3 (1.6)
	Central Leaflet	Shape		Oval
		Length (mm)		9.2 (1.4)
		Width (mm)		6.1 (0.7)
		Size (mm)		55.6 (8.5)
		Hairiness		Short and sparse
	Flower	Color of the petals		White
		Color of the standard		White
		Outer base of the standard		Smooth and greenish
		Bracteole	Shape	Cordate
			Size	Medium
		Inflorescence		Multinode
	Pod	Length (mm)		169.4 (9.45)
		Width (mm)		13.3 (1.2)
		Height (mm)		5.7 (0.6)
		Weight (g) of ten pods		57,9
		Seeds per pod		4.8 (0.4)
		Thread		Highly present
		Pick origin		Placental
		Shape of pick		Bent
		Pods per plant		24 (5.5)
	Seed	Weight (g) of 100 seeds		56.7 (5.5)
		Width (mm)		9.8 (0.8)
		Length (mm)		18.46 (1.3)
		Height (mm)		5.98 (0.7)
		Shape		Rhombohedric
		Brightness		Bright
		Veins		Absent
		Color		White
		Pattern		Absent
Phaseolin seed protein				B
Yield (Kg/Ha)				2976 (639)
Cooking time				60'-70'

*Singh et al. (1991)

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PRELIMINARY QTL ANALYSIS FOR WHITE MOLD RESISTANCE IN A BLACK BEAN X WILD MEXICAN BEAN INBRED BACKCROSS MAPPING POPULATION

K.A. Terpstra and J.D. Kelly, Department of Crop and Soil Sciences, Michigan State University, East Lansing, MI 48824

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 recently been reported in diverse bean genotypes (Ender and Kelly, 2005; Kolkman and Kelly, 2003; Miklas et al., 2001; Park et al., 2001). The present work was undertaken to identify novel QTL from undomesticated 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 some level of white mold resistance and PI 318695 has shown resistance to white mold in greenhouse straw tests. Greenhouse straw tests were also conducted on the population in 2005. The straw tests were rated on a scale of 1-9 as described by Petzoldt and Dickson (1996). Phenotypic data was also collected on a subset of 30 lines in 2004 and 2005 in naturally infected field plots rated when the plants reached physiological maturity. In the field, disease was rated on a scale of 1 to 9 as described by Miklas et al. (2001).
The population was genotyped using both SSR and SRAP markers (Blair et al., 2003; Gaitan-Solis et al., 2002; Li and Quiros, 2001; Yu et al., 2000). 167 SSR primer combinations were screened in the parents and 37 SSR combinations were found polymorphic and genotyped in the population. 28 polymorphisms were genotyped in the population from 11 SRAP primer combinations. The markers were mapped using Joinmap and single marker analysis and composite interval mapping was conducted using QTL Cartographer.

Results and Discussion

22 SSR and 14 SRAP markers were mapped to nine known and three unanchored linkage groups of common bean. One QTL for resistance to white mold in the greenhouse straw test was identified on B9 near the SSR markers BM114 and PV-at007. In the 2004 field season a QTL on unanchored linkage group A was identified near SRAP marker TE1/EM7 (270 bp). This QTL reappeared in 2005 along with another QTL on unanchored linkage group C near SRAP marker ME1/EM5 (310 bp). The QTL on

linkage group A had R^2 values of 17 and 47% in 2004 and 2005, respectively. The data from both field seasons could not be combined in the QTL analysis due to differences in disease ratings between the two years.

The QTL on linkage group A, however, remains of significant interest because of its appearance in both 2004 and 2005. Both QTL identified in the field are from the parent 'Tacana.' The QTL on B9 that was identified in the straw test is from PI 318695. This is notable because this parent was originally identified as having resistance to white mold using the straw test, and no prior studies have identified QTL for white mold resistance on B9. Work will continue to place additional markers on the linkage map for this population to ensure more complete genome coverage and to identify the location of unanchored linkage groups. The population will also be planted and rated for white mold resistance in the field in 2006.

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DISTRIBUTION AND PATHOGENICITY OF BEAN COMMON BACTERIAL BLIGHT IN THE SEMIARID HIGHLANDS OF MEXICO.

R. Navarrete-Maya[1], J.A. Acosta-Gallegos[2], F.J. Ibarra-Pérez[2]*, R. Zandate-Hernández[2], P. Fernández-Hernández[2], J. Navarrete-Maya[1] and J. D. Kelly[3].

[1]UNAM, FES-Cuautitlán, UNIGRAS. Apdo. Postal 25. C. Izcalli, Edo. de México, CP 54700, [2]INIFAP, Apdo. Postal 112, Celaya, Gto. CP 38110, [3]370 Crop and Soil Science Department, Michigan State University, E. Lansing, MI 48824. rnavarre@servidor.unam.mx,

INTRODUCTION

Bean common bacterial blight (CBB) is an important damaging disease in the semiarid highlands of Mexico (SAHM) and elsewhere. The causal agent (*Xanthomonas campestris* pv. *phaseoli*) (*Xcp*)) is seed transmitted (6). Mexico is one of the primary centers of origin and diversification of beans (*Phaseolus* spp.) (3), and of the companion pathogens that are highly variable (1). Our objectives were to study the distribution of CBB in the semiarid highlands of Mexico and to detect the pathogenic diversity of the *Xcp* isolates from the region.

MATERIALS AND METHODS

Diseases leaves, pods and bean seeds were collected in 2004 from 200 sites in the SAHM. *Xcp* was isolated by disinfecting the plant material in 1% NaClO for one min, maceration in sterile H_2O, and striating on Nutrient Agar at 26 ºC. The identification of *Xcp* was based on colony color (yellow colonies on Yeast Dextrose Calcium carbonate) and on biochemical tests (6). Eight isolates of *Xcp* were inoculated on detached leaves of Pinto Bayacora $3X10^7$ cfu/ml from plants at R_5 growth stage, 10 leaves/ isolate (Table 1) inoculated leaves were kept at 26 ºC, >80% R.H., and 12/12 h day/night duration. Severity assessments were done eight days after inoculation, with a visual scale (from 1 to 9) (7).

RESULTS AND DISCUSSION

Bean common bacterial blight was widely distributed in the SAHM which comprises more than 1.2×10^6 ha sown to beans. 200 commercial and experimental fields across the region were sampled (Fig. 1). All cultivars being grown in the region, both landrace and improved, were indistinctly affected by CBB during the 2004 season, the incidence being more uniform on improved genotypes. The eight isolates of *Xcp* tested on Pinto Bayacora displayed differences in severity, six isolates caused an intermediate reaction and two were highly virulent (Fig. 2). Those isolates were chosen to geographically represent the region. In a second trial using a set of 36 cv inoculated with five of the eight isolates, results indicated the existence of highly virulent isolates which severely damaged all genotypes, while others damaged only few cultivars, and others provoked a slight reaction on all of them (data not shown). Thus variation in the aggressiveness of *Xcp* from the SAHM was observed on common bean as it has been mentioned by other authors in different countries (2, 4, 5, 8). Since the use of certified seed in the region is low, the use of grain that is probably infected, is a widespread practice in the region.

This is partially due to the fact that rain-fed bean production is an un-secure activity. On the other hand, the pathogenic variation of *Xcp* could be due to the mono-cropping of bean in the same areas for many years, and the unrestricted movement of the seed/grain throughout the production region. The pathogenic variation and aggressiveness of *Xcp* hampers the development of resistant cultivars, in addition, sound cultural practices should be encouraged in the region to help control the problem

Fig. 1 Frequency distribution of 200 samples of bean infected with *X. campestris* pv. *phaseoli* per commercial bean class sampled in the semiarid highlands of Mexico

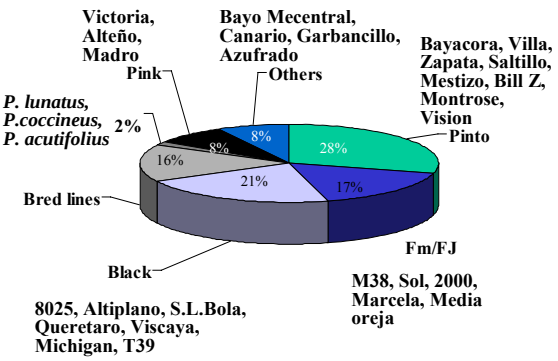
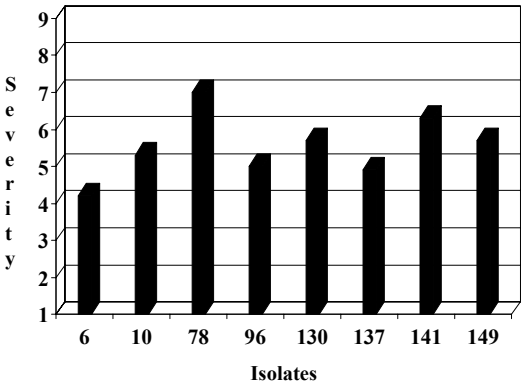


Fig. 2 Severity of eight isolates of *Xcp* on detached leaves of cv Pinto Bayacora, inoculated with twin razor blade



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INTROGRESSED GENOTYPES TO IMPROVE COMMON BEAN

Santalla, M., M. Lema, M. Pérez-Barbeito, M. De La Fuente, S.P. Singh & A.M. De Ron

Plant Genetic Resources Department, Misión Biológica de Galicia-Consejo Superior de Investigaciones Científicas (MBG-CSIC), P.O. Box 28, 36080 Pontevedra, Spain

Introduction

Progress in common bean breeding requires the exploitation of genetic variation that is present among races or through introgression across gene pools. Iberian Peninsula is considered as a secondary center of genetic diversity (Santalla et al., 2002). Introgression from the Middle American to the Andean gene pool appears to be relatively common in Andean zones, while Middle American accessions from the Iberian Peninsula exhibit evidence of introgression from Andean beans (Chacón et al., 2005; Paredes and Gepts, 1995). This introgression was assumed to result from spontaneous outcrossing in farmers' fields, based on segregation found previously in farmers' varietal mixtures. Studies employing allozymes and DNA-based markers have revealed dozens of instances of natural introgression in plants, and morphological intermediary and molecular confirmation of introgression go hand by hand. The presence of crop-specific alleles in intermediate populations can help to provide strong evidence for a history of hybridization. The objective of the investigation presented here was to quantify the degree of spontaneous introgression on the phenotype of Mesoamerican landraces.

Material and Methods

One-hundred and sixty nine great northern cultivars were chosen for this study. The 23 parental cultivars, their 137 breeding lines and 9 controls were planted in four environments in the northwest of Spain during 2003-2004 seasons. Morphological, agronomical and seed quality traits were measured. Phaseolin seed protein, allozymes and microsatelites were studied. Principal component and canonical discriminant analysis were performed, and the classification criterion used was the allozyme cluster membership (Singh et al., 1991; Santalla et al., 2002).

Results and Discussion

Results from the multivariate analyses (Figure 1) consistently identified fifth internode length, number of nodes to first flower, leaflet length, seed yield and seed weight as major traits separating lines of Mesoamerican origin. The proportion of introgressed accessions in the Mesoamerican germplasm studied (33 out of 137, or 24%) was similar to the Middle American accessions with Andean phaseolin (33%) in the Middle American region but higher than the proportion of introgressed accessions (13%) in the Andean region. These great northern types (> 40 g/100 seeds) are common in the Iberian Peninsula and

are surrounded by landraces that are genetically Andean American. There is a higher probability that any outcrossing of these Mesoamerican types would involve Andean American germplasm. This might explain the surprisingly high frequency (24%) of introgressed Middle American types in this secondary center of diversity. Microsatellite approach seems to identify a molecular marker for these accessions. The introgressed genotypes are sufficiently productive to have survived in farmers' systems, possibly due to more effective disease resistance. These may represent unique genetic recombination events that could be of utility to breeders seeking to improve common bean.

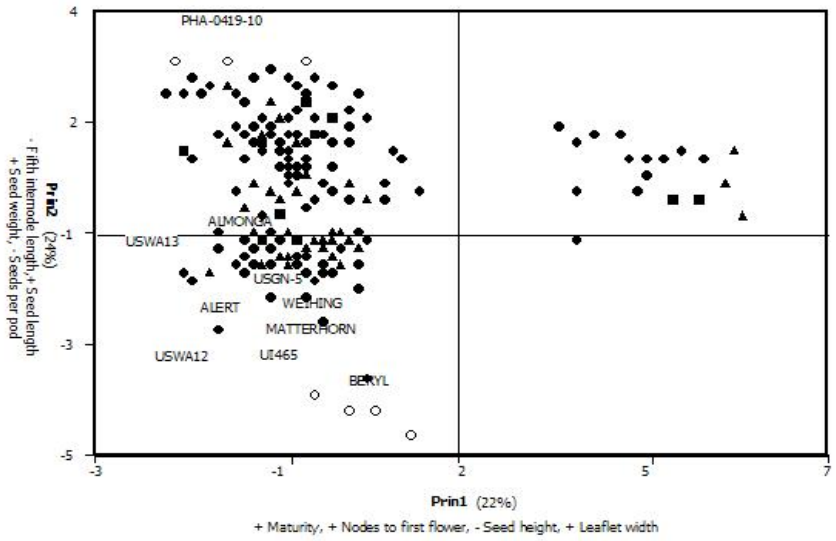

Fig. 1. Principal component analysis of diversity for Mesoamerican accessions.

Acknowledgements

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VARIABILITY IN SYMBIOTIC NITROGEN FIXATION IN COMMON BEAN

A. P. Rodiño[1], M. Santalla[1], A. M. González[1], J. J. Drevon[2] and A. M. De Ron[1]
[1]Plant Genetic Resources Department, Misión Biológica de Galicia-CSIC, P.O. Box 28, 36080, Pontevedra, Spain. [2] Rhizosphère et Symbiose. UMR 1222. INRA-ENSAM, 1 Place Viala, 34060, Montpellier, France.

Introduction

Yield responses of bean are often limited by the nitrogen deficiency, being the limiting factor more common for the growth of the plants. Common bean (*Phaseolus vulgaris* L.) is often considered as a poor N_2-fixing legume. Thus, it is often cultivated with a complement of mineral nitrogenous fertilization to correct this deficiency and to raise the yields. The symbiotic nitrogen fixation (SNF) provides an ecologically acceptable alternative to the high applications of nitrogenous fertilizers, especially in Europe, and an economic alternative to the limited access to these fertilizers in the developing countries. The objectives of the work are i) to study the genotypic diversity represented by local populations of bean, ii) to identify those populations who can be useful to improve SNF potential as well as iii) to determine the degree of genetic diversity of the native populations of rhizobia that nodulate *P. vulgaris* in the soils of Galicia and their potential to fix N_2 with local populations of bean.

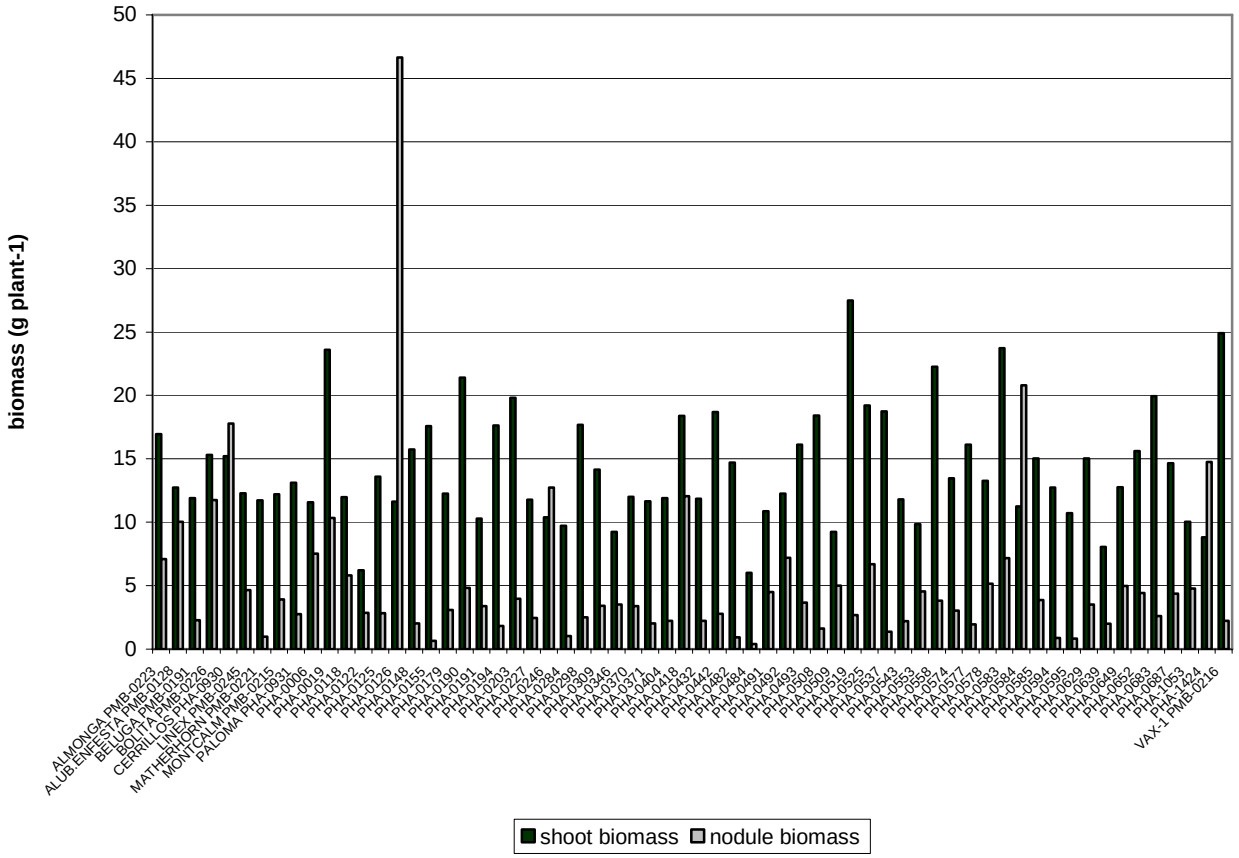
Material and Methods

Sixty four landraces from the common bean collection of the Misión Biológica de Galicia-CSIC were chosen after to screen the nodulation of beans in glasshouse hydroponic-culture. The evaluation in the field were carried out in 2004 and in three locations, Pontevedra (42º 25'N, 8º 38'W 20 masl), Xinzo (42º 5'N, 7º 43'W, 620 masl) and Ponteceso (43º 16'N, 8º 44'W, 400 masl). The experimental design was a randomized complete block with two replications. Each plot consisted of a single 5 m row, with row spacing of 50 cm and plant spacing of 20 cm. At flowering stage, five plants were collected by excavating 20 cm around the root system. The plant was separated immediately from surrounding rhizospheric soil. For each individual plant, the shoot was separated from the root at the cotyledonary node; the number of nodules was recorded and the nodule and shoot dry weight were measured after drying at 80 ºC. At maturity, yield components were measured. Rhizobial strains used in this study were isolated from root nodules from plants grown on soil samples. Pure strains were usually cultivated on YEM media (Vincent, 1970) and they were stored at -20 ºC in 50% YEM glycerol for future studies.

Results and Discussion

There is an important genotypic variability associated with SNF potential. The data in the figure 1 shows that the landraces PHA-0519, PMB-0216, PHA-0583, PHA-0019, PHA-0558 and PHA-0190 had a highest shoot dry weight, while PHA-0126, PHA-0584, PHA-0093, PHA-1424, PHA-0246, PHA-0418, PMB-0226, PHA-0019, PMB-0128 had the highest nodule dry weight. 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 symbiotic N_2 fixation among bean landraces opens a real possibility for enhancing N_2 fixation through selection and breeding. An extensive analysis of genetic polymorphism in the bean crop and in their nodule bacteria will enable to characterize the genetic potential to be used for

improvement of symbiotic N_2 fixation. These results indicated that the following accessions had the best characteristics for SNF: PHA-0019, PHA-0190 and PHA-0583. Those varieties that stand out will be able to be incorporated into programs of genetic improvement, having an important role in the future of the agriculture, as parents in breeding programs. This work can help to improve the growth of legumes along with reducing the costs of production and preserving the environmental quality (Vance et al., 2000). Besides, it contributes to food quality for health and to new cropping systems including for the agriculture in less favoured regions and adverse environmental conditions.



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PRELIMINARY SCREENING OF RUNNER BEAN FOR TOLERANCE TO LOW TEMPERATURE AT EARLY STAGE

A. P. Rodiño, M. Santalla, and A. M. De Ron
Plant Genetic Resources Department, Misión Biológica de Galicia - CSIC, P.O. Box 28, 36080 Pontevedra, Spain

Introduction

The runner bean generally requires moderate temperatures for optimum germination and growth. Thus, temperature is a limiting factor for bean production, and low temperatures at sowing delays both germination and plant emergence, lengthening the crop cycle and increasing production costs. Therefore, to make maximum use of the available growing period, an alternative is to use cultivars which are more tolerant to low temperature at the germination and emergence stages (Revilla et al., 2005). The identification of potential germplasm with tolerance to sub-optimum temperatures during early seedling growth may be of considerable value in the improvement of runner bean cultivars. The objective of this research was to identify potential runner bean germplasm with tolerance to low or sub-optimal temperature during germination.

Materials and Methods

Sixteen runner bean accessions were evaluated in climatic chamber (14-hours light day at 14 ºC and 60 % of relative humidity and 10-hours night at 8 ºC and 80 % relative humidity) for their tolerance to low temperature during early seedling growth. The following traits were determined: emergence score (100 x ∑(number of plants emerged at time i/time from planting)/time from planting to end of emergence), proportion of emergence (%), emergence (days from sowing to hypocotyls emergence), shoot dry weight (determined after the plants have grown at least 20 days, in grams after drying at 80 ºC during 48 h). Seed colour, seed size (g 100 seeds $^{-1}$) and yield (g plant^{-1}) were determined in previous studies.

Results and Discussion

The table 1 shows the seed colour and weight and yield. The runner bean cultivars maintain a high level of diversity since they are a cross-pollinated species with medium to high variation within populations (Zeven et al. 1993). The table 2 shows the mean values and range of variation of the agronomic traits evaluated. The emergence score indicates the emergence speed of accessions along the timeand it had a maximum value of 5.82 (PHA-0133). This rapid emergence under stressful cold conditions would result in an early development of the plants. The proportion of emergence had an average value of 80.7% and a minimum value of 53.3% which indicates that in general all the accessions were able to germinate under cold experimental conditions. The emergence of all the accessions under the experimental conditions with a minimum value of 22.7 days (PHA-1025) were delayed very much compared to the usual values for the north of Spain (commonly 8-12 days). In the field, the seeds are not able to germinate after 20 or more days inside the soil. The shoot dry weight permits an approach to the estimation of vigour. The most vigorous were PHA-0311 (0.60 g) and PHA-0011 (0.56 g). These results indicate four accessions that had the best performance under the experimental cold conditions in the growth chamber: PHA-0011 PHA-0311, PHA-1018 and PHA-1031. These accessions have a large seed size and yield. Besides, they present a high proportion of emergence, earliness and a good growth under cold conditions. Thus, these landraces merit special attention for commercial use and as genetic material for breeding.

Research was supported by the projects AGF2000-1613 and RF03-024-C6-2 from the Spanish Government.

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Table 1. Type of germination, seed colour, seed weight and yield of the runner bean accessions

Accession[(1)]	**Seeed colour**	**Seed weight (g 100 seeds^{-1})**	**Yield (g plant^{-1})**
PHA-0011	white	167.3	-
PHA-0127	white	78.3	54.6
PHA-0133	white	90.7	-
PHA-0163	white	150.0	120.5
PHA-0166w	white	96.7	94.0
PHA-0166c	violet, speckled	102.3	94.0
PHA-0311	white	155.0	198.8
PHA-0322	white	151.3	95.1
PHA-0352	white	114.7	211.7
PHA-0409	white	116.3	77.9
PHA-0469	white	186.0	91.6
PHA-0605	white	84.0	-
PHA-1018	white	138.7	107.7
PHA-1023w	white	180.7	71.9
PHA-1023c	violet, speckled	135.3	71.9
PHA-1025	white	133.0	99.6
PHA-1031	white	285.3	157.3
PHA-1029	white	116.3	106.5

Table 2. Mean values and range of variation of agronomic traits from runner bean accessions under cold conditions.

Accession	Emergence score	Proportion emergence (%)	Emergence (days)	Shoot dry weight (g)
PHA-0011	5.74	96.7	26.1	0.56
PHA-0127	3.12	53.3	31.4	0.28
PHA-0133	5.82	100.0	25.0	0.37
PHA-0163	4.91	86.7	24.4	0.41
PHA-0166c	5.65	86.7	28.0	0.47
PHA-0166w	3.74	56.7	28.4	0.33
PHA-0311	5.39	93.3	24.8	0.60
PHA-0322	4.57	73.3	26.8	0.48
PHA-0352	4.95	83.3	25.6	0.27
PHA-0409	4.64	73.3	30.4	0.47
PHA-0469	5.49	90.0	26.2	0.47
PHA-0605	4.16	63.3	28.3	0.26
PHA-1018	4.18	76.7	23.4	0.54
PHA-1023c	5.31	86.7	26.3	0.45
PHA-1023w	5.05	83.3	26.1	0.43
PHA-1025	5.11	96.7	22.7	0.38
PHA-1031	4.71	70.0	29.0	0.53
PHA-1029	5.26	83.3	27.7	0.45
Mean	**4.36**	**80.7**	**26.7**	**0.43**
Minimum	**3.12**	**53.3**	**22.7**	**0.26**
Maximun	**5.82**	**100.0**	**31.4**	**0.60**

AGRONOMIC PERFORMANCE OF FLAGEOLET BEANS IN SPAIN

A. M. De Ron, A. P. Rodiño, S. Rodríguez, M. Santalla

Plant Genetic Resources Department, Misión Biológica de Galicia–CSIC. Pontevedra, SPAIN

The flageolet is a type of common bean that was originally developed in France, in the XVIIIth century. It is known as Flageolet "vert" or Green Flageolet and it was first obtained by a French grower called Chevrier, in Brétigny-sur-Orge, a suburb of Paris, around 1878 (Porcher, 2005). Its most remarkable feature is its seed, smaller than other kidney bean seeds, and of an attractive pale green color, a rare trait within the common bean germplasm. In order to obtain fresh green colored seeds the pods have to be harvested when well filled but still immature. In northern Spain the flageolet is an heirloom variety demanded for niche markets. In these areas the flageolet bean is named as "Verdina" or "Faba do Marisco" often incorporated to local recipes cooked with seafood.

The objective of this research was to evaluate the agronomic performance of local varieties of flageolet bean as the beginning of a breeding program focused to the selection of breeding lines to be grown by farmers in northwestern Spain as new commercial varieties under the regulation of "Designation of Origin - Lourenzá" (Anonymous, 2004).

Material and Methods

Fifteen varieties (Table 1) were evaluated in field trials in two locations in Northwestern Spain, Pontevedra (40 masl, 46º 26' N, 8º 38' W) and Ponteceso (400 masl, 43º 16' N, 8º 44' W) in 2004. To obtain semi-dried seeds the whole plant was pulled up and hanged into a drying chamber at 25ºC in darkness for 21 days. The dried seeds had the pale green color demanded by the market. The commercial quality of seeds was evaluated at this stage.

Table 1. Flageolet bean varieties evaluated in two environments.

VARIETY	NAME	ORIGIN
PHA-1054, PHA-1422, PHA-1423, PHA-1424, PHA-1841, PHA-1842, PHA-1843, PHA-1844, PHA-1845	Faba do Marisco	Local farmers. Lourenzá, Spain
PHA-1402	French flageolet	Washington State University, US
PHA-1405	Verdina	CRF-INIA. Madrid, Spain
PHA-1406	Verdina	CRF-INIA. Cantabria, Spain
PHA-1407	Verdina	CRF-INIA. Palencia, Spain
CO-025	Flageolet	Commercial. Sprout master, Canada
CO-032	Chevrier vert	Commercial. Thomas Etty, UK

Results and Discussion

The Table 2 shows the analysis of variance for the traits used in the evaluation of the flageolet bean varieties. The environmental effect and/or the interaction variety by environment were significant for first flower, pods/plant, yield, seed weight and water absorption. Miles (2003) evaluated the varieties French Flageolet, French Shell Flambeau and Nugge and found also environmental variation for earliness, yield and seed size. Average value for yield in the varieties evaluated was higher that those by Miles (2003) whereas the seed size was slightly lower.

Seeds/pod, seed length, water absorption and seed coat showed significant differences among varieties. Based on the results, these germplasm is being evaluated in more environments (years and/or locations) to allow a distinct identification of valuable varieties as source of genetic material for the selection of breeding lines.

Table 2. Mean squares in the analysis of variance for eight quantitative traits in the flageolet bean varieties evaluated in two environments.

Origin of variation	df	First flower (days)	Pods plant^{-1}	Seeds pod^{-1}	YIELD (g plant^{-1})
Environments - E	1	749.1 **	149.6 **	0.01	329.8 **
Replications (E)	2	3.3	2.7	0.07	6.8
Varieties – V	14	91.4	24.3	1.76 **	15.7
V × E	14	39.4 **	12.3 **	0.41	13.2 **
Error	28	2.7	2.7	0.21	2.8
Mean		44.1	9.8	4.46	77.7
SE		1.6	1.63	0.46	1.7
Minimum		42.5	6.2	3.55	4.9
Maximum		47.0	16.6	5.85	12.0
CV (%)		3.7	16.6	10.4	21.9

Table 2. Cont.

Origin of variation	df	Seed length (mm)	Seed weight (g 100 seeds^{-1})	Seed water absorption (%)	Seed coat (%)
Environments - E	1	0.77	2.1	411.2 *	1.34
Replications (E)	2	0.10	2.2	23.5	2.12 *
Varieties – V	14	2.40 **	18.5	426.1 *	3.02 **
V × E	14	0.38	13.2 **	117.7	0.73
Error	28	0.28	3.3	91.0	0.53
Mean		12.51	23.9	131.9	9.00
SE		0.53	1.8	9.5	0.73
Minimum		10.75	18.6	118.9	7.82
Maximum		13.52	26.7	151.2	11.39
CV (%)		4.2	7.6	7.2	8.1

*, ** Significant at P≤0.05 or 0.01, respectively; SE= standard error; CV= coefficient of variation

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MODIFIED PETZOLDT AND DICKSON SCALE FOR WHITE MOLD RATING OF COMMON BEAN

Henry Terán[1], Margarita Lema[1*], Howard F. Schwartz[2], Robert Duncan[3], Robert Gilbertson[3], and Shree P. Singh[1].
[1]University of Idaho, Kimberly, ID; [2]Colorado State University, Fort Collins, CO; [3]University of California, Davis, CA.

Introduction

Direct (Petzoldt and Dickson, 1996) or indirect (Kolkman and Kelly, 2000; Miklas et al., 1992) greenhouse tests for detection of physiological resistance to white mold have been developed. One of the most used is the straw test proposed by Petzoldt and Dickson (1996). Moreover, they suggested a rating scale to discard susceptible and allow developing the resistant genotypes. Sometimes this scale cannot discriminate between intermediate and resistant, and intermediate and susceptible genotypes. We propose a modified Petzoldt and Dickson scale that facilitates a better separation between resistant and intermediate and intermediate and susceptible classes (Table 1).

Material and Methods

More than 2000 bean plants were evaluated in the greenhouse at Kimberly, Idaho between October 2004 and May 2005. The inoculum was grown on PDA plates for 48 hours before the inoculation and only the newest part of the mycelial culture was used. Plastic tips (e.g. Eppendorf tips) carrying the inoculant were utilized. The cut-stem and cut-branch methods were used.

The modified scale

The modified scale for the greenhouse straw-test is the following:

Resistant (scores 1-3)

1. No sign of stem/branch infection adjacent to agar inoculant when straw/pipette tube is removed for inspection.
2. Stem/branch infected but invasion of the first internode <1 inch.
3. Stem/branch invasion of the first internode >1 inch but not reached the first node.

Intermediate (scores 4-6)

4. Stem/branch invasion reached the first node, but no further.
5. Stem/branch invasion passed the first node, but invasion of the second internode <1 inch.
6. Stem/branch invasion of the second internode >1 inch but not reached the second node.

Susceptible (scores 7-9)

7. Stem/branch invasion reached the second node, but no further.
8. Stem/branch invasion passed the second node, but invasion of the third internode <1 inch.
9. Stem/branch invasion of the third internode >1 inch leading to plant death.

Table 1. Mean white mold score for selected common bean using the Petzoldt & Dickson and modified scales in the greenhouse at Kimberly, Idaho in 2005.

Genotype	Petzoldt and Dickson Scale	Modified Scale
A 195	4.8	2.8
B 7354	6.7	6.0
G 122	7.5	5.7
ICA Bunsi	7.8	6.8
I 9365-25	8.7	5.7
MO 162	6.7	3.8
NY 6020-4	6.2	3.7
WM 32	6.6	4.4
WM 35	6.7	4.0
92 BG7	6.7	3.3
Othello (susceptible)	8.6	7.6
LSD ($P \leq 0.05$)	1.2	1.8

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ON-FARM DRY BEAN BREEDING FOR HIGH- AND LOW-INPUT CONVENTIONAL AND ORGANIC FARMING SYSTEMS

Shree P. Singh[1*], Henry Terán[1], Dale Westermann[2], Richard Hayes[1], Carlos German Muñoz[1], Margarita Lema[1], Marie Dennis[1], David Fullmer[3], Richard Parrott[4], Kenneth Mulberry[5], and Jay Smith[6].

[1]University of Idaho, Kimberly, ID; [2]USDA-ARS-NWISRL, Kimberly, ID; [3]High-input Conventional Farmer, Kimberly, ID; [4]Low-input Organic Farmer, Twin Falls, ID; [5]High-input Organic Farmer, Kimberly, ID; [6]Low-input Conventional Farmer, Kimberly, ID.

Introduction

Knowing the performance of dry bean cultivars and landraces and selection in different production systems is essential for measuring gains from selection and developing high yielding cultivars for low- and high-input sustainable organic and conventional production systems. Our objectives were to (1) evaluate 16 medium-seeded cultivars and landraces of great northern, pinto, and red market classes for biomass and seed yield, harvest index, 100-seed weight, days to maturity, and plant and seed uptake of 15 nutrients in seven production systems in southern Idaho, and (2) select for seed yield in on-farm high- and low-input organic and conventional production systems and at REC in conventional production system.

1. Selection in landraces and cultivars

Materials and methods

Four great northern, seven pinto and five red cultivars were evaluated in three production systems on REC, namely conventional (RFC), continuous bean cropping (RCB) and drought (RDT). They were also tested in four on-farm production systems, namely conventional (OFC), low fertility soil (OLF), organic low-input (OGL) and organic high-input (OGH) in the Magic Valley in southern Idaho in 2003 and 2004. A randomized complete block design with four replicates was used, and each plot consisted of four or eight rows, 25 or 50 ft long. The spacing between rows was 22 inches, and the RFC, RCB, RDT, OGL, and OLF trials were grown on the residual soil fertility. The OGL plots received only three irrigations until July 15 (i.e., middle of the growing season) in 2003, and both OGL and OGH had exceptionally high populations of more than six weed species approximately four weeks after emergence. Also, the OFC plots had excess salt content and suffered moderate water stress in 2003 and water logging in 2004. Data were analyzed separately for each production system and year. Subsequently, combined analysis was performed after testing for the homogeneity of variances. Genotypes and production systems were considered fixed effects and replicates and years as random effects.

RESULTS AND DISCUSSION

The effects of production systems were highly significant ($P < 0.01$) for all traits including seed yield, 100-seed weight and plant and seed uptake of nutrients. Highly significant differences were observed among cultivars in all production systems. Moreover, production system and cultivar interactions were also highly significant for most traits, indicating that the rank-order of cultivars and landraces changed from one production system to another. Therefore, evaluation and identification of high yielding cultivars specific to each production system would be needed. Furthermore, selection in contrasting production systems may be required for identification of broadly adapted high yielding cultivars across production systems. The mean seed yield of 16 dry bean landraces and cultivars was the lowest in the OGL and the highest in OGH production system. The mean seed yields were reduced by 62% in RDT and by 43% in RCB compared to the RFC in 2003. These reductions were moderate in 2004 because of more favorable weather conditions. Plant and seed uptake of most nutrients were also adversely affected by OFC, OGL, RCB and RDT production systems.

2. Selection in Multiple-parent populations.

Materials and Methods

Two hundred and twenty $F_{2:3}$ (2002), $F_{2:4}$ (2003) and $F_{2:5}$ (2004) breeding lines along with five parents from each of two multiple-parent populations, namely 1WS and 2WS were evaluated for seed yield in OGH, OGL, OFC, and RFC production systems. Each plot consisted of four rows 10 ft long with two replicates in 2004. Subsequently 44 $F_{5:7}$ breeding lines from the highest yielding families and the five parents from each population and production system were evaluated for seed yield in their respective system in 2005. Each plot consisted of a single row 10 ft long with three replicates.

Results and Discussion

Mean seed yields were the highest in OFC and lowest in OGL. The RFC yields were positively correlated with OGH, and OFC yields were positively correlated with OGL yields in one population. In the second population all correlation coefficients were positive and highly significant except for yield between OGL and OGH. The yield trials of $F_{5:7}$ breeding lines will be repeated in 2006 for identification of 11 highest yielding breeding lines from each production system for subsequent comparison across production systems.

ANALYSIS OF IRON CONTENT IN DIFFERENT CULTIVARS, TISSUES AND DEVELOPMENTAL STAGES OF COMMON BEAN.

Espinosa-Huerta E., Acosta-Gallegos J. A., Guzmán-Maldonado H.S., Mora-Avilés M.A.*

Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias, Km. 6.5 Carr. Celaya-San Miguel de Allende, Apdo. Postal 112, Celaya, Guanajuato 38010, México.
*mora.alejandra@inifap.gob.mx

INTRODUCTION

Common bean is one of the main iron sources, which is a very important microelement in human diet. Iron deficiency is a serious nutritional problem around the world reaching close to 30% of world population and is related to a reduced cognitive development as well as anemia. The analysis of iron content in bean has been oriented mainly to seeds; however, understanding of iron content in other bean tissues would provide the actual behavior of this important element to nutritional breeding programs making available alternative iron sources. Our objective is to know the iron content in different common bean cultivars, plant tissues and plant developmental stages as part of ferritin genetic analysis in common bean.

MATERIALS AND METHODS

Plant Material. Leaves, stems, roots, pods and seeds of 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 (I), beginning of pod filling (II), full pod filling (III) and physiological maturity (IV).

Growing Conditions. Plants were germinated in Sunshine 3® and fertilized every week with SUPER TRIPLE 00-46-00, with no iron supply. Growing conditions were 22-25°C and luminosity 170-285 $\mu mol/m^2/seg$ until seed production.

Sample Size. One hundred seeds were germinated for each variety and distributed in 20 pots (5/pot). Each pot was an experimental unit from which, all tissues were collected at different developmental stages. Sampling was done in four replicates.

Sample Preparation. All tissues were lyophilized at -50°C and $50x10^{-3}$ M BAR and each sample ground in a stainless steel grinder.

Analysis of iron content. A tissue extract for mineral solution was prepared by the humid digestion method (8, 3). Iron detection was performed by spectrophotometer of atomic absorption (7).

RESULTS AND DISCUSSION

Leaf and root tissues showed the highest iron contents above pods, seeds and stems levels, regardless the cultivar. Leaves tissue had 9 to 12-fold higher iron content than seeds, followed by roots with 3 to 4-fold above seed levels (Table1).

Iron content in leaves showed statistical differences among cultivars ($p=0.001$) being PV the cultivar with the highest iron content followed by FJM, AH and NJ (Table 1). High iron contents suggest that leaf tissue can be used as a natural iron source, since some biofortification bean programs have reported leaf consumption as vegetable. Additionally, leaves are a good vitamin C source, which improves iron absorption and limits the activity of absorption inhibitors (polyphenols and phytates), increasing the bioavailability (1, 2)

Table 1. Iron content in different common bean cultivars and tissues.

CULTIVAR	TISSUES	IRON[1] (PPM±SE)
AH	Leaf	500.4±23.7
	Root	166.9±24.7
	Seed	52.2±2.1
	Stem	41.4±3.4
	Pod	49.9±11.8
FJM	Leaf	515.5±38.9
	Root	165.5±19.4
	Seed	52.8±3.4
	Stem	43.9±2.1
	Pod	44.1±2.7
NJ	Leaf	439.0±34.7
	Root	156.0±14.2
	Seed	51.3±2.2
	Stem	47.0±8.7
	Pod	59.0±6.7
PV	Leaf	642.8±38.4
	Root	189.2±23.1
	Seed	51.2±3.2
	Stem	57.8±4.9
	Pod	54.9±2.1

[1] Values represent average iron content of all developmental stages.

There was no difference in leaf iron content among developmental stages (p=0.527) therefore, leaves used as iron source could be consumed at any developmental stage. Iron content in roots did not show differences among cultivars (p=0.718), however, it did for developmental stages (p=0.035); FJM and AH showed an increasing iron content pattern starting at the pod filling (II), whereas NJ and PV showed an inconsistent pattern.

For seeds, stems and pods the iron contents were similar among them in all cultivars and in all developmental stages (p=0.962). Iron levels in seeds for all cultivars is considered average and was found to be consistent to previously reported values (4).

Preliminary studies in our lab showed that NJ ferritin gene relative expression in leaves had the highest expression followed by PV, AH and FJM. Ferritin gene expression and iron content did not have a consistent pattern for the four cultivars; however, no conclusions can be made yet since complete analysis is in progress. Some authors mention that there are differential mRNA expression patterns for different tissues because of different protein sub units complex (4, 6). We recognize the need to study ferritin gene expression and protein to understand the differential in iron content among cultivars (in progress).

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ORGANOGENIC PLANT REGENERATION SYSTEM FOR THE COMMON BEAN (*PHASEOLUS VULGARIS L*).

Saucedo-Ruiz, M., Delgado-Sánchez, P., Guzman-Maldonado, S.H., Villordo-Pineda, E., Acosta-Gallegos, J.A. and Mora-Aviles, M.A.*

Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias, Km. 6.5 Carr. Celaya–San Miguel de Allende, Apdo. Postal 112, Celaya, Guanajuato 38010, México.
*mora.alejandra@inifap.gob.mx

INTRODUCTION

Common bean (*Phaseolus vulgaris L*.) cultivars are planned to have good agronomic characteristics (high nutritional quality, resistance to pests and diseases and climatic conditions, among others). However, when genes that provide these characteristics are not available or modification of gene expression is needed, gene insertion represents a suitable approach. For this, it is important to comply with basic requirements such as a consistent in vitro regeneration system which, up to now, has been difficult to establish due to the recalcitrant characteristic of this crop. Here we present a regeneration protocol established in two commercial common bean cultivars.

MATERIALS AND METHODS

Plant Material. Embryonic axis isolated from matured seeds of Flor de Junio Marcela (1) and Flor de Mayo Anita (2) were used as initial explants. Seeds were surface sterilized with the vapours produced by the combination of commercial chlorine (Cloralex®) and HCl 12 N (v/v 5: 0.16) in a vacuum chamber during 17 hrs. Seeds were then soaked in double distilled sterile water for 24 hours for embryo extraction.

Culture media. Basic medium consisted on MS medium (3), amended with myo-inositol 100 mg/l, thiamine 1 mg/l, sucrose 3 %, agar (SIGMA®) 6.8 g/l and pH 5.8 Regeneration induction media consisted of six treatments combining adenine (A) (0, 20, 40 mg/l) and benzyl-amino-purine (BAP) (5 and 10 mg/l).

Elongation medium consisted of the basal MS medium with no hormones and Rooting medium was 50% MS medium. Ten embryonic axis of each cultivar were cultured in three replicates for each of the 6 treatments.

Growing conditions. Explants were incubated in a growth chamber under 16 hrs light with a light intensity of 45 mmol/m^2 /seg. Plantlets obtained were transferred to soil and hardened for further development.

RESULTS

The regeneration response in both cultivars consisted of a bud cluster formation. FJM formed this organogenic structures 18 days after initial culture mainly at the internodal and apical areas, whereas FMA formed organogenic clusters 13 days after initial culture only at the apical area of the embryo. Explants were transferred to fresh medium every two weeks for further bud differentiation. Clusters were excised from the embryo after 3 transfers and subdivided in 5 mm segments. Shoot development was obtained after 60 days of initial culture.

FMA formed one bud cluster form every 10 embryonic axes, whereas FJM regenerated 6.3 to 9 bud clusters from the same number of embryonic axes (Table 1). No difference was

observed in FJM shoot formation when adenine was included either with 5 mg/l BAP (P=1.0) or 10 mg/l BAP (P= 0.385). However, presence of adenine was required for FMA shoot formation combined with 10 mg/l BAP (Table 1).

Bud clusters were sub-cultured on elongation medium; these clusters developed at least 1-2 plantlets showing leaves and stems well differentiated. Organogenic shoot and callus formation in hypocotyls and embryonic axes have been reported in *P. acutifolius* and *P. vulgaris* (4, 5, 6, 7, and 8). In this study, bud clusters corresponded to deep green, compact and well developed structures such as leaves and stems.

Individual plantlets were excised from the cluster after 40 days in elongation medium. Finally, they were transferred into rooting medium and incubated for 25 days. Whole plant formation efficiency was 83% for FJM and 50% for FMA (Table 1).

Table 1. Regeneration efficiency of common bean cv. Flor de Junio Marcela (FJM) and Flor de Mayo Anita (FMA).

BAP mg/l	A mg/l	Bud cluster induction[1]		Number of shoots[2]		No. Of whole plants (Efficiency %)[3]	
		FJM	FMA	FJM	FMA	FJM	FMA
5	0	6.3	0.5	13	6	8 (61.5	0
5	20	6.6	1.5	15	10	9 (60.0)	0
5	40	6.6	1.8	12	11	8 (66.6)	3 (28)
10	0	8.6	0.3	14	5	10 (71.4)	0
10	20	8.3	1.2	15	10	9 (60.0)	5 (50.0)
10	40	9	1.3	12	8	10 (83.3)	2 (25.0)

[1] Values represent an average of three Petri dishes with 10 embryos each and three replicates.
[2] Values represent average of shoot formation in 20 bud clusters.
[3] Number of regenerated whole plants / Number of induced shoots x 100.

CONCLUSIONS

- A consistent organogenic regeneration protocol is reported for *Phaseolus vulgaris* from embryonic axes.
- High BAP concentration was determinant for the novo shoot regeneration.
- Shoot formation and whole plant development was adenine dependant for FMA, whereas this cytokinine was not required form FJM shoot regeneration.
- This protocol will be used in transformation experiments due to its convenient regeneration system.

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EFFECT OF COMMON BEAN (*PHASEOLUS VULGARIS*) CONSUMPTION ON COLON CANCER IN SPARGUE-DAWLEY RATS

M.C. Ríos-Uglade[1], R. Reynoso-Camacho[1], I. Torres-Pacheco[2], J.A. Acosta-Gallegos[2], A.C. Palomino-Salinas[2], M. Ramos-Gómez[2], E. González-Jasso[3], and S.H. Guzmán-Maldonado[2]

[1]PROPAC Universidad Autónoma de Querétaro, Centro Universitario, Querétaro, México. [2]Biotechnology Unit of the Experimental Station El Bajío, National Research Institute for Forestry, Agriculture and Livestock (INIFAP). Km. 6 Carretera Celaya-San Miguel Allende. Celaya, Guanajuato. 38000, México. [3]Centro de Investigación en Ciencia Aplicada y Tecnología Avanzada-IPN. Querétaro, México. *Corresponding author: e-mail:guzman.horacio@inifap.gob.mx.

Introduction. Besides proteins, carbohydrates, vitamins and minerals, common bean also contain other components that play an important role in the prevention of disease. Those components, known as phytochemicals, include phenolics, resistant starches, oligosaccharides and phytic acid, among others [1,2]. It has been demonstrated that common bean phytochemicals display antioxidant and antimutagenic activity [3-6], induce apoptosis [7], inhibit cellular proliferation [8] and reduce insulin requirements and triglyceride concentration in the plasma [9]. The objective of this research was to determine the effect of feeding the seed of four different common bean cultivars on colon cancer in rats and to determine the amount of phytochemicals in the seed.

Materials and Methods. Four bean cultivars, three of common bean Flor de Junio Marcela, Flor de Mayo Anita and Pinto Zapata, as well as one of *P. coccineus* Blanco Tlaxcala were used. Sprague-Dawley rats were fed diets either with or without bean. Diets with beans contained 99.4 g commercial diet plus 0.6 g cooked bean of each bean cultivar; an extra treatment had 25 g commercial diet plus 75 g Flor de Junio Marcela cultivar. After the rats had consumed diets with or without bean, the colon carcinogen 1-2 dimetilhydracine was injected (21mg/kg body wt) once a week for 8 weeks. Bean components that have been related to the prevention of colon cancer and other diseases, including tannins [12], soluble sugars [13], anthocyanins [14] and phenolic acids (HPLC) were determined. The diets were fed 21 weeks. At seventeen weeks after the first carcinogenic injection, the rats were killed by ether inhalation. The colon was removed and rinsed with a saline solution so that it could be examined visually for tumors. All plaques and suspected tumors were excised, weighed and sized.

Results and Discussion. In regard to phytochemical characterization, seed of Flor de Junio Marcela along with Pinto Zapata, had the highest tannin content (Table 1). Flor de Junio Marcela showed the highest anthocyanin content. The soluble sugars content in Blanco Tlaxcala was higher compared to the other cultivars. The soluble sugars represent the oligosaccharide content along with other soluble disaccharides, glucose and fructose. For the phenolic acids, siringic acid, vanillin and catechin, little amounts were detected (Table 1).

Weight gain and rat survival. During the experiment, the rats consumed 23±3 g of feed per day (equivalent in weight proportion to 11 kg of beans per adult per year). There were no significant differences in weight gain among the rat groups that were fed different bean cultivars, with exception of Flor de Junio Marcela. Also, 65% of the rats feed with Flor de Junio Marcela at the highest rate (75% bean) died after 10 weeks of initiated the experiment. It is possible that the high content of tannins in Flor de Junio Marcela was the contributing factor. The other rat diets resulted in different levels of rat survival.

Table 1. Phytochemical characterization of the grain in four common bean cultivars and commercial rat feed.

Compound	Commercial feed	Pinto Zapata	Flor de Mayo Anita	Flor de Junio Marcela	Blanco Tlaxcala
Tannins (mg EC1/100 g)	ND	197.90c	75.6b	689.4d	0.60a
Anthocyanins (mg EC3G[1]/100 g)	ND	1.77b	---	3.75c	1.29a
Soluble sugars[2] (g EG[1]/100 g)	1.59a	2.20b	---	1.64a	4.20c
Phenolic acids (mg/100 g)					
Siringic acid	ND[3]	0.10a	ND	ND	ND
Vanillin	ND	0.10a	0.15b	0.19c	0.09a
Catechin	ND	0.37c	0.19b	ND	0.15a

[1]EC=Catechin, EC3G=Cianydin 3-glucoside, and EG=Glucose equivalents. [2]As glucose, fructose and sucrose, oligosaccharides and other minor components. [3]Not detected. Significance level $p<0.05$.

Table 2. Number and volume of tumors and reducing tumor incidence

Cultivar	Number of tumors/rat	Tumor volume (cm^3)	Tumor Incidence (%)	Reduced incidence (%)	Rat survival %
Control[1]	4	0.071c	100	0	58
Pinto Zapata[2]	1	0.043a	64	36	68
Flor de Mayo Anita[2]	4	0.055b	100	0	78
Flor de Junio Marcela[2]	4	0.095e	90	10	52
Blanco Tlaxcala[2]	3	0.084d	89	11	93
Flor de Junio Marcela[3]	2	0.082d	88	12	35

[1]Commercial feed without bean
[2]0.6 g of bean plus 99.4 g of commercial feed
[3]75 g of bean plus 25 g of commercial feed.

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PHASEOLUS ACUTIFOLIUS AS A POTENTIAL BRIDGE SPECIES IN THE HYBRIDIZATION OF *P. VULGARIS* AND *P. ANGUSTISSIMUS*

Gurusamy Valarmathi, Jocepascual Martinez Rojo*, Kirstin Bett & Bert Vandenberg. Department of Plant Sciences, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK S7N 5A8. e-mail: jom822@mail.usask.ca

INTRODUCTION

Phaseolus angustissimus, a wild bean, has been shown to be more tolerant to frost than common bean. As a means of introgressing frost tolerance from *P. angustissimus* into *P. vulgaris*, several F_1 hybrids were produced through embryo rescue (Balasubramanian, 2002). Backcrosses with these hybrids using *P. vulgaris* pollen were unsuccessful due to early embryo abortions (~72h after pollination). Thus, a new strategy for introgressing the frost tolerance from *P. angustissimus* is necessary. *P. acutifolius* was chosen for an initial attempt as a bridge species to introgress frost tolerance from *P. angustissimus* into *P. vulgaris*. Thus the objectives of this study include developing hybrids between *P. acutifolius*, *P. vulgaris* and *P. angustissimus*, determining the pollen fertility of the resulting F_1 hybrids and evaluating the cytoplasmic effects on pollen viability and pollen size using reciprocal crosses.

MATERIALS AND METHODS

Two accessions of *P. acutifolius,* PI 430219 (*P.ac 1*) and WP 15578 (*P.ac 2*) were crossed with *P. angustissimus* PI 535272 (*P.a*) and *P. vulgaris* cv ICA Pijao (*P.v*). Parents and hybrids were grown in controlled growth cabinets. A total of 711 crosses were made using *P.v*, *P.ac 1* and *P.ac 2* as the female parent and *P.a* as the pollen donor. Embryos were rescued ~7-10 days after pollination as per the protocol described in Schryer et al. (2005). Only a few hybrid seeds were collected at maturity. Pollen fertility of all the hybrids was estimated using 1-% acetocarmine. The size of the pollen grains was measured using an ocularmicrometer (Nikon microphot-FXA EPI-FL3 13161 microscope) and the average was estimated for each cross combination along with their parents.

RESULTS AND DISCUSSION

Table 1. Number of embryos rescued, seeds obtained and total hybrids established

Cross combination	# embryos rescued	# seeds obtained	# hybrids established
P.ac 2 x *P.v*	-	19	19
P.ac 1 x *P.v*	-	13	13
P.ac 2 x *P.a*	192	-	17
P.ac 1 x *P.a*	151	-	29
P.v x *P.ac1*	26	-	5
P.v x *P.ac2*	23	-	4

The morphology of the hybrids between *P.ac1* or *P.ac2* with *P.v* were intermediate. Abnormalities such as determinant plant type, leaf wrinkling and rosate branching was observed in these hybrids. No abnormality in plant type and flowering was noticed in hybrids between *P.ac1* or *P.ac2* with *P.a*.

The percentage of pollen fertility and the average pollen grain size of the bridge hybrids along with their parents are shown in Fig.1 A and B respectively. The fertility of most of the hybrids from the cross combinations listed in Table 1 was less than 20%. However, the hybrids with the *P.v* cytoplasm had higher fertility (35-50%, Fig.1) indicating a cytoplasmic influence on pollen fertility. These combinations will be used in future crossing. The size of the pollen grains of the hybrids was significantly smaller compared to that of their parents probably due to the genomic imbalance in the hybrids.

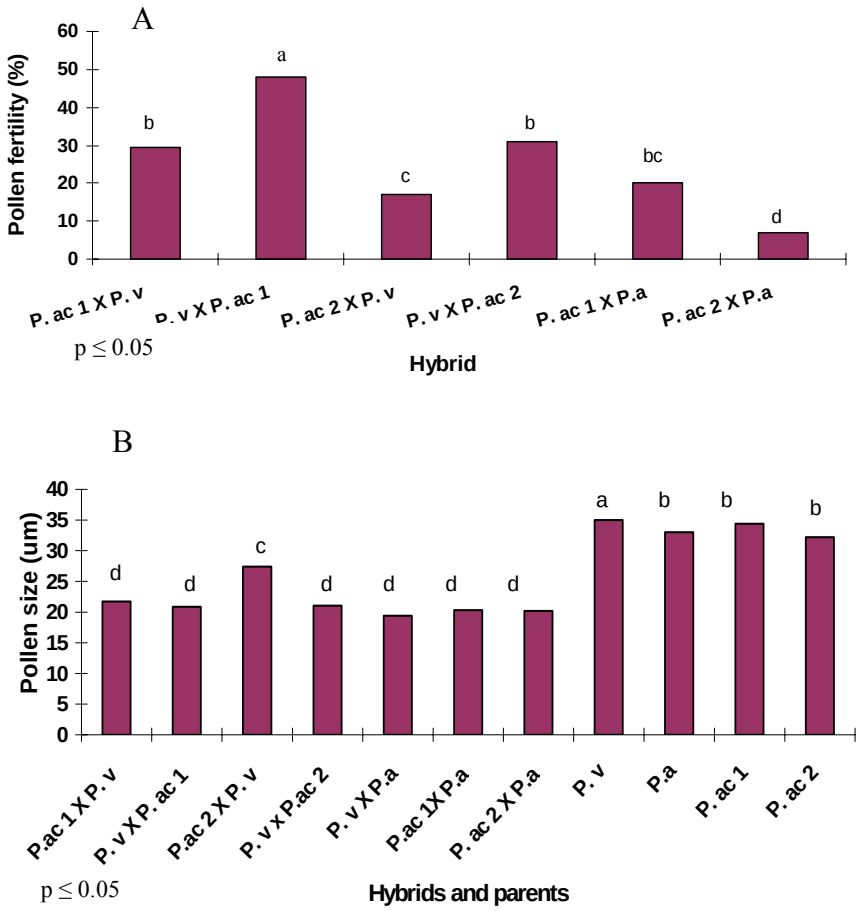

Figure 1. A. Percent pollen fertility in various *Phaseolus* interspecies hybrids. B. Pollen grain size in the hybrids and parents

FUTURE WORK

The cytoplasmic influence on fertility of the hybrids will be explored further. Chromosome doubling of the bridge hybrids is underway and the doubled F1s will be intercrossed and congruity backcrossing to *P.a* and *P.v* will begin. Physiological and chemical characterization of the hybrids and parents under chilling and sub-zero conditions will also be studied.

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AGRONOMIC DIVERSITY OF WILD *PHASEOLUS* SPECIES IN CENTRAL MEXICO[1].

Jorge A. Acosta-Gallegos[2], Francisco Manuel Mendoza-Hernandez[2], Alejandra Mora-Aviles[2] , Rosa Navarrete-Maya[3], Bismarck Aguilar-Garzon[2] and Salvador H. Guzman-Maldonado[2].

[1] Research partially funded by the SINAREFI, SNICS-SAGARPA Project 045

[2]Campo Experimental Bajio of INIFAP, Apartado Postal 112, Celaya, Gto. 38110, Mexico, jamk@prodigy.net.mx. [3]FES-Cuatitlan, Universidad Nacional Autónoma de México, Apdo. Postal 25, Cuautitlán Izcalli, Edo. de México, CP 54700, Mexico.

Mexico is the main center of diversity of the genus *Phaseolus*. Most of the species in this genus grow in Mexico and some of them are endemic to the country (Freytag and Debouck, 2002). Several national and foreign institutions have collected numerous accessions of wild species of the genus in Mexico; however, a bottleneck that has hampered the study of the diverse species is the lack of sufficient seed and the little characterization carried out (Acosta *et al*., 1996). The objective of this research was the seed increase and agronomic characterization of accessions from different wild species of *Phaseolus* under field conditions.

One hundred and thirty accessions of 15 *Phaseolus* species were established in the field at Celaya, Guanajuato during the months of May and June, 2005 using 60 scarified seeds per accession. Annual species were direct planted while perennials were transplanted after germinating them in the greenhouse. Irrigation was applied to both as needed. Species included: *P. vulgaris* (34 accessions), *P. acutifolius* (9), *P. microcarpus* (17), *P. coccineus* (28), *P. maculatus* (9), *P. lunatus* (10), *P. leptostachyus* (8), *P. polymorphus* (4), *P. xantotrichus* (4), *P. tuerkemii* (2), *P. grayanus* (1), *P. polyflorus* (1), *P. macvaguii* (1), *P. salicifolius* (1), and *P. xolocotzii* (1). Each accession was planted in two 4m rows separated by 80 cm, and two to four seeds were spaced planted at 50 cm within the row, three rows were left empty between accessions. Trellises were installed for each accession before flowering. Data were taken on the beginning of flowering and the time when the first ripe pod was observed, disease incidence, 100-seed weight and yield per plot. After scoring the disease reaction at the beginning of flowering of most species, diseases and insects were controlled with commercial agrochemicals at reduced rates, since rates used with domesticated common bean have been observed to cause damage to some wild species.

The main diseases attacking some of the wild species were halo and common blight, powdery mildew and rust (Table 1). The incidence of rust was low, probably because of the scarce and erratic rainfall pattern during the growing season, in total 290 mm were registered from June to December at the experimental site. The species not included in the tables did not produce seeds and did not display disease symptoms. The higher disease scores were registered with common blight on *P. vulgaris* and *P. acutifolius*, although some accessions were not uniformly attacked by those diseases and have few healthy plants. Root-rots elicited by *Fusarium* spp and *Rhizoctonia solani* were observed damaging all accessions of *P. acutifolius* and *P. microcarpus* and to lesser extent some of *P. coccineus.*

Eight of the fifteen species produced some yield; many of these that did not produce seeds flowered late or did not flower at all. The species that displayed high seed yield included *P. coccineus, P. vulgaris, P. maculatus, P. lunatus* and *P. polymorphus* (Table 2); of these, *P. polymorphus* has the smaller seed size, while the rest have accessions with relatively large seed size, particularly *P. maculatus* and *P. coccineus*. In *P. vulgaris* many accessions had low seed yield, particularly those from the southern part of the country, this response was probably due to specific photoperiod-temperature requirements. In the *P. vulgaris* and *P. coccineus* groups some accessions displayed larger seed size than true wild and were

considered as weedy (data not shown). All *P. lunatus* accessions flowered from November onwards and were damaged by frost, but still managed to produce seed from January to March. Some of the perennial species that did not set seeds may do it during the second year after regrowth, a second alternative to get them to set seed may be by sowing them several months in advance in the greenhouse before transplanting them to the field. Many accessions produced enough seed for further studies.

Table 1. Disease incidence on accessions of wild *Phaseolus* species grown under field conditions at Celaya, Guanajuato, Mexico in 2005.

Species	Halo blight[1]	Common blight	Rust	Powdery mildew
P. vulgaris	1 - 3	1 - 7	1 - 4	1 - 4
P. coccineus	1 - 2	1 - 3	1	1 - 3
P. microcarpus	1 - 2	1 - 2	1	1 - 4
P. lunatus	1	1	1	1
P. leptostachyus	1	1	1	1
P. maculatus	1	1	1	1 - 4
P. acutifolius	1 - 3	5 - 6	1	2 - 5
P. polymorphus	1	1	1	1

[1] 1 to 9 scale, where 1 = symptomless and 9 = maximum severity (Shoonhoven and Pastor-Corrales, 1987).

Table 2. Number of accessions established and harvested, seed yield and 100-seed weight of wild *Phaseolus* species grown under field conditions at Celaya, Guanajuato, Mexico in 2005.

Species	Accesions established	Accesions harvested	Range in yield per plot -g-	Range in 100-seed weight -g-
P. vulgaris	34	33	1 - 1085	2.20 – 8.42
P. coccineus	28	21	4 - 1034	6.10-12.14
P. microcarpus	17	16	3 - 66	0.83 – 1.86
P. lunatus	10	7	4 - 1061	5.24 -9.87
P. leptostachyus	9	4	3 - 27	0.97 – 1.29
P. maculatus	9	9	30 - 1765	12.7 – 19.5
P. acutifolius	8	8	20 - 370	1.56 – 2.67
P. polymorphus	4	2	375 - 872	0.96 – 1.23

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EVALUATING HEIRLOOM DRY BEAN VARIETIES AS A NICHE MARKET CROP IN THE MARITIME NORTHWEST

Jennifer Wagner[1], Carol Miles[1], and Phil Miklas[2]. [1]Washington State University, jjwagner@wsu.edu, [2]U.S. Department of Agriculture – Agriculture Research Service, Prosser, Washington

Introduction

"Niche market" is a general term that describes bean varieties not in the major market classes. Not only are there niche market classes, there are also niche market varieties within the main market classes, some of which are classified as heirlooms. Although the definition for an heirloom variety is often debated, most people agree to the term "heirloom" if the variety has been either passed down for several generations or has been grown for a certain period of time. In addition, an heirloom plant must produce seeds that create a plant genetically identical to its parents. Heirloom varieties are increasing in popularity. Many people select them for taste, while others feel as though they are connecting with their heritage or the heritage of other cultures. It is important to research heirloom varieties not only as a potential niche market crop, but also for the sake of germplasm conservation.

The purpose of this research is to determine which niche market varieties are suitable for production in the maritime Northwest. To investigate the fitness of these varieties in this region, trials were conducted at the Washington State University Vancouver Research and Extension Unit and on farms in the region over two years, 2004 and 2005. Entries included standard varieties, breeding lines, plant introductions (PIs), new releases, and niche market varieties including heirlooms.

Methods

Replicated field trials were conducted in 2004 and 2005 at Washington State University Vancouver Research and Extension Unit. A total of 34 bean entries were included in this study both years, and included 7 standard varieties, 10 niche market/heirloom varieties, 1 niche market/non heirloom variety, 9 breeding lines, 4 PIs, and 3 new releases. Dry bean entries were planted in mid May both years in a randomized complete block design with four replications. Plots measured 2 rows wide and 3 meters long, and spacing between rows was 0.6 m. The field was certified organic and maintained accordingly. Plants were harvested from the center of each plot, for a total harvest area of 3 m per plot. Whole plants were harvested, dried, threshed and cleaned by hand.

Results and Discussion

2004: The overall mean yield was 458 g per plot (3 m row) and there was a significant difference among entries (data not shown). The range was 148-653 g and the lowest yielding entry was W614733 (148 g), while the highest yielding entries were CELRK, H9673-87, Othello, and Burke which all averaged over 600 g per 3 m row. The mean yield of the niche market/heirloom

varieties was 424 g, and except for Brown Dutch, Magpie, and Pinto, all varieties yielded lower than the overall mean (Figure 1).

2005: The overall mean for all entries was 616 g per plot (3 m row) and there were significant differences among entries (data not shown). Yield of heirlooms Pinto (908 g) and Red Mexican (868 g), along with breeding line USRM-20 (894 g) were all significantly greater than the overall mean. The four lowest yielding entries included heirlooms Calypso (413 g), Molasses Face (409 g), Maine Yellow Eye (370 g), and a new release Blush (383 g). Mean yield of the niche market/heirloom varieties (611 g) was slightly lower than the overall mean, while five out of the 11 niche market/heirloom varieties yielded above the overall mean (Figure 1). There was a significant difference among entries between 2004 and 2005.

Conclusions

The results from 2004 and 2005 indicate that the yield of the majority of heirlooms was below the overall mean yield of standard varieties, breeding lines, new releases, and plant introductions in this study. Yield is one aspect of niche market/heirloom varieties that could be improved through breeding, including screening and selecting for yield components such as number of pods per plant. Disease resistance/tolerance should also be investigated as not much is known regarding heirloom varieties. The variation among heirloom varieties in the two years of this study suggests that further studies are needed to better understand the yield potential of niche market/heirloom varieties.

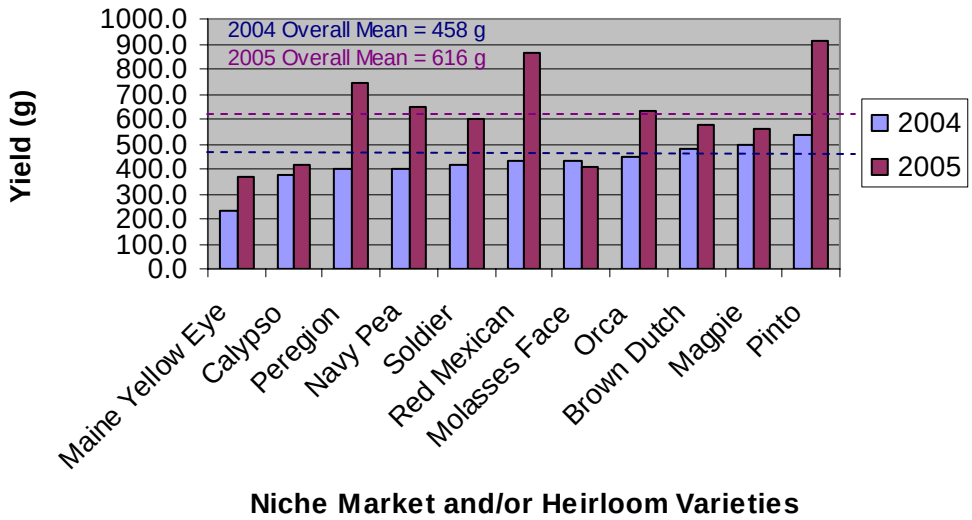

Figure 1. **Mean yield (g) of niche market and heirloom varieties grown in Vancouver, WA in 2004 and 2005.**

GENETIC DIVERSITY AMONG COMMON BEAN (*PHASEOLUS VULGARIS* L.) ACCESSIONS BASED ON RAPD MARKERS

Marco Antonio Aparecido Barelli, Maria Celeste Gonçalves-Vidigal, Cláudia Thomazella, Pedro Soares Vidigal Filho and Carlos Alberto Scapim
Departamento de Agronomia, Universidade Estadual de Maringá, Av. Colombo, 5790, 87020-900, Maringá, Paraná, Brasil

Introduction

Information about genetic divergence inside of the same species is essential for rational genetic sources (Loarce et al., 1996), beyond its importance for breeding programs success practically in all economical important characters (Ramalho et al., 1993). The characterization of genetic diversity among common bean accessions from Andean and Mesoamerican gene pools, using RAPD molecular markers, has been revealed to be efficient to group the different genotypes from these gene pools (Franco et al., 2001). The present study had the objective to evaluate the genetic divergence among 30 common bean traditional cultivars from Mato Grosso do Sul utilizing RAPD molecular marker.

Material and Methods

Thirty common bean accessions from Mato Grosso do Sul state (Germoplasm Bank of the Empresa Brasileira de Pesquisa Agropecuária - Embrapa), were analyzed for the genetic diversity with RAPD markers. The DNA extraction method followed the procedure of Afanador et al. (1993). Amplification reactions were performed similarly to that described by Young and Kelly (1996) using the following primers: OPA18, OPAA3, OPC08, OPF06, OPF10, OPG19, OPH20, OPI03, OPY20, OPZ04 (Operon Technologies, Alameda, Calif.). The amplification was carried out in a thermal cycle (MJ Research Inc., Waltham, MA). The genetic distance between the pairs was estimated based on the arithmetic complement of the Jaccard index (Cruz and Carneiro, 2003). The analyses were performed with the software Genes (Cruz, 2001).

Results and Discussion

A total of 97 polymorphic bands were amplified, which the sizes varied from 300 to 1850 pairs of bases. The results allowed to determinate the genetic distances among the accessions, providing a composition of four distinguished clustering (Figure 1). The first clustering was subdivided in three subgroups, in which one of them showed only accessions of Andean origin. The accessions Mulatinho Vagem Roxa B and Jalo were the most divergent (91.80 %), whereas Rosinha B and Rosinha Opaco demonstrated to be the most similar (18.67 %). The use of molecular markers to evaluate common bean cultivars gives precise information for genetic divergence analyses, in this case it provided the distinction of Andean and Mesoamerican genotypes, and allowed the identification of presence of RAPD molecular markers that are possibly linked to genes of resistance to anthracnose in 22 out of 30 accessions. The results indicated that accessions from Mato Grosso do Sul have wide genetic diversity based on RAPD markers.

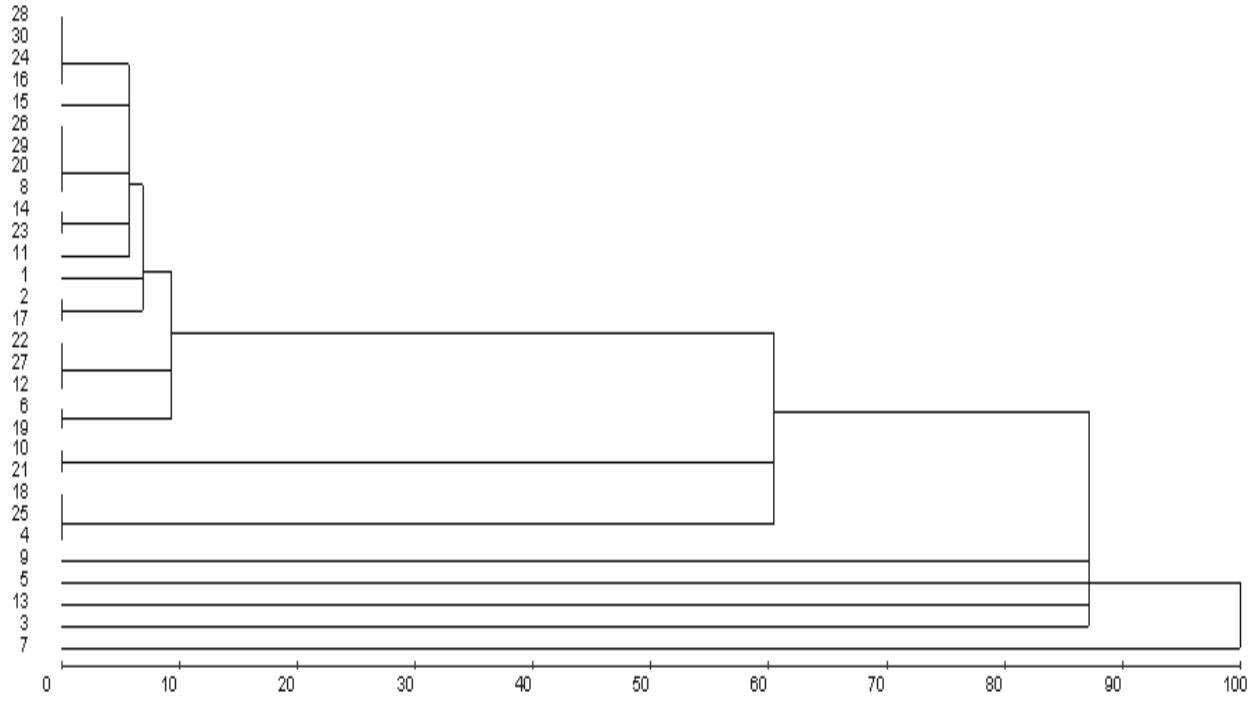

Figure 1. Dendrogram of genetic dissimilarity among 30 common bean accessions obtained through Nearest Neighbor clustering method. Identification of the accessions: 1, Bolinha, 2, Manteiguinha de Cipó; 3, Rosinha B; 4, Mulatinho Vagem Roxa B; 5, Rosinha Opaco; 6, Rosinha C; 7, Cara Suja; ,8, Manteigão; 9, Rosinha Guaicurus; 10, Rosinha D; 11, Manteiga; 12, Roxinho Mineiro; 13, Bico de Ouro B; 14, Manteiga com Cipó; 15, Rosinha sem Cipó; 16, Carioca com Cipó; 17, Preto Guamirim; 18, Manteiga sem Cipó; 19, Chita Bonita; 20, Bodoquena; 21, Carioca sem Cipó; 22, Jalo sem Cipó; 23, Carioca Novo; 24, Uberabinha Preto; 25, Manteiguinha; 26, Jalo; 27, Carioca Vagem Rosada; 28, Mulatinho Vagem Roxa A; 29, Bico de Ouro A; 30, Mulatão Lustroso.

Acknowledgements

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GENETIC DIVERGENCE IN COMMON BEAN (*PHASEOLUS VULGARIS* L.) LANDRACES FROM PARANÁ STATE

L. P. Bonett[1], M. C. Gonçalves-Vidigal[2], A. R. Schuelter[1], P. S. Vidigal Filho and J. P. Klasmann[1].

[1]Universidade Paranaense; [2]Departamento de Agronomia, Universidade Estadual de Maringá, Brazil.
E-mail: mcgvidigal@uem.br

Introduction

The common bean (*Phaseolus vulgaris* L.) is one of the most consumed crop in the world and its growth area is concentrated in Latin America, where is also registered the highest consumption. Brazil is the major producer and consumer of *Phaseolus vulgaris* L., and Paraná state is responsible for most of this production (25%). The landraces of common been have been showing wide genetic variability for seed color, shape, brightness, size (in general large seeded cultivars are preferred), among other characteristics of cultivars used by farmers. These cultivars have demonstrated adaptability to several environmental conditions, which can be observed through the resistance to diseases and elevated yield potential (Rodiño et al., 2003). Multivariate statistical methods have been used previously to analyze patterns of genetic diversity (Pereira et al., 1992). Due to its importance on Brazilian diet, the collection and evaluation of germplasm are very important to expand genetic basis of cultivars using genetic sources available. This work had the objective to quantify genetic variability in landraces cultivars collected in Paraná bean areas producer.

Material and Methods

The genetic divergence in 63 cultivars of common bean was evaluated using 11 morphoagronomic characters through multivariate statistics techniques. A randomized complete block design with four replications was used. The experimental unit was made up of four rows with 5 meters of length, spaced at 0.5 meters. The useful plot area consisted on two central rows, leaving a total of 5.0 m^2, with 48 plants. Genetic divergence was determined through multivariate techniques; the means were compared by the Scott-Knott's test, at 5% probability. The grouping method used was the Mahalanobis' generalized distance ($D_{ii'}^2$), and for clustering the Tocher's method and the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). The relative contribution of each trait to diversity was determined by the Singh's method (Singh, 1981). The analyses were carried out with the Genes Software (Cruz, 2001).

Results and Discussion

The results demonstrated that the first three canonic variables were the ones which contributed most for total variation of 82.88%, being them: plant height, number of days to flowering, and mean number of pod per plant. Using Tocher method, landraces and additional controls formed nine groups, being group I subdivided in seven subgroups (Figure 1). It was observed one subgroup composed mainly by cultivars of Carioca group. The UPMGA method clustered cultivars of different types, as Carioca and Jalo in distinguished groups. The most

divergent cultivars were Carioca Pitoco and Jalo vermelho, whereas the most similar were Carioca Pitoco and Carioca. In this study, the morphoagronomic characteristics proved to be efficient on discriminating landraces of common bean into Andean from Mesoamerican groups. These findings show the large genetic diversity of the Paraná landraces. Therefore, in the interpopulational selection is recommended to produce segregant populations from the crosses of Carioca and Jalo cultivars. These results evidenced the existence of genetic variability in bean cultivars used by farmers and multivariate analyses methods demonstrated efficiency to detect it separating in different groups the cultivars Carioca and Jalo. The cultivar Carnaval (33), Carioca Pitoco (16), Pérola (14) and Carnaval (27), due to the presence of highest productivity and being divergent, were indicated to generate populations in interpopulational selection programs.

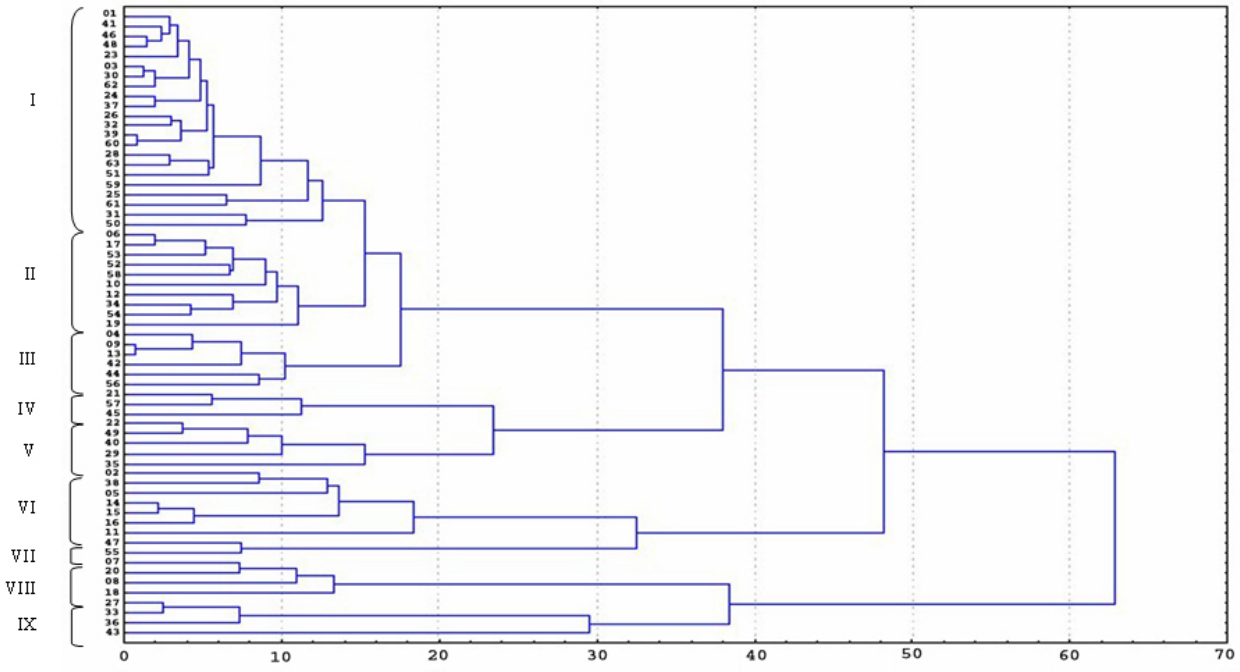

Figure 1. Dendrogram of the 58 landraces and five cultivars (controls) of common bean, based on UPGMA method, using $D^2_{ii'}$ as a measure of dissimilarity.

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EVIDENCE OF GENE FLOW AMONG BEAN SPECIES OF SECTION *PHASEOLI* IN COLOMBIA AND COSTA RICA USING MICROSATELLITE MARKERS.

R.I. González-Torres[1], M. Carvajal[1], O. Toro[1], M.C. Duque[2], R. Araya[3] & D.G. Debouck[1]
CIAT [1]Genetic Resources Unit, [2]Biotechnology Research Unit, AA 6713 Cali COLOMBIA;
[3]EEFBM Universidad de Costa Rica, Apartado postal 2645-300 Heredia COSTA RICA.

Gene flow events in common bean have been reported in several parts of the Americas where wild and cultivated forms are sympatric, often distant from a few to dozens of meters. After using microsatellite markers to successfully establish gene flow events in weedy forms and to indicate pollen direction from the wild into the cultivated and vice versa (González-Torres et al. 2004), we were interested in testing the hypothesis of participation of alien species into such a flow. Studies (Schmit et al. 1993; Llaca et al. 1994; Delgado-Salinas et al. 1999) using neutral molecular markers have shown that among the dozens of species described (Freytag & Debouck 2002) a small group of species including the common bean – section *Phaseoli* – share the same lineage. These species are sympatric with wild *P. vulgaris* in several mountainous areas of tropical America (Debouck 2000): *P. albescens* in western Mexico (Ramírez-Delgadillo & Delgado-Salinas 1999), *P. costaricensis* in central Costa Rica (Araya-Villalobos et al. 2001), and *P. dumosus* in several parts of Central America and the northern Andes (Schmit & Debouck 1991).

We tested microsatellites screened at 67 loci (Gaitán-Solís et al. 2002) to evaluate the level of participation of nuclear genes in six rare forms possibly resulting from interspecific hybridizations in natural conditions of Colombia (in contact with *P. dumosus*) and Costa Rica (with *P. costaricensis* and *P. dumosus*). The analysis involved these species as well as *P. coccineus* and *P. albescens* as controls (Table). The atypical materials were selected because of growth abnormalities often seen in artificial interspecific hybrids (shriveled seeds, ovule abortion, crippled plants) (Hucl & Scoles 1985).

CIAT Identification	Collector Identification	Species	Country	Department	Biological Status
G24765 (Pop9072)	OT-453	P. *x* vulgaris	Colombia	Boyacá	Weedy
G24666A (Pop9077)	OT-229	*P.* x *vulgaris*	Colombia	Cundinamarca	Weedy
FI7031 (S34124)	DGD-3149	*P.* x *vulgaris*	Costa Rica	Cartago	Weedy
FI7033 (S34124)	DGD-3149	P. *x* vulgaris	Costa Rica	Cartago	Weedy
FI7034 (S34124)	DGD-3149	P. *x* vulgaris	Costa Rica	Cartago	Weedy
FI7035 (S34124)	DGD-3149	P. *x* vulgaris	Costa Rica	Cartago	Weedy
S29699	DGD-2102	*P. costaricensis*	Costa Rica	San Jose	Wild
G36285 (Coc-1718)	DGD-3087	*P. coccineus*	Guatemala	Quezaltenango	Wild
G36290 (Coc-1440)	OT-811	*P. dumosus*	Colombia	Caldas	Cultivated
PL3592	ROL-141	*P. albescens*	Mexico	Jalisco	Wild
G23418 (FI6846)	DGD-2111	P. vulgaris	Costa Rica	Cartago	Wild

Data analysis was carried out considering each band as an allele, using multiple correspondence analysis (MCA), also in order to understand the population structure and individual dispersion. The resulting graphic representation allowed to locate the materials according to their genetic similarity in a multidimensional plane using CORRESP module of NTSYS v.2.10Y.

Results and Discussion

The microsatellites were powerful enough to separate the species though they belong to the same evolutionary phylum (Fig. 1a). The characterization of the different bean species through microsatellite *loci* evaluation according to Gaitán-Solís et al. (2002) is confirmed and extended to *P. albescens* and *P. costaricensis* for the first time (Fig. 1a, 1b). These molecular markers indicated that the putative natural

interspecific hybrids actually were hybrids (Fig. 1b, 1c). The data indicate that the evaluated hybrids result from gene flow between the common bean and *P. dumosus* (as pollen donor) in the Central Valley of Costa Rica as well as in Boyacá, Colombia (Fig. 1c). A different allelic frequency, namely of *P. dumosus*, suggests that the hybrids are of different generations (Fig. 1b). The cluster obtained using MCA established the hybrid group structure. This group is spatially near to *P. vulgaris* and *P. dumosus* indicating that the evaluated *loci* have been a recombination among these species by gene flow events. The natural interspecific hybrids are rare, and their reduced fertility might imply that the species of this *phylum* are "good biological species". They can be valued as natural genetic bridges in improvement programs (Singh 2001).

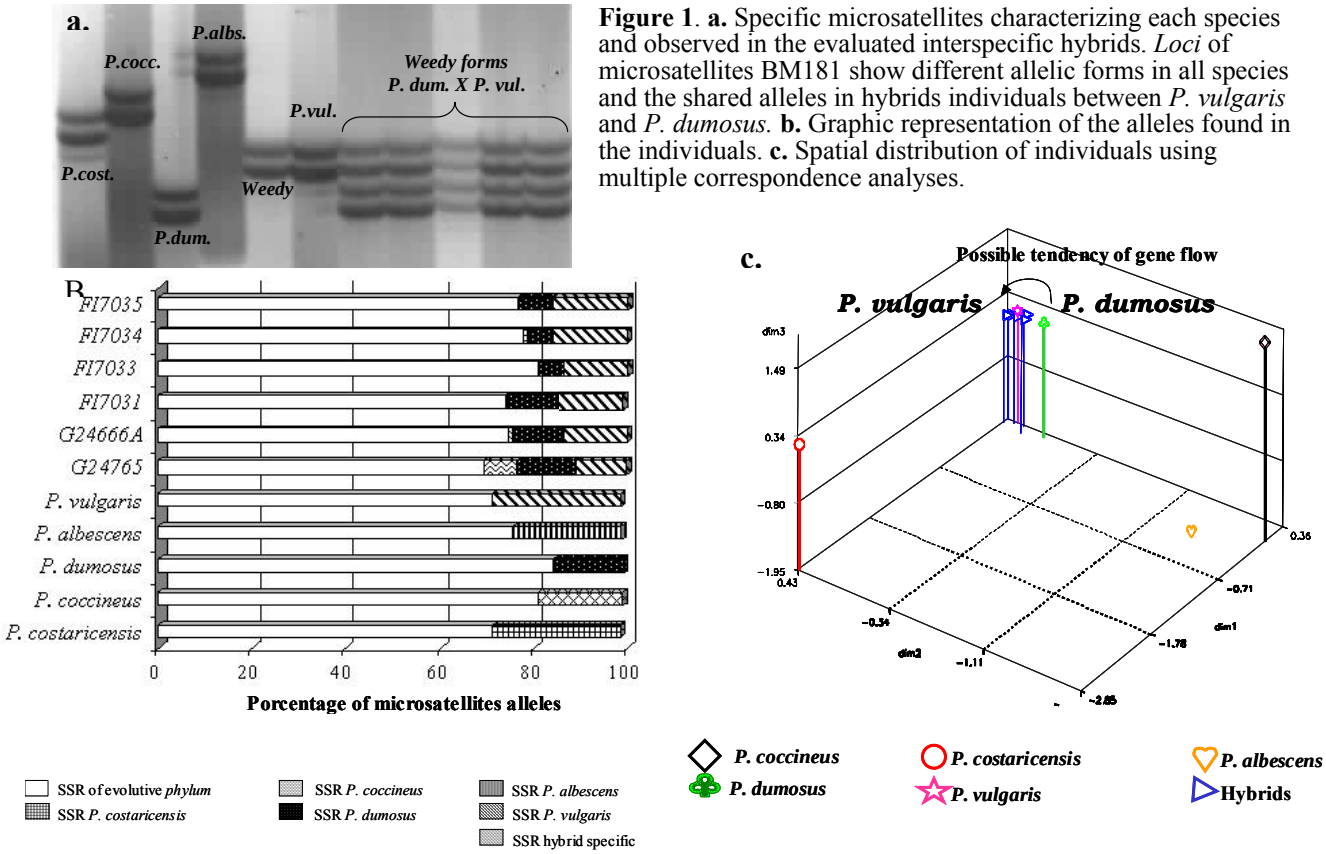

Figure 1. **a.** Specific microsatellites characterizing each species and observed in the evaluated interspecific hybrids. *Loci* of microsatellites BM181 show different allelic forms in all species and the shared alleles in hybrids individuals between *P. vulgaris* and *P. dumosus*. **b.** Graphic representation of the alleles found in the individuals. **c.** Spatial distribution of individuals using multiple correspondence analyses.

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FRIMEX: A COMMON BEAN DATA BASE WHICH INCLUDES OLD AND RECENT BRED MEXICAN CULTIVARS

Rigoberto Rosales-Serna[1], Juan Manuel Hernández-Casillas[1], Octavio Salvador Magaña-Torres[1] and Horacio González-Ramírez[2]

[1]INIFAP-Campo Experimental Valle de México. Apdo. Postal 10. C. P. 56230. Texcoco, Edo. de México. MÉXICO. e-mail: rigoberto_serna@yahoo.com; [2]INIFAP-Campo Experimental Valle de Guadiana. Apdo. Postal 186. C.P. 34000. Durango, Dgo. MÉXICO.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is a world important crop originated in the Americas. In México common bean breeding started in 1943, since then to 2005 the Bean Breeding Program of The Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (The National Research Institute For Forestry, Agriculture and Livestock; INIFAP) from México developed 144 common bean bred cultivars for all the Mexican production areas. Developed cultivars are diverse in growth habit, maturity and seed type, and some of them are actually used in breeding programs around the world. The knowledge of genetic diversity patterns can increase the efficiency for conservation, utilization and genetic improvement of common beans (Singh, 2001; Rosales *et al*., 2003). The development and availability of data bases will increase our knowledge about bean cultivars, improve the efficiency of breeding programs and contribute to increase common bean production.

The **FRIMEX** data base includes information on the common bean bred cultivars developed by INIFAP since 1940's. Information is available on plant traits, pedigree, breeding method, average yield, photoperiod response, market class and adaptation to different producing areas. The data is contained in sets as:

Cultivar description traits, list of cultivars released by INIFAP's breeding program, cultivars actually recommended by INIFAP and approved by the Sistema Nacional de Inspección y Certificación de Semillas (National Seed Inspection and Certification Service; SNICS) and crop management recommendations are also included.

Cultivar description traits: This set contains the description on the breeding methods, selection criteria, breeding working scheme and genealogy records plus nomenclature used by INIFAP's common bean breeding program. Information is also available for the year of release, ID number given by SNICS, agronomic and phenotypic plant atributes, commercial class and main and secondary recommendation areas.

Complete list of cultivars: Includes all the known cultivars developed by INIFAP's common bean breeding program trough the period 1943 to 2005. Cultivars are displayed in alphabetical order and specific information on pedigree and morpho-agronomic traits is provided and a magnifying image of the seed is available.

Recommended cultivars: In this section a map of México is displayed in which eight ecological regions considered by INIFAP can be observed. For each region a list of current cultivars recommended by INIFAP-SNICS is displayed and for each cultivar there is specific information for agronomic, phenotypic, commercial and developing traits.

Crop management recommendations: This section contains information about crop management practices. Information is available for soil preparation practices, planting density, fertilizer doses, solutions for pre and post planting problems, pests and weed control, harvest methods used in México and seed selection and grain storage.

Acknowledgements: A tribute is made for many Mexican and international breeders who contributed to common bean improvement in México. The contributions of individual researchers and institutions were considered in developing the data base. Breeders at international centers and universities, working along with their colleagues in Mexican national programs, produced new recombinant common bean genotypes that surpassed the yield of landraces by far. Modern common bean cultivars show disease resistance, shorter growth period, and better cooking and commercial quality (Acosta *et al*., 2002)

Accessing the data base

A Spanish version on CD of the **FRIMEX** data base is available at the main author's e-mail. **FRIMEX** data base will be available at INIFAP's web site http://www.inifap.gob.mx from July 2006 onwards and a English version will be prepared and released in September 2006, and it also will be available at the INIFAP's web site.

Data base implications

There is the expectation that this data base could contribute towards the conformation of an international data base (IBIS; International Bean Information System) which will enhance our knowledge on common bean germplasm around the world. Common bean breeding has contributed to improve human health and to alleviate the increasing hunger problems in the world.

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PREDICTING THE GENETIC POTENTIAL IN SEGREGATING POPULATIONS OF COMMON BEAN (*PHASEOLUS VULGARIS* L.)

Carlos Lásaro P. de Melo[1,2]; José Eustáquio de S. Carneiro[2]; Pedro Crescêncio S. Carneiro[3]; Lêlisângela C. da Silva[2]; José Eduardo V. Cintra[1,3]; Alisson C. Pereira[1,2]; Maurílio A. Moreira[1,4] and Everaldo G. Barros[1, 3]*

[1]Instituto de Biotecnologia Aplicada à Agropecuária (BIOAGRO), [2]Departamento de Fitotecnia; [3]Departamento de Biologia Geral; [4] Departamento de Bioquímica e Biologia Molecular. Universidade Federal de Viçosa (UFV), 36570-000, Viçosa, Minas Gerais, Brazil; *Corresponding author: *ebarros@ufv.br*

A number of studies have been conducted in order to evaluate the procedures for discarding undesirable populations already in the first breeding generations (Singh & Urrea, 1995; Oliveira et al., 1996; Abreu et al., 2002). One of these procedures (Jinks and Pooni, 1976) is based on the behavior of the bulk. This study was conducted to evaluate the genetic potential in 29 segregating populations of common bean, based on the average and variance of the F_3 generation, in order to select the most promising lines.

The method by Jinks and Pooni (1976) estimates the probability that one population will originate lines that would overcome a certain control line. This probability would correspond to the area to the right of a given value *x* in the abscissa of the normal distribution curve. Twenty-nine bean segregating populations from crosses between "carioca-type" cultivars and the "carioca-type" line Rudá-R (containing a pyramid of genes for resistance to anthracnose, rust and angular leaf spot) were evaluated in the F_3 generation. Three "carioca-type" cultivars, were used as controls. The experiment was conducted in Coimbra County, Minas Gerais state, in 2005, using a triple lattice (6x6) with plots consisting of four rows with 5m long each. Grain yield was determined for each plot and plant. Based on the methodology by Jinks and Pooni (1976), the probability for each population to originate lines superior than Talismã (control) was estimated as follows: $Z_i = \left(\bar{L} - \bar{F}_{3i} \right) \Big/ \sqrt{1.33\sigma^2_{P_{3i}} - 0.33\sigma^2_{E_i}}$; where $\sigma^2_{P_{3i}}$ is the phenotypic variance and σ^2_{Ei} is the environmental variance. σ^2_{Ei} was obtained by the average of the phenotypic variances of the cultivars Talismã, Pérola and Rudá-R which was the same for all populations.

The population showing the highest probability for producing superior lines was OPS-82 x Rudá-R; the worst one was FT-Bonito x Rudá-R (Table 1). According to the results, the line OPS-82 showed the best genetic complementation with the line Rudá-R. Although the population Carioca 1070 x Rudá-R presented the highest phenotypic variance, it was classified as the third better population. This might have been partially due to an average with intermediate value, compared to other populations. Cultivars Pérola and Talismã are among the main "carioca-type" cultivars grown in Brazil. However, populations generated from crosses between these cultivars and Rudá-R yielded low averages and variances. The methodology by Jinks and Pooni (1976) provides an excellent alternative for selecting segregating populations of autogamous species, as it allows the discarding of undesirable populations in the first breeding generations. In this work it was possible to obtain twelve populations with high averages and satisfactory genetic variability, therefore with high potential to produce lines that would be superior to cv. Talismã (Table 1). Five of these populations were selected to be used in the BIOAGRO bean breeding program which uses molecular markers to develop cultivars with gene pyramids for resistance to anthracnose, rust and angular leaf spot.

Table 1. Average yield in the F_3 generation ($\overline{F}_{3i}$), in g/plant, estimates of phenotypic variance (σ^2_{pi}), genetic variance (σ^2_{Gi}), and probability (PST) for obtaining lines superior to bean cultivar Talismã.

Crossings and controls*	$\overline{F}_{3i}$	σ^2_{pi}	σ^2_{Gi}	PST (%)[1/]
OPS-82 x Rudá-R	14.2	99.30	38.43	46.41
UTF 0013 x Rudá-R	13.8	90.49	29.62	44.43
Carioca 1070 x Rudá-R	13.5	105.41	44.54	43.64
CNFC 8017 x Rudá-R	13.5	85.74	24.87	43.25
LP 98-20 x Rudá-R	13.7	76.32	15.45	42.47
CNFC 9437 x Rudá-R	13.0	92.84	31.97	41.68
UTF 0030 x Rudá-R	13.1	88.65	27.78	41.68
GEN 12-2 x Rudá-R	13.1	82.32	21.45	41.29
GEN 12 x Rudá-R	13.3	74.16	13.29	41.29
VC 5 x Rudá-R	13.3	72.11	11.24	41.29
UTF 0037 x Rudá-R	13.7	68.64	7.77	40.52
LH-11 x Rudá-R	13.0	74.41	13.54	40.13
UTFB 0018 x Rudá-R	12.3	103.75	42.88	39.36
Pérola x Rudá-R	13.2	58.00	-2.87	39.36
CNFC 9500 x Rudá-R	12.5	90.53	29.66	39.36
LP 98-76 x Rudá-R	12.4	86.89	26.02	38.97
Vi 4899 x Rudá-R	13.3	48.06	-12.81	38.97
LP 98-31 x Rudá-R	12.8	65.98	5.11	38.59
UTF 0019 x Rudá-R	12.6	71.98	11.11	38.59
Talismã x Rudá-R	13.3	44.58	-16.29	38.59
VC 2 x Rudá-R	12.9	59.19	-1.68	38.21
Vi 0699 x Rudá-R	12.3	75.97	15.10	37.45
UTF 0031 x Rudá-R	12.7	55.16	-5.71	37.07
UTFB 0022 x Rudá-R	11.4	111.03	50.16	36.69
VC 4 x Rudá-R	11.4	89.43	28.56	35.19
IAPAR 81 x Rudá-R	11.7	70.15	9.28	34.46
Vi 4599 x Rudá-R	12.6	44.34	-16.53	34.09
UTF 0029 x Rudá-R	12.1	48.99	-11.88	32.27
FT-Bonito x Rudá-R	10.9	45.59	-15.28	25.46
Rudá-R*	12.0	58.81	-2.06	33.72
Talismã*	12.6	58.75	-2.12	36.69
Pérola*	10.9	65.05	4.18	29.81

[1/] Probablility for obtaining lines superior in yield in relation to cv.Talismã.

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RELATIVE HETEROSIS IN COMMON BEAN (PHASEOLUS VULGARIS L.) GENOTYPES

Lucas Silvério, M. C. Gonçalves-Vidigal, P.S. Vidigal Filho, C. A. Bastos Andrade and J. P. Poletine
Departamento de Agronomia, Universidade Estadual de Maringá, Brazil. E-mail: mcgvidigal@uem.br

Introduction

The genetic variability of Brazilian germplasm is very large due to the environmental conditions of cultivation, the innumerable plant introductions made and because it was maintained by the diversity of consumer and farmer preferences as well. This large variability is essential for the success of breeding programs for practically all the important economically characteristics (Ramalho et al., 1993). Greater bean yield could be obtained through hybridizing superior cultivars. The diallel cross is a viable alternative because it allows a wide recombination of the genomes with greater chances of generating superior cultivars in segregant generations (Ayele, 1994; Cruz et al., 2004; Barelli et al., 2000). Therefore, the objective was to estimate the relative heterosis among six common bean cultivars to assess the general and specific combining abilities and discriminate the superior parents and hybrid combinations, as an initial step in developing a breeding program for the species to increase yield.

Material and Methods

The cultivars BRSMG-Talismã, IPR Uirapuru, FT Soberano, BRS Campeiro, IAC Tibatã and IPR Juriti were crossed to obtain a complete diallel without reciprocals. The populations (six parents and 15 F_1's) in a total of 21 treatments were evaluated in 2003 at the experimental area of the Empresa de Pesquisa de Santa Catarina, Brazil. A randomized complete block with three replications was used and the experimental unit was made up of two rows of plants spaced of 0.5 m and with 4.0 m of length, totaling a useful plot area of 3.0 m^2. Six morphoagronomic characteristics were evaluated according to Griffing's (1956), Method 2 (parents and F_1 hybrids) Model 1 (fixed effects). The percentage of heterosis was obtained in a relation from parents, for each characteristic evaluated.

Results and Discussion

The Table 1 shows that for yield grain characteristic all combinations presented values of positive relative heterosis (H_{MP}). The positive values of specific combining ability effects observed for the characteristic number of seeds per plant (NSP) demonstrated the existence of genetic divergence among parents. This fact justifies high magnitude heterotic value (H_{MP}) for combinations 'BRS Campeiro x IPR Juriti' (48.65), being combination of 'IPR Uirapuru x FT Soberano' also positive sign. In relation to characteristic of mean weight of 100 seeds (WS_{100}), it was observed that for the whole combinations (Table 1), high magnitudes and positive heterotic values (H_{MP}) except 'BRS Campeiro x IPR Juriti'. The hybrids composed through crossing of 'IPR Uirapuru x IAC Tibatã' (138.94) and 'BRSMG-Talismã x IAC Tibatã' (98.35) pointed out among the others. The characteristic yield grain demonstrated heterotic positive values obtained

among all combinations, presenting mean productivity superior to all parents. The crosses, 'IPR Uirapuru x IAC Tibatã' and 'IPR Uirapuru x FT Soberano' gave the best hybrids, exhibiting very high heterosis for yield as well as significant specific combining ability effects, capable of giving maximum transgressive effects. Therefore, these hybrids are recommended in the choice of superior genotypes.

Table 1 - Mean[1] and percentage heterosis in relation to parent mean (H_{MP}), for six characteristics[2] evaluated in a diallel combination, among six common bean parents, 2004

Genotypes	ALTP		TNPP		NSP		MNSP		WS_{100}		MYG	
	Mean	H_{MP} (%)	Mean	H_{MP} (%)	Mean	H_{MP}(%)	Mean	H_{MP} (%)	Mean	H_{MP} (%)	Mean	H_{MP} (%)
1	0.83	-	14.59	-	59.50	-	4.24	-	8.92	-	1,375.00	-
2	0.67	-	17.50	-	79.33	-	4.54	-	5.44	-	1,137.04	-
3	0.68	-	13.73	-	57.5	-	4.18	-	9.75	-	1,466.67	-
4	0.65	-	14.51	-	54.87	-	3.79	-	11.12	-	1,573.15	-
5	0.81	-	15.47	-	67.5	-	4.34	-	5.23	-	931.48	-
6	0.71	-	16.33	-	56.53	-	3.49	-	9.54	-	1,431.48	-
1x2	0.74	-1.77	13.73	-14.39	54.01	-22.18	3.94	-10.14	11.99	67.03	1,722.22	37.18
1x3	0.78	3.30	11.78	-16.78	50.31	-14.00	4.31	2.33	12.91	38.32	1,728.70	24.67
1x4	0.71	-3.58	11.10	-23.72	46.14	-19.30	4.19	4.27	15.00	49.70	1,814.85	23.12
1x5	0.97	17.81	12.24	-18.54	42.77	-32.65	3.49	-18.57	14.03	98.35	1,600.00	38.72
1x6	0.75	-3.22	13.43	-13.15	47.94	-17.36	3.57	-7.58	13.06	41.43	1,680.56	19.76
2x3	0.74	9.09	15.54	0.47	77.87	13.81	5.02	15.34	9.41	23.98	1,955.56	50.22
2x4	0.62	-7.03	13.79	-13.82	62.15	-7.37	4.50	8.08	10.14	22.40	1,676.85	23.74
2x5	0.72	-2.23	11.88	-27.87	52.76	-28.13	4.44	0.04	12.75	138.94	1,787.04	72.78
2x6	0.85	23.26	13.86	-18.09	62.64	-7.79	4.52	12.58	8.69	16.06	1,409.26	9.73
3x4	0.73	9.23	11.63	-17.67	52.80	-6.12	4.53	123.76	12.68	21.53	1,792.59	17.94
3x5	0.78	4.23	11.23	-23.06	54.76	-12.38	4.88	14.55	9.64	28.72	1,401.85	16.91
3x6	0.78	11.90	11.46	-23.79	50.04	-12.23	4.37	13.90	11.02	14.22	1,803.70	24.47
4x 5	0.66	-9.54	12.70	-15.26	82.80	-17.17	3.99	-1.93	13.12	60.42	1,759.26	40.48
4x6	0.72	5.11	19.59	27.07	48.94	48.65	4.24	16.34	8.79	-14.92	1,941.67	29.24
5x6	0.77	0.65	12.85	-19.18	53.09	-21.08	3.79	-3.15	11.73	58.77	1,431.48	28.76

[1/] 1 = BRSMG-Talismã; 2 = IPR Uirapuru; 3 = FT Soberano; 4 = BRS Campeiro; 5 = IAC Tibatã; 6 = IPR Juriti;
[2/]ALTP = mean final plant height; TNPP = total number of pods per plant; NSP = number of seeds per plant; MNSP = mean number of seeds per pod; WS_{100} = mean weight of 100 seeds, and MYG = mean yield of grains.

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DNA SEQUENCE POLYMORPHISMS AMONG COMMON BEAN GENES

Melody McConnell, Sujan Mamidi, Rian Lee, and Phillip McClean
North Dakota State University, Fargo, ND 58105

Abstract

Limited genomic resources exist for *Phaseolus vulgaris* L. (common dry bean). By making use of over 2600 *P. vulgaris* TC (Tentative Consensus) sequences recently generated by Ramirez et al. (2005), we have expanded the genomic resources of common bean by gene-by-gene sequencing. For sequencing, *P. vulgaris* TCs were chosen that were homologous to 1) *Arabidopsis* genes in which the mutant displays an obvious phenotype; 2) maize genes that were under selection during domestication: 3) plant genes that are involved in vital biochemical pathways; and 4) other random genes. Sequence data for 322 genes was collected from BAT93 and Jalo EEP558. We have analyzed these polymorphisms in regards to their type, location, and distribution. In total, over 260 kilobases of DNA sequence data was collected from the two genotypes. 1003 polymorphisms were discovered, 858 SNPs and 145 indels. 70% of the genes with BAT93 and Jalo EEP558 data were polymorphic. This data will be useful for the development of a gene-based linkage map for common bean that will have applications for molecular breeding and diversity and evolutionary analyses.

Materials and Methods

Genes were selected based on similarity in a BLAST search. TC sequences (Ramirez et al 2005) were used as a query for an all-against-all blastp analysis against individual databases containing *A. thaliana* genes with mutant phenotypes (Meinke et al 2003), genes under selection during domestication in maize (Wright et al 2005), *Arabidopsis* genes involved in biochemical pathways, and all *Arabidopsis* genes. A gene was selected for sequencing if had at least 100 nucleotides in the 3' UTR and an E-value of <e-30 with the top hit. Primers were designed with Primer3 (Rozen and Skaletsky 2000) with a target TC fragment size of 450-500 nucleotides, primer size of 18-28 nucleotides, and T_m of all primers about 58^{o}C. The 3' primer was targeted to a location 150 nt downstream of the putative stop codon. Fragments were amplified from BAT93 and Jalo EEP558 genomic DNA and directly sequenced.

Results and Discussion

This report provides data from an on-going project to uncover diversity levels in common bean. Here we report data from a DNA sequence comparison of BAT93 and Jalo EEP558, the parents of the community-wide mapping population. Our blastp analysis found that 81% of the 2686 TC sequences were homologous to an *Arabidopsis* gene at an e-value of <e-30, and 65% had sufficient UTR sequence for our analytical purposes. Of the 852 genes analyzed to date, DNA sequence data for the two genotypes were obtained for 322. Of these, 222 genes were polymorphic, and 100 were monomorphic. A total of 1003 polymorphisms were detected, and of these 85.5% were SNPs. On average, one SNP was detected every 151 nt, and one indel was observed every 897 nt. 44.1% of the polymorphisms were located in introns, 38.7% in exons,

and 17.0% in the 3' UTR. SNPs were evenly distributed between introns and exons, whereas indels were largely found with introns. In regards to SNPs, the number of transitions and transversions was roughly equal, and transitions were evenly distributed between exonic and non-exonic regions, while about 63% of transversions were present in non-exonic regions. 37.4% of the SNPs present in exons were non-synonymous substitutions. The polymorphism data is summarized in Table 1.

Table 1. Polymorphism statistics between BAT93 and Jalo EEP558 among 222 polymorphic common bean (*Phaseolus vulgaris* L.) genes. The average number of SNPs and indels per gene are calculated based on the 322 genes.

Data	Value
Number of polymorphisms	1003
Polymorphism frequency (nucleotides/polymorphism)	129.6
Number of SNPs	858
Number of genes containing SNPs	214
SNPs per gene	2.7
SNP frequency (nucleotides/SNP)	151.5
Number of indels	145
Number of genes containing indels (excluding 1 nt indels)	56
Number of indels per gene	0.25
Indel frequency (nucleotides/indel)	896.6
Percentage of polymorphisms in introns	44.1
Percentage of polymorphisms in exons	38.8
Percentage of polymorphisms in UTR	17.0
Percentage of SNPs in introns	39.6
Percentage of SNPs in exons	43.3
Percentage of SNPs in UTR	17.0
Percentage of indels in introns	76.0
Percentage of indels in exons	9.3
Percentage of indels in UTR	14.7
Percentage of nonsynonymous SNPs	37.4
Percentage of synonymous SNPs	62.6
Percentage of nonsynonymous SNPs that are transitions	43.0
Percentage of nonsynonymous SNPs that are transversions	57.0
Percentage of synonymous SNPs that are transitions	63.8
Percentage of synonymous SNPs that are transversions	36.2

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SERINE PROTEINASE INHIBITORS IN COMMON BEAN (*PHASEOLUS VULGARIS* L.): PRELIMINARY INVESTIGATION ON GENOME ORGANISATION

Incoronata Galasso[1], Angela R. Piergiovanni[2], Lucia Lioi[2], Bruno Campion[3], Francesca Sparvoli[1] and Roberto Bollini[1]

[1] Istituto di Biologia e Biotecnologia Agraria, IBBA-CNR, Milano (Italy)
[2] Istituto di Genetica Vegetale, IGV-CNR, Bari (Italy)
[3] Istituto Sperimentale per l'Orticoltura, CRA, Monatanaso Lombardo, Lodi (Italy)

The Bowman-Birk inhibitors (BBI) are double-headed serine proteinase inhibitors with a low molecular mass (8–9 kDa) and a high cysteine content. The great interest on BBIs is due to the several roles attributed to them: reduction of protein digestibility, storage of sulphur amino acids in plant tissues, active role against pest and diseases (Ryan 1990) and effectiveness in preventing or suppressing carcinogenic processes in both *in vitro* and *in vivo* models (Kennedy 1998).
The control of antitryptic activity is very important since the selection for low activity is considered a desirable trait in breeding programmes aimed to increase the nutritional value of animal feed. In seeds of common bean, quantitative variation in trypsin inhibitor (TI) activity has been well documented. Indeed, an extensive study involving several genotypes belonging to the Mesoamerican and Andean gene-pools, grew for three years in the same environment, revealed a wide variation in seed TI content from 19.5 to 25.4 TIU/mg dry matter (Piergiovanni and Pignone 2003). In addition, it has been demonstrated that drought and thermal stress during the vegetative growth stage may favour an increase of TI expression. However, the extent of TI increase appears to be genotype dependent. On the light of this, it is very important to understand and define the relationship between the protease inhibitory activity and the allelic variation of genes encoding for such inhibitors.
Recently, we evaluated the seed trypsin inhibitor activity in a number of bean genotypes and isolated the corresponding genes by PCR on genomic DNA (Piergiovanni and Galasso, 2004). Preliminary analysis on the primary structure of the genes isolated from several cultivated and wild genotypes (showing high and low antitryptic activity) evidenced that polymorphisms were present both outside and within the enzyme binding loops, but apparently no relationship could be established between these polymorphisms and the quantitative variation of the inhibitory activity. Most likely, as demonstrated in pea (*Pisum sativum* L), also in bean a combinatorial variation in coding and promoter sequences of trypsin inhibitor genes could be associated to the variation of the inhibitory activity (Page et al., 2004). In order, to explore this hypothesis we started with the isolation and sequencing of the promoter region of trypsin inhibitor, using our BAC library from *Phaseolus vulgaris* accession G12949, (CIAT, Cali, Colombia). To identify all BAC clones containing BBI genes, the entire library (consisting of 30,720 clones), was gridded onto two high-density filters double-spotted in a 4 x 4 array and probed with a previously isolated trypsin inhibitor gene (Piergiovanni and Galasso, 2004). After filter hybridisation a second screening was carried out by PCR, using specific BBI-primers. Three positive BAC clones were identified after filter hybridisation and PCR. Southern profiles obtained after digestion with selective restriction enzymes of positive BAC clones probed with labelled-BBI, suggest a complex genome organisation. Results confirmed that BBI are coded by a multigene family constituted by at least three types of double-headed inhibitors.

The following combinations of active sites were found: Trypsin/Trypsin, Trypsin/Chymotrypsin and Elastase/Trypsin. Two of them (Trypsin/Chymotrypsin and Elastase/Trypsin) appear to be located at the same locus, since they were found in a single BAC clone of about 130 Kbp. An *Eco*RI digestion of this BAC clone was then used to get the upstream region of BBI genes. In order to do this, all positive *Eco*RI fragments in the range of 3-4 Kbp were cloned and sequenced and putative promoter regions have been identified and are currently under analysis.
The following step of this study will be the design of specific PCR primers in the upstream and downstream regions of the BBI, to explore and compare both coding and promoter regions in genotypes showing low and high antitryptic activity.

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TOWARD PRODUCTION OF GENETICALLY MODIFIED COMMON BEAN VIA AGROBACTERIUM-MEDIATED TRANSFORMATION

Mohamed F. Mohamed [1,2], Jun Cao[1] and Elizabeth D. Earle[1]
[1]Department of Plant Breeding and Genetics, Cornell University, Ithaca, NY 14853; [2]Department of Horticulture, Assiut University, Assiut 71526, Egypt. E-mail:mofouad@yahoo.com

Abstract: An attempt was undertaken to transfer a synthetic *Bacillus thuringiensis (Bt) cry1C* gene (controlling Lepidopteran insect pests) to common bean (*Phaseolus vulgaris* L.) 'Xan-159' via *Agrobacterium tumefaciens*-mediated transformation of cotyledonary explants. Hygromycin resistance served as the selective marker. Shoots were regenerated either via direct organogenesis or through intermediate meristematic callus. Putative transformed shoot initials (1-2 mm) exhibited resistance to hygromycin (10 mg/l) level that killed all the control cultures. The detected amount of Cry1C proteins in tissues of bulked groups of these shoots ranged from 10.2 to 79.5 ng *Bt* protein/mg total soluble protein. Although they remained green for more than 2 months, these shoots failed to elongate and establish plants. It is suggested that *Agrobacterium*-mediated transformation of common bean is attainable but requires technical modification to allow shoot development from minimal tissues.

INTRODUCTION

Agrobacterium-mediated transformation is considered the best method available to produce transgenic plants providing that the target species is susceptible to *Agrobacterium* and recipient tissues are competent. Susceptibility of common bean to *Agrobacterium* has been shown (McClean et al., 1988). Current tissue culture methodology allows production of common bean plants directly from cultures of excised meristems and adventitiously via organogenesis in primary explants (cotyledons, seedling nodes and embryonic axes) and derived meristematic callus (Mohamed, 1997). However, there are still major difficulties in producing stable transgenic bean cultivars (Mohamed, 1997). In contrast, stable transformation of tepary bean (*P. acutifolius* A. Gray), a close relative of common bean, has been demonstrated (Dillen et al., 1997). Capability to produce transgenic tepary may be due to its ability to proliferate shoots from minimal differentiating tissues (Mohamed, 1997; Mohamed et al., 1993). This study was conducted to test this factor as limiting the development of transgenic common bean.

MATERIALS AND METHODS

Plant material and explant preparation: Surface sterilized seeds (common bean line 'Xan-159') were placed on germination medium (GM) [Gamborg's B5 (Gamborg et al., 1968), pH 5.5, 3% sucrose and 6 g/l Phytagar (GibcoBRL)] in Magenta boxes. Five days after incubation under 16/8 h (light/dark) photoperiod, cotyledonary nodes (CN) with 2-3 mm cotyledons attached were dissected. Preexisting axillary shoots-buds and surrounding meristematic tissues were gently interrupted using a needle.

Transformation vector and co-cultivation: Transformation was performed using *Agrobacterium tumefaciens* strain ABI (*A.t.)* with the binary vector pNS6 (Fig. 1, Strizhov et al. 1996) at concentrations of 0.6-0.8 at OD600. Wounded CN meristematic areas were individually inoculated using a needle dipped in *A.t* suspension. Control explants were treated without *A.t.* Explants were incubated in light for 2, 3 or 4 d. Co-cultivation medium was GM medium with 200 mg/l ascorbic acid (ASA) added.

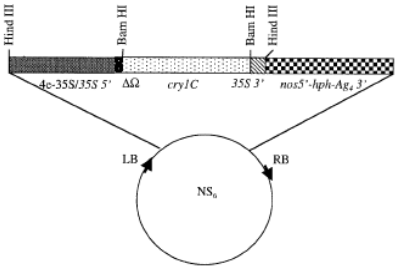

Fig. 1 The binary vector pNS6.

Selection procedure and Bt protein assays: After co-cultivation, explants were washed twice in B5 liquid medium containing 300 mg/l Timentin (SmithKline Beecham) and blotted dry. They were then placed on co-cultivation medium plus 300 mg/l Timentin and either Benzyladenine (BA, 0.5 mg/l) for

shoot induction or thidiazuron (TDZ, 0.1 mg/l)/Indole-3-acetic acid (IAA, 0.05 mg/l) for meristematic callus induction. One week later, cultures were transferred onto selection medium that additionally contained 10 mg/l hygromycin (selective agent). One set of control explants was continuously grown on medium without hygromycin while the other was grown on hygromycin selection medium. After 2 weeks, both *A. tumefaciens*- treated and control explants grown on selection medium containing BA (0.5 mg/l) were subcultured on fresh selection medium with 0.1 mg/l BA for shoot proliferation. Subsequently these cultures were transferred onto the respective fresh medium at 2-3 week intervals. Those on selection medium with TDZ (0.1mg/l)/IAA (0.05 mg/l) were transferred to fresh medium for meristematic callus proliferation. After two subcultures, callus was placed on medium with 0.1 mg/l BA. Subsequently all cultures developing shoots were transferred regularly every 2-3 weeks to fresh selection medium lacking BA for shoot elongation. Cry1C proteins produced were monitored by ELISA using kits from EnviroLogix Inc. (Portland, ME.). Proteins from untransformed explants were used as the control.

RESULTS AND DISCUSSION

All control cultures on selection medium with 10 mg/l hygromycin died in 2-3 weeks. Controls on medium without hygromycin developed shoots either from primary explants (70%) on BA-containing medium or from induced meristematic callus (80%) when transferred to BA-medium. Explants co-incubated with *Agrobacterium* survived and differentiated (50%-60%) shoot initials (1-2 mm long, Fig. 2) on hygromycin selection medium. Although these cultures contained green shoots for several subcultures, the shoots did not elongate further. However, ELISA assays of extracts from bulk shoots per explant ranged from 10.2 to 79.5 ng *Bt* protein/mg total soluble protein. Bulk tissues were used due to limited shoot growth, and higher protein may have been present in some individual shoots. It was observed that when similar size shoot initials of control cultures were separated and placed individually on medium lacking hygromycin, 90% of them failed to elongate. However, when control cultures were kept intact, at least one shoot elongated in each culture. The mass of explant tissue surrounding the shoot may enable increased nutrient supply to the shoot initials, which enhanced their growth and elongation. Shoot initials of *Agrobacterium*-treated explants grown on BA selective medium was surrounded by brown dead tissues. Callus induced on TDZ/IAA selective medium was nodular but friable and readily dissociated during subculture giving small separate pieces with shoot initials. Modifications in nutrient supplements in the medium to support growth of minimal sized shoots may be useful to develop excisable transgenic shoots.

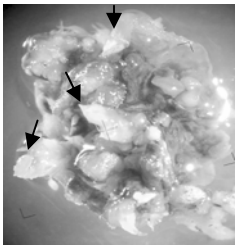

Fig. 2. Putative transformed shoot initials developed from 'Xan-159' common bean primary nodal explant derived meristematic callus.

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SCREENING *PHASEOLUS VULGARIS* L. EMS MUTANTS TO ISOLATE PLANTS FAILING IN SEED DEVELOPMENT AND TO STUDY GENETICS OF EMBRYOGENESIS

S. Silué[1], P. Lariguet[2], C. Pankhurst[2], J.M. Jacquemin[3], W. J. Broughton[2] & J. P. Baudoin[1]

[1]Faculté des Sciences Agronomiques de Gembloux, Unité de Phytotechnie Tropicale et d'Horticulture. 2 passage des Déportés, BE-5030 Gembloux, BELGIQUE.

[2]LBMPS, Université de Genève. 30 quai Ernest-Ansermet, 1211 Genève 4, SUISSE.

[3]CWRA, Chaussée de Charleroi, 234 BE-5030 Gembloux, BELGIQUE.

Introduction

In the genus *Phaseolus*, 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 abortion of immature embryos, mainly at the globular stage, particularly when the donor parents are used as female (Baudoin *et al.*, 2004). These abortions can be caused by the disruption of major genes involved in embryogenesis process, as it is shown in studies using model plants embryogenesis (Devic, 1995; Elster *et al.*, 2000). In order to study genes implied in *P. vulgaris* embryogenesis, an ethyl methane sulphonate (EMS)-induced mutant collection of common bean (Pankhurst & Broughton, 2004) was screened to isolate plants which failed in seed development.

Materials and Methods

Forty grammes of BAT93 seeds were treated with 200 ml of 30mM EMS overnight (approximately 16 hours) at the room temperature with slow shaking. Seeds were rinsed with sterilized water and sown. M1 (first generation) and M2 (second generation) mutants were screened for changes in seed development. Crosses were attempted between interested mutants (i.e. defective plants in seed development) and the original variety BAT93, in order to estimate the genetic determinism of these mutations in progenies.

Results and discussion

M2 mutant seeds were harvested from M1 plants divided into two groups: one which produced mainly empty pods and a second group which produced mature pods containing a high number of degenerated seeds. In total, 343 M2 mutants were tested from 62 lines. Seeds of four plants belonging to a first line (490) failed to develop normally while seeds of two mutants from a second line (522) aborted within 15 to 25 days after anthesis. Embryos of this line failed to grow normally and showed abnormalities at the two heart and cotyledon developmental stages (Figure 1). Abnormalities appeared in the suspensor and the cotyledons. The suspensor plays a role in pushing the embryo into the endosperm to facilitate its nutrition. Therefore defects in the suspensor can disrupt embryo nutrition and induce their abortion (Lecomte, 1997).

Crosses between these two types of mutants and the original variety were carried out to estimate the genetic transmission of the selected mutants in F1 and F2 progenies. F1 seeds were obtained when the original variety BAT93 was used as female parent in the crosses with the two

mutants. In reciprocal crosses, F1 mature seeds were obtained with the mutant line M2490, while crosses with line M2522 as female parent lead to the abortion of immature embryos within 15 to 25 days after pollination. All supposed F1 hybrid mature seeds were sown for phenotypal screening among F2 plants.

Simultaneously with this investigation, we study through semi-quantitative RT-PCR the expression of some major genes (i.e. Lipid Transfer Protein, Heat Shock Protein, KNOX, Leucine Zipper genes, etc.) involved in model plants embryogenesis, during the BAT93 wild-type seed development . This study will be extended to other genes (MONOPTEROS, GNOM, KNOLLE, TWN1, GURKE, etc.) known to be involved in plant embryogenesis. The expression of such genes in the control material (BAT93) will be compared with that observed during the

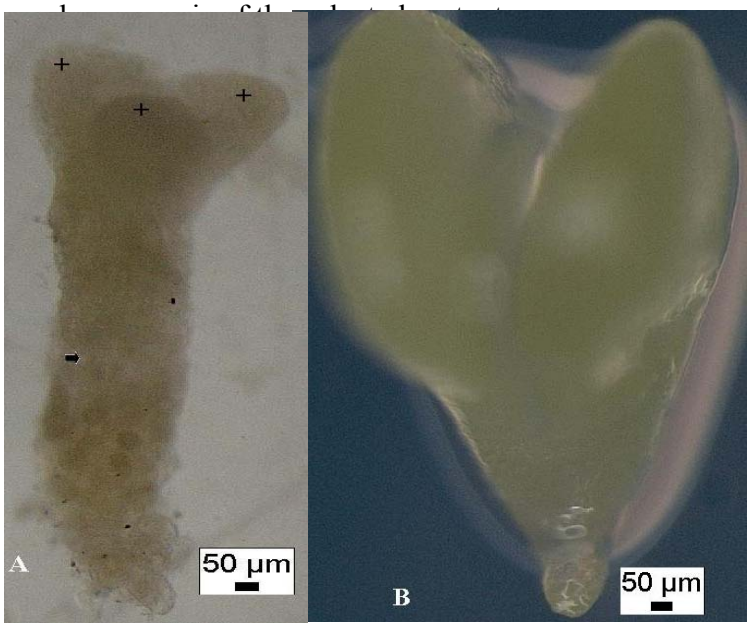

Figure 1. *Phaseolus vulgaris* embryos extracted from ovules 20 days after anthesis.
A: Defective embryo from mutant line 522 with abnormal suspensor (➡) and three cotyledons (+) instead of two. **B**: Wild-type embryo with normal cotyledons.

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TANNIN CONTENT OF COMMERCIAL CLASSES OF COMMON BEAN.

M.W. Blair, G. V. Caldas, P. Avila, C. Lascano

CIAT – INTERNATIONAL CENTER FOR TROPICAL AGRICULTURE - A. A. 6713, CALI, COLOMBIA

Introduction: Common bean seed (*Phaseolus vulgaris* L.) is a significant source of protein, calories, vitamins and minerals to diets of Latin America and Africa. However, seeds of some varieties of beans may contain high amounts of tannins, which adversely affect the nutritional quality of beans (House et al., 2002). Tannins are important because of their ability to interact with proteins and to chelate minerals resulting in reductions in protein digestibility and mineral bioavailability. Tannins are derived from phenolic compounds and contribute to the coloring found in the seed coats of common beans (*P. vulgari*s) and their relatives. They can be divided into hydrolizable / soluble tannins (derived from Gallic acid) and condensed tannins / proanthocyanidins (derived from polymerized flavonoids), which are measurable by different techniques. Bean genotypes that differed in their apparent ability to accumulate minerals have been selected from the CIAT core collection and evaluated for tannins (House et al., 2002). In that study, tannin content was determined using the vanillin method (Desphande et al., 1987), methanol extraction and a commercial standard for the calibration curve. While this previous method was useful for general analysis we have implemented a butanol-HCl method using purified tannin from beans as a more appropriate standard than any of the commercially available ones. Because of the differences between methodologies we decided to evaluate the same genotypes as House et al. (2002) along with an additional set of low tannin white seeded varieties for tannin content using the new method.

Materials and Methods: A total of 42 accessions all from the CIAT core collection were selected to represent a variety of commercial classes and seed colors. Of these accessions, 21 had been analyzed previously by House et al. (2002) (Table 1) and 21 were chosen to further study of the white and dual white and non-white patterned seed classes (data not shown). Seed coats were peeled from the grain and ground into a fine powder by hand to use in all subsequent analysis. To facilitate seed peeling, the seeds had been soaked in n-heptane for 24 hours. A total of 10 mg of ground seed coat and three replicates were used per seed coat sample. Total condensed tannin extraction and analysis of soluble and insoluble condensed tannins were as reported last year. Photometric tannin analysis was realized with the Butanol-HCl method and the blank was a butanol-water (5%) mix. The calibration curve used was chosen from a variety of curves described in a previous section of this annual report made for determining differences among purified tannins from bean seeds of different color. The absorbance of the samples was determined at 550nm in a spectrophotometer Shimadzu UV-1601.

Results and Discussion: The amount of soluble and insoluble condensed tannins in the beans that were analyzed ranged from a minimum of 0.00 % (for almost all the white bean accessions, data not shown) to maximums of 22.8% and 4.3%, respectively for non-white beans (Table 1). The genotype with the highest soluble condensed tannins was a cream mottled bean (G21242) while the genotype with the highest insoluble condensed tannins was a yellow bean (G13220). Black, purple and black mottled beans although they are the darkest in the color range have intermediate to intermediate/high amounts of total tannins. Cream beans especially lighter, non-

mottled cream beans had low soluble and total tannins. Correlation between soluble and insoluble tannins was high (r=0.861, P>.001) across all genotypes. Among the 15 white accessions analyzed only one, G18372, contained tannin in its seed coats and this was due to insoluble tannins. On the other hand, white beans with colored patterns, heretofore called white mottled beans, had varying amounts of tannin depending on the proportion of the seed coat that contained color and the proportion lacking color as well as the type of color present with white and red combinations having more tannins than white and black, white and brown or white and cream beans (data not shown). Although it seems unlikely that it would be possible to transfer the null or very low tannin trait from white beans into other colors without also transferring white seededness, the apparent variability within other color classes establishes the possibility of altering the tannin content of future varieties in non-white seeded beans. Because the pigmentation plays an important role in breeding, the study of the relationships between seed coat color and tannin content is an important step in the elucidation of genes from the biosynthetic pathway and in the effort to improve the bioavailability of mineral content, which is affected by tannins. It will be interesting to determine if tannin content and seed coat color are correlated in common beans. Overall, tannin measurements were consistent between repetitions showing that the accuracy range of the Butanol-HCl method was good. Compared to the Vanillin HCl method carried out previously by House et al. (2002), the Butanol method was more accurate as the ranges coincided with values previously reported for common bean of 0% to 18% tannin per seed coat weight (Ma y Bliss, 1978). With both methods there was more variation within color classes than between classes.

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Table 1. Least significant difference (LSD) Comparisons of a) soluble and b) insoluble tannin content in seed coats of 21 common bean genotypes with different seed colors.

Seed color	Genotype	Mean	Homogeneus group
cream/purple	G21242	22.825	A
cream/white	G21078	17.275	B
red	G11350	16.825	B
cream/brown	G4825	15.55	C
brown	G3971	15.44	C
cream	G734	15.403	C
cream	G11419	15.293	C
pink	G16267	14.897	C
red	G15137	14.007	D
cream-ray	G2774	13.694	D
cream	G21725	12.715	E
yellow	G13220	11.913	EF
purple	G23063	11.675	FG
cream-yellow	G5034	11.385	FGH
black	G5706	11.215	FGH
brown	G14519	10.88	GHI
cream-black	G19022	10.505	HI
purple mottled	G1678	10.15	I
black mottled	G3096	8.545	J
cream	G1844	8.0933	J
cream	G12610	5.6567	K

Seed color	Genotype	Mean	Homogeneus group
yellow	G13220	4.3252	A
cream/purple	G21242	4.1517	AB
cream	G21725	3.9466	AB
cream/brown	G4825	3.7867	B
cream	G734	3.2948	C
cream-yellow	G5034	3.2084	C
black	G5706	2.9651	CD
cream-black	G19022	2.9295	CDE
cream	G1844	2.9151	CDE
brown	G3971	2.7457	DEF
purple mottled	G1678	2.6872	DEF
cream	G12610	2.6309	DEF
cream	G11419	2.6147	DEFG
cream/white	G21078	2.5435	EFGH
brown	G14519	2.3308	FGHI
pink	G16267	2.2286	GHIJ
purple	G23063	2.221	GHIJ
cream-ray	G2774	2.2037	HIJ
black mottled	G3096	2.0271	IJK
red	G15137	1.9208	JK
red	G11350	1.8013	K

LOW PHYTIC ACID (*LPA*) MUTANTS IN COMMON BEAN (*PHASEOLUS VULGARIS* L.): MUTANT ISOLATION AND MOLECULAR ANALYSIS OF OF MYO-INOSITOL-3-PHOSPHATE SYNTHASE

E. Doria*, B. Campion**, M. Fileppi**, I. Galasso***, R. Bollini***, E. Nielsen** and . Sparvoli***

*) Department of Genetics and Microbiology, University of Pavia

**) Istituto Sperimentale per l'Orticoltura, Monatanaso Lombardo, Lodi,

***) Istituto di Biologia e Biotecnologia Agraria, CNR, Milan, Italy Italy

Phytic acid, myo-inositol 1,2,3,4,5,6-hexakisphosphate (InsP6), is the major storage form of phosphorus in the seed, where it is deposited in protein bodies as a mixed salt of mineral cations, such as potassium, magnesium, iron, and zinc called phytin. Both phosphorous and cations bound to phytin are hardly or not absorbed in the intestine and are largely excreted, thus affecting the nutritional quality of food. In humans, seed based diets can lead to deficiency of micronutrients, particularly iron and zinc, just due to the binding of mineral cations by phytic acid. The problem of mineral bioavailability is of particular concern in developing countries. Therefore, modulation of phytic acid content in the seed is one of the major goals in seed crop genetic improvement.

Recently mutant lines with greatly reduced levels of phytic acid have been generated by random mutagenesis in various crops (Hitz et al. 2002, Pilu et al. 2003). Reduction of phytic acid resulted in an increase of phosphate level in the seed. The fact that these mutants were recovered and can be seed propagated showed that phytate-eliminating mutations did not compromise seed viability. Therefore, modulation of phytic acid content in the seed can be a major goal in seed crop genetic improvement.

The main aim of this work was thus to isolate low phytic acid (lpa) bean mutants and to characterize them at genetic, biochemical and molecular level. In addition, we aimed to evaluate the possibility of their exploitation for human and, possibly, animal consumption, as well as of using them as tools to provide insight into the physiology of seed and seedling development.

Material and methods

EMS MUTAGENESIS

Bean seeds were treated with 48mM of EMS (ethyl methane sulfonate) mutagen for 12 hours. M1 bean plants were grown to maturity for seeds harvest.

PI ASSAY AND RAFFINOSE CONTENT

Flour from pools of M2 seeds of each M1 plant and single seeds from positive pools were analysed for phosphate content by the molybdate staining assay and for raffinose content by the Megazyme assay kit.

ANALYSIS OF MIPS DNA SEQUENCES

The isomerase D-myo-inositol-1-phosphate synthase (MIPS) was isolated from genomic DNA of wild type and putative low phytic acid (lpa) seeds, using specific MIPS-PCR primers (Fileppi et al 2004).

Results

Samples	Free P mgP/g	Phytic acid P mgP/g	FreePi/Phytic P
average W.T.	**0.42** ± 0.08	**1.70** ± 0.05	0.25
280	**0.76** ± 0.03	**1.38** ± 0.18	0.55
447	**0.69** ± 0.06	**1.31** ± 0.08	0.53
514	**0.70** ± 0.07	**1.74** ± 0.02	0.4
639	**0.73** ± 0.04	**1.75** ± 0.15	0.42
652	**0.66** ± 0.06	**1.69** ± 0.04	0.39
657	**0.69** ± 0.03	**1.72** ± 0.18	0.4
868	**0.74** ± 0.04	**1.59** ± 0.03	0.47
916	**0.84** ± 0.06	**1.87** ± 0.08	0.49

lpa mutant. A population of mutagenized common bean was produced by incubating the seeds with EMS. These M1 seeds were planted and self-pollination of the M1 plants yielded 1774 M2 seed families. The first screening for the high level of free phosphate (HIP-phenotype) carried out on the flours obtained from a pool of 15 M2 seeds/family allowed to identify eight putative *lpa* mutant plants (Fig. 1). Among them, plant 280 showed the highest content of free Pi (181% of the wt value) and also a decreased amount (81% of the wt value) of phytic acid P (Fig. 1). Therefore, we focused on L280 plant and analysed the free phosphate as well as the phytic acid levels in the flours obtained from 34 single M2 L280 seeds: results revealed in one of them an eight fold increase, compared to wild-type, in free Pi, and a contemporary 2.25 fold decrease in phytic acid P. 29 (M2) L280 seeds were planted and the HIP phenotype was once more screened in the flours from 16 single M3 seeds produced by each plant. In two plants 1 seed out of 16, in a third plant 4 seeds out of 16, in a fourth plant (L280-10) all 16 seeds displayed the HIP phenotype. In the L280/10 seeds, a reduction (of 30%) in the content of raffinose respect to the wild-type (5.6 mg/g vs. 8.4 mg/g) was also observed.

MIPS DNA sequence. Sequence analysis of the genomic form of the first enzyme of the pathway (MIPS), isolated from the wild type and the *lpa* line showed no differences among the two coding regions while one difference was observed among introns. Therefore, we are now analysing the promoter regions and in the same time the other two enzymes (inositol polyphosphate kinases) involved in later steps of plant phytate biosynthetic pathway.

Conclusions

Following chemical mutagenesis, we have isolated viable seed mutants showing an increased inorganic P, a decreased phytic acid, and a decreased total raffinose content. The isolation of common bean low phytic acid mutants will provide a novel approach to study this biochemical pathway and to overcome the human and animal nutritional problems associated with this compound.

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IRON, ZINC AND PROTEIN CONCENTRATION IN AFRICAN BEAN CULTIVARS

[1]P. M. Kimani, [2]E. Karuri and [2]S. Mwaura
[1]Dept of Crop Science ; [2]Dept of Food Science and Nutrition, University of Nairobi
P.O. Box 29053, Nairobi, Kenya

Introduction

Iron and zinc deficiency in diets is widespread in Sub-Saharan Africa, affecting mainly the poor particularly pregnant women and children. Micronutrient rich cultivars of common bean offers unique ways of contributing to the alleviation of iron and zinc deficiency in Africa because bean is widely grown and consumed particularly by medium and low income households which are the majority in the region. It is relatively cheap compared to animal sources of micronutrients. It is a highly sustainable food based approach to resolving micronutrient deficiency problem since there is no additional cost to consumers and hardly requires any changes in die-hard eating habits. This report presents some preliminary data on variation in iron and zinc concentration among bean cultivars grown in East and Central Africa as part of larger effort of developing micronutrient dense bean cultivars.

METHODS

Bean samples were obtained from Congo, Rwanda, Ethiopia, Sudan, Uganda and Kenya. They included over 70 locally popular cultivars, landraces or selections from segregating populations and commercial cultivars released by national programs. The samples were washed with double distilled water, dried overnight in an oven and ground in stainless steel hammer mill. To compare mineral recovery levels, samples were prepared by (a) either ashing followed by acid dissolution of the ash, or (b) digestion with a mixture of perchloric and nitric acid prior to elemental analysis (Zarcinas et al, 1987; AOAC, 1981). Elemental analysis was done by atomic absorption technique (AAS). Zinc concentration was determined by wet digestion only. Nitrogen was determined by standard Kejdhal digestion

RESULTS AND DISCUSSION

Zinc concentration varied from 62 ppm in VNB 81010 to 12 ppm in M211. Mean concentration was 31 ppm. Mean of 1500 varieties at Colombia was 35 ppm with a high of 52 ppm in G11350 (Beebe et al, 2000). These results are comparable. In both analyses at Kabete and Colombia Vunikingi had a concentration of 35 ppm(Table 1). The top five varieties for zinc were VNB 81010, MLB-49-98A, LIB 1 and Kiangara and A 620. These had 38 ppm and above. All others showed rather ordinary levels.

Iron was determined by both wet digestion and by ashing. The range was 147 to 68 ppm for the first method and 131 to 59 ppm in the second. The mean was 96.1 ppm for wet digestion and 94.1 ppm for ashing method (Table 1). These means are higher than 55 ppm reported in Colombia. A high of 102 ppm was reported in Colombia analyses. This may be due to influences of soil type and location (and perhaps other environmental influences). Effect of environment was clearly demonstrated by Beebe et al (2000).They concluded that superior mineral content of a line selected at one experimental site would not be lost when the materials are planted at other sites, although the degree of expression of the trait will vary.

The most promising cultivars for iron content are AND 620, GLP 2, MLB-49-98A, VCB 87013, G59/1-2, Naindeky and Kiangara with more than 100 ppm. These were among the top seven lines for iron concentration by both methods. The results of wet digestion and ashing were highly correlated (r=0.8**). AND 620, MLB-49-98A (the black seeded root resistant cultivar popular in western Kenya) and Kiangara combined high levels of both zinc and iron and represent three seed types consumed in the region. Cultivars with high protein levels were VCB 87012, Awash Melka, K131 and Awash (Table 1). Protein concentration varied from 13 % in Roba-1 to 26.4% in VCB 87012.This indicated the potential for protein improvement in bean.

The results indicated that considerable potential exists for improving the micronutrient and protein nutrition by promoting consumption of bean cultivars rich in these nutrients. Other popular bean cultivars low in these nutrients can be improved through breeding.

Table 1. Iron and zinc concentration in bean cultivars grown in East, Central and Southern Africa.

Cultivar	Origin	Growth habit	Seed colour	Seed size	Wet digestion (ppm)		Ashing (ppm)	Protein (%)
					Zinc	Iron	Iron	
MLB-49-98A	Congo	bush	black	small	55	124	131	-
Maharagi Soja	Congo	bush	yellow	small	23	97	107	20.1
VCB 87013	Congo	climber	white	small	25	122	109	19.4
Ituri Matata	Congo	bush	white	large	35	87	87	16.2
Vunikingi	Rwanda	climber	brown	small	35	88.5	76	20.1
MLV-6-90B	Congo	climber	brown	small	26	96	96	18.8
M'Mafutala	Congo	bush	brown	small	28	95	102	-
VNB 81010	Congo	climber	black	small	62	77	70	-
GLP 24	Kenya	bush	red	large	35	93	99	18.0
GLP 1127	Kenya	bush	Mwezi Moja	large	29	91	88	
GLP X 92	Kenya	bush	pinto	medium	16	68	68	16.3
G59/1-2	Congo	climber	brown	large	24	106	115	-
Kiangara	Congo	climber	brown	small	44	104	117	20.1
M211	Congo	climber	pinto	small	12	94	92	
VCB 81012	Congo	climber	brown	medium	32	86	74	26.4
Simama	Congo	bush	calima	large	13	78	68	19.4
AND 10	Congo	climber	sugar	large	30	80	90	18.9
LIB 1	Congo	climber	yellow	medium	52	94	105	20.8
Naindeky	Congo	bush	white	small	30	106	105	21.4
GLP 2	Kenya	bush	calima	large	28	124	115	16.2
AND 620	Congo	bush	calima	large	38	147	121	20.4
M'Sole	Congo	bush	brown	small	22	99	61	22.2
Nakaja	Congo	bush	brown	small	20	74	96	20.1
Kirundo	Congo	bush	yellow	large	31	76	59	17.1
Awash Melka	Ethiopia	bush	white	small	28	-	65	25.3
K 131	Uganda	bush	carioca	small	31	-	32	25.0

THE EFFECT OF SOAKING AND COOKING ON THE OLIGOSACCHARIDE CONTENT OF RED KIDNEY BEANS (*PHASEOLUS VULGARIS L.*)

G.Nyombaire[1], M. Siddiq[1], K.D.Dolan [1,2]

[1]Department of Food Science and Human Nutrition, [2]Department of Biosystems and Agricultural Engineering, Michigan State University, East Lansing, MI, 48824

Introduction

Legumes, especially beans are considered an important and inexpensive source of protein, dietary fiber, vitamins, essential minerals (Fe, Zn, Ca, etc) and bioactive compounds such as folates, saponins, and phenolics, in many developed and developing countries. In addition, beans are very low in sodium, cholesterol, and saturated fatty acids but rich in unsaturated fatty acids such as linoleic acid. Albeit beans are very important to human health, several factors distract from their full nutritional potential, such as the presence of antinutritional factors that cause flatulence. Oligosaccharides of the raffinose family (raffinose, stachyose, and verbascose) cannot be digested because of lack of α-1,6-galactosidase activity in the mammalian intestinal mucosa. These sugars are therefore fermented anaerobically by microorganisms on the wall of large intestines to produce carbon dioxide, hydrogen and methane gases. The major objective of this study was to evaluate the effect of soaking at different lengths of time and the effect of sugar-coating on the oligosaccharide contents in red kidney beans. Sugar-coating of beans was done to produce a potential value-added snack product.

Materials and Methods

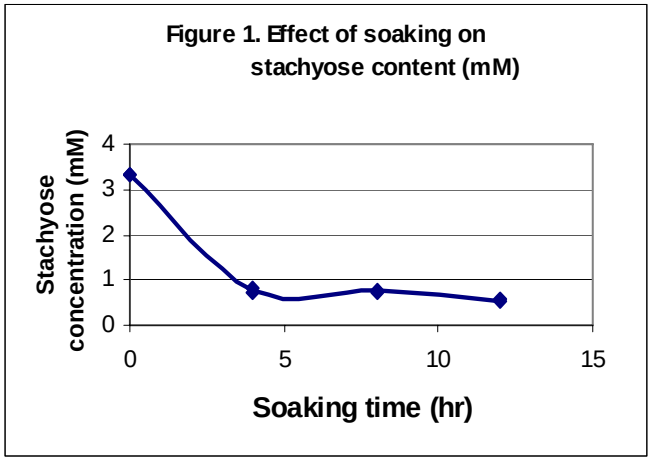
Dry red kidney beans (*Phaseolus vulgaris L*) used as research material were purchased from Bayside Best Beans, LLC (Sebewiang, MI). The damaged beans were segregated from the main population, and then raw bean material were mixed in duplicate with distilled water at 1:5 (w/v) at an initial temperature of 77^{o}C containing approximately 0.08 and 0.06% of sodium carbonate and sodium polyphosphate respectively. Samples were collected after 4, 8, and 12 hours of soaking. After 12 hours soak, the beans were cooked in water for 14 minutes at 98^{o}C containing 0.06 and 0.04% sodium bicarbonate and sodium polyphosphate respectively. Beans were further sugar-cooked in 20, 35 and 50% sugar/water solution at 70^{o}C for 45 minutes. After, the samples were analyzed for raffinose and stachyose using High Performance Liquid Chromatography (HPLC). Analysis of variance was done using JMP IN 5.1 software to determine and compare the differences in stachyose and raffinose contents. Tukey-Kramer Honestly Significant Difference Test was performed for comparisons. Significant differences were established at α = 0.05.

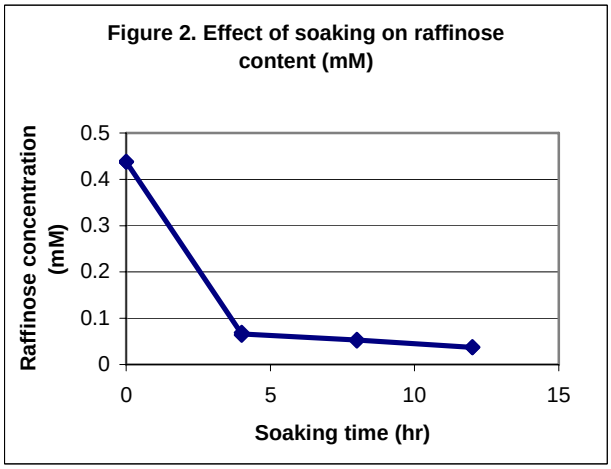
Results and Discussion

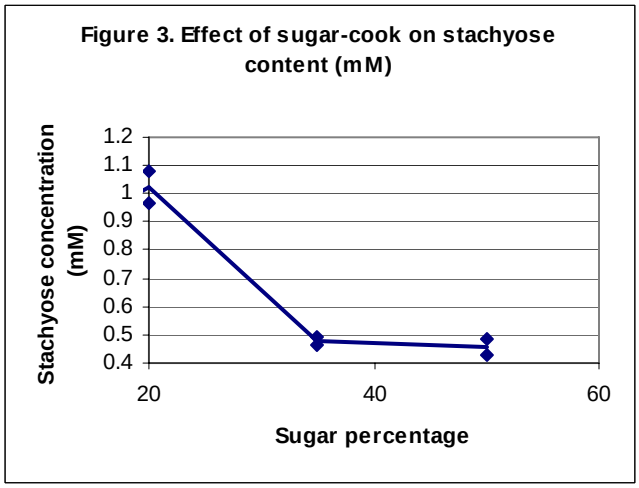
Bean soaking

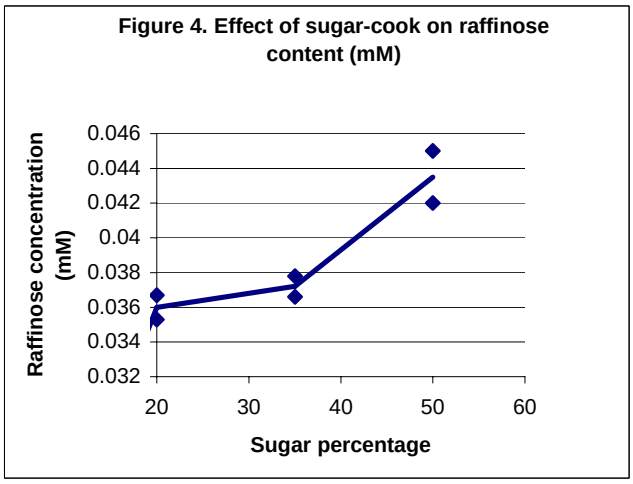
Four hours of soaking resulted in greater than 76 and 84% of stachyose and raffinose reduction respectively (α = 0.05, Figures 1 & 2). These results show that it may not be necessary for to

soak beans for 12 hours if the aim is to reduce flatulence-producing oligosaccharides. Complete removal of flatus-producing oligosaccharides is not advisable as they have health benefits.









Sugar-cook

The amount of stachyose decreased ($\alpha = 0.05$) with increase in sugar concentration (Figure 3). Conversely, raffinose concentration increased with increase ($\alpha = 0.05$, Figure 4) in sugar concentration. It is unclear why raffinose concentration increased slightly with increase in sugar concentration.

Acknowledgement

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EFFECT OF SOAKING ON THE OLIGOSACCHARIDE CONTENT IN COMMON BEANS

Carmen Jacinto-Hernández[1]; Marcela E. Blancas-Carrazco[2]; Samuel Sánchez-Domínguez[2]; J. Enrique Rodríguez-Pérez[2] ; Ramón Garza García[1]; Irma Bernal-Lugo[3]

[1]Laboratorio Calidad de Frijol y Programa de Frijol INIFAP, CEVAMEX, Correo-e: carmenjh8@yahoo.com.mx. [2,] Departamento de Fitotecnia, Universidad Autónoma Chapingo. Estado de México, 56230. México. [3]Laboratorio de Bioquímica, Facultad de Química, UNAM. Ciudad Universitaria, D. F. l04510 México.

INTRODUCTION

Common bean, is a good source of dietary protein, vitamin B, folic acid, among other essential nutrients for humans; however a significant problem is poor consumer acceptability due to gastrointestinal discomfort and flatus production associated to α-oligosaccharides of the raffinose family. However, a number of health benefits results from ingestion of oligosaccharides, such as reduction of toxic metabolites, reduction of serum cholesterol, and they may have anticancer effects (Tomomatsu, 1994). Besides, there is evidence that α-oligosaccharides play an important role in the acquisition of seed desiccation tolerance and also as a substrate for embryo growth during germination To satisfy market demands it is necessary for bean plant breeders to know the genetics, environmental, and processing factors affecting this trait to attain a balance between expected benefits and negative factors of grains. Since, it is known that sucrose and the α-galactosides raffinose, stachyose, and verbascose (RFOs) in beans decrease during soaking and germination, the objective of this work was to find out the efficiency of soaking grains for removal of these sugars from 13 common bean genotypes.

MATERIAL AND METHODS

We choose 13 genotypes from four market classes to represent a significant genetic diversity for RFOs. The oligosaccharides content in dry as well as in soaked in water (12 h at 23 °C) grains, was analyzed. Water absorption (%) was measured. Cotyledons were ground and then homogenized with aqueous ethanol (15%, 30 ml) with an internal standard (melezitoze) for 2 h at 50-60 °C. The mixture was centrifuged at 12000 x g and supernatants recovered and passed through Waters nylon filters. Samples (20 µl), sugars were analyzed with a Waters (Milford MA) HPLC a Waters 2414 refractive index detector. A Waters WAT044355 column was used with acetonitrile:water (75:25 v/v). All analyses were made with three replications. A one-way analysis of variance (ANOVA) and tukey (α 0.05) test were performed.

RESULTS AND DISCUSSION

Considerable variability existed among common bean genotypes in oligosaccharide content, as well as in sucrose content (table 1). Stachyose was the main α-galactoside with values between 9.4 and 36.7 mg g^{-1} while raffinose was between 1.63 and 7.04 mg g^{-1}. There was not a clear difference in RFO content among market classes. Content of raffinose and stachyose decreased with increase in seed size (-0.65**, α= 0.001) which is similar to results observed by Kosson (1989) in 16 Polish cultivars.

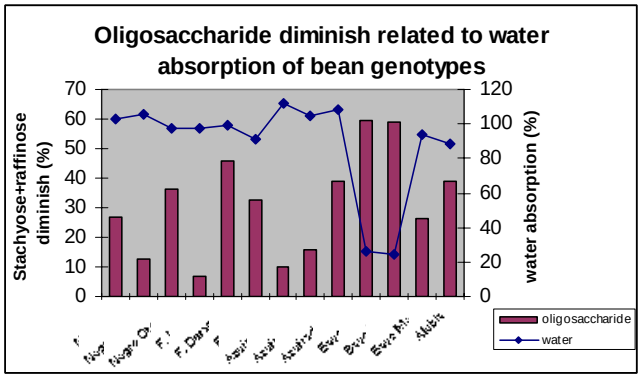
The amount of RFOs removed through soaking was since 7 % of raffinose+stachyose in Flor de mayo M-38 up to 59-60 % in Bayo Zacatecas and Bayo Victoria (DM basis). During soaking, bean proteins become hydrated and their enzymatic activity regained. Therefore, the differences in soaking effectiveness for RFO removal from different cultivars may be due to variable levels of α-galactosidase activity which selectively cleaves galactose from raffinose, stachyose and verbascose leaving behind sucrose. More over, the fact that also sucrose decreased during soaking of all

the bean seeds indicated that sucrose, in addition to α-galactosides, can be degraded to release energy for the germinating embryo.

It is remarkable that Bayo Zacatecas and B. Victoria, genotypes adapted to semiarid conditions with the lowest water absorption had the highest reduction in oligosaccharide after soaking. This characteristic could be desirable, because it allows RFOs to have a positive role during the growing season and later the seed is more digestible for consumers. Varieties such as Bayo Zacatecas and Bayo Mecentral would be the best option for consumers who experience serious problems associated with consumption of sources of oligosaccharides, especially for children. However it would be necessary to determine soluble fiber because it is believed that this fraction is also related to flatulence. In general soaking beans overnight before cooking is a recommended practice to shorten cooking time and eliminate some of the RFOs. In addition, discarding soaking water gives an increase in apparent digestibility of beans protein Cárdenas *et al.* (2000).

Grain weight and soluble sugar content in common bean varieties

Genotype	100 grain weight (g)	Dry grain			Soaked grain		
		Sucrose	Raffinose	Stachyose	Sucrose	Raffinose	Stachyose
		Concentration (mg g^{-1})			Concentration (mg g^{-1})		
Bayo Victoria	54.72 a	43.90 a	4.43 abc	36.66 a	26.07 abc	2.57 cde	14.08 ab
Azufrado Higuera	54.32 a	34.56 bc	1.63 c	26.98 cde	28.50 ab	1.94 de	22.14 ab
Flor de Durazno	47.18 b	31.66 bcd	2.20 abc	31.33 abc	14.66 d	1.61 e	16.48 abcd
Azufrado Peruano	41.78 bc	34.43 bc	2.06 bc	34.27 ab	20.66 bcd	1.73 e	20.41 ab
Bayo Zacatecas	40.20 c	33.25 bcd	5.39 ab	26.39 cde	18.80 bcd	2.08 cde	10.76 cd
Azufrado Reg.	37.66 cd	28.83 bcd	1.72 c	23.32 de	27.30 ab	1.82 e	20.32 ab
Bayo Mecentral	33.38 de	26.36 cd	6.16 a	9.43 f	18.99 bcd	3.36 bcde	8.03 d
Flor de Junio M.	31.28 e	35.47 b	5.38 ab	28.06 bcd	28.43 ab	3.78 bc	18.75 abc
Negro Otomí	28.90 e	34.67 bc	3.87 abc	26.76 cde	23.19 abcd	2.45 cde	16.36 abcd
Flor Mayo M38	28.72 e	30.40 bcd	3.94 abc	26.50 cde	30.08 a	3.74 bcd	24.48 a
Alubia	28.62 e	25.37 d	5.65 a	20.56 e	15.92 cd	3.13 bcde	12.82 bcd
Negro Jamapa	18.52 f	32.72 bcd	7.04 a	35.22 a	28.43 ab	6.00 a	24.76 a
Negro 8025	18.30 f	27.72 bcd	6.55 a	23.62 de	19.70 bcd	4.73 ab	21.53 ab



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INTERACTION OF STORAGE CONDITIONS ON THE LOSS OF BEAN QUALITY

Carmen Jacinto-Hernández,[1] Lucero Mendoza-Bahena[2] Irma Bernal-Lugo[3] y Ramón Garza-García[1]

[1] Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias. CEVAMEX. A. Postal 10. Chapingo, edo. México C.P. 56230. (México). carmenjh8@yahoo.com.mx [2] Universidad Autónoma Chapingo, Departamento de Ingeniería Agroindustrial. [3]Departamento de Bioquímica, Facultad de Química, UNAM. Ciudad Universitaria, D. F. l04510 México.

INTRODUCTION

Ageing of common beans is a challenge for farmers and bean traders. Beans subjected to long periods of storage undergo gradual loss of quality, such as changes of the seed coat color, soaking characteristics and cooking time (Liu, 1995, Jacinto *et al.,* 2004). The objective of the study was to detect significance and interaction of storage factors in the changes of the main characteristics of common bean quality.

MATERIAL AND METHODS

Eleven common beans (*Phaseolus vulgaris* L.) genotypes four with cream coat and seven with black coat were evaluated. Genotypes were grown at Santa Lucía de Prías, state of México during 2002 crop season. The seeds were cleaned, grain moisture content was adjusted to 12%, separated into nine lots and each lot was submitted to different storage conditions: combining two conditions of temperature (30 and 40 ºC), two conditions of Relative Humidity, HR, (32.5 and 75%) in containers with saturated $MgCl_2$ and NaCl solutions respectively, and two periods of storage (14 and 28 days) in a factorial arrangement getting a total of nine treatments including a control sample of each variety was kept in polyethylene bags at 5 °C until analyzed. Coat color was measured with a Hunter Lab MinSanXEPlus L 50. One hundred seed wt. and volume was measured. Seed wt after soaking for 20 h in distilled water was measured to evaluate water absorption capacity, and cooking time was evaluated using a sensorial method (Guzman *et al.,* 1995). Data were processed through an analysis of variance.

RESULTS AND DISCUSSION

The more evident changes by ageing were the darkening of seed coat (Hunter L value) in cream coat beans, although a change in color was also observed in black beans; Significant sources of variation for darkening (Hunter L) of cream coat genotypes were cv., temperature, RH, and also their interactions (Table 1). For black beans, sources of variation for Hunter L value were cv., RH, and interactions of this with the other variables. The difference between the cream and the black group is that while interaction cv. x time of storage affects Hunter L value in the cream it is non significant in the black group. The Hue (tone) and chroma (intensity or purity) values were also more significant in cream than in the black group (table 1).

Other changes observed in stored bean seeds were decrease in water absorption, increase in coat percentage and the hardening of beans measured as cooking time. For these factors the significant sources of variation were cv, RH, and time of storage, temperature and interactions among them. An association was observed between cooking time and storage temperature (r=0.45**) and also with storage time (r=0.41**). While, for grain and volume wt. as well as for solid content in broth only cv., and interaction of cultivar x time of storage were significant sources of variation (Table 2).

The results demonstrate that there are significant differences in response to storage conditions between cream and black beans. Even though water absorption was affected by RH conditions (r= -0.38**), no association was detected between water absorption and cooking time.

Table 1. Mean square of the analysis of variance of color variables

		Cream coat				Black coat		
Source of variation	**DF**	**L**	**Hue**	**Chroma**	**DF**	**L**	**Hue**	**Chroma**
	3	205.98**	0.05**	37.53**	6	12.19**	3.56**	3.20**
	4	1.52**	0.00 NS	2.31 NS	7	0.07 NS	0.46 NS	4.39**
Cv*Temp	3	7.33**	0.01 NS	4.78 NS	6	4.40**	0.98 *	0.01NS
Cv*RH	3	22.01**	0.02 NS	9.83 NS	6	3.68**	0.48 NS	0.01 NS
Cv*time	3	8.16**	0.03 NS	25.66*	6	0.21 NS	1.35**	0.03 NS
Cv*Temp*RH	3	9.935**	0.01 NS	3.76 NS	6	4.05 **	0.53 NS	0.03 NS
	3	4.05**	0.01 NS	11.76 NS	6	3.59 **	1.93**	0.06**
Cv*HR*time	3	15.97**	0.01 NS	14.96 NS	6	1.22 **	0.23 NS	0.02 NS
Cv*Temp*RH*time	3	1.26**	0.02 NS	9.35 NS	6	2.38 **	0.68 NS	0.01 NS
Error	24	0.19	0.01	6.91827.34	42	0.14	0.40	0.01
Total	71				125			
CV (%)		0.98	8.90	14.9		2.38		33.6

** Highly significant difference (P≤0.01). * Significant difference (P≤ 0.05). NS not significantly different
Only part of the sources of variation are presented

Table 2. Mean square of the analysis of variance for physical and physicochemical characteristics.

Source of variation	**DF**	**Grain wt**	**Grain vol**	**coat**	**Water abs**	**Cooking**	**Solids**
Cv	10	604.55**	277.16**	5.54**	64.69**	4906.33**	0.0381**
	11	10.96*	6.56*	0.02 NS	61.66 NS	3.36 NS	0.00 NS
Cv*Temp	10	2.15 NS	3.44 NS	0.09**	171.83**	695.24**	0.0126 NS
Cv*RH	10	2.02 NS	4.81*	0.12**	1021.67**	1488.10**	0.0135 NS
Cv*time	10	9.60**	19.19**	0.06**	352.43**	1093.44**	0.0232**
Cv*Temp*RH	10	1.33 NS	2.72 NS	0.13**	194.56**	469.60**	0.0148 NS
Cv*Temp*time	10	1.34 NS	3.56 NS	0.08**	138.75**	251.82**	0.0105 NS
Cv*RH*time	10	2.20 NS	6.59**	0.15**	261.74**	437.06**	0.0111 NS
Cv*Temp*RH*time	10	2.40 NS	2.97 NS	0.07**	120.83**	324.29**	0.0084 NS
Error	66	3.37	2.02	0.02	41.72	23.44	0.00
Total	197						
CV (%)		0.17	5.6	1.51	7.12	5.57	23.33

** Highly significant difference (P≤0.01). * Significant difference (P≤ 0.05). NS not significantly different
Only part of the sources of variation are presented

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EFFECT OF STORAGE ON THE ANTIOXIDANT ACTIVITY OF COMMON BEANS (*PHASEOLUS VULGARIS* L.)

Patricia Vargas Vázquez and Yolanda Salinas Moreno
Campo Exp. Valle de México INIFAP-SAGARPA. PO Box 10, Chapingo, México, 56230

Introduction

The seed coat anthocyanins has been studied in beans of different colors; these compounds together with condensed tannins (proanthocyanidins) and flavonols are responsable of almost all the antioxidant activity that seed coat bean methanol extracts exhibit (Beninger and Hosfield (2003). Recent studies have been reported that concentrations of some flavonols of seed coat beans can change during storage (Beninger *et al*. 2005). The objective of this work was to evalute the antioxidant activity of seed coat in 13 accessions of aged and non-aged purple beans.

Materials and Methods

The accessions were selected from the mexican bean core collection of Instituto Nacional de Investigaciones Forestales, Agricolas y Pecuarias (INIFAP). A sample of 200 g of seed stored since 1984 under low temperature (1 to 7°C) (aged) and the same amount of seed harvested in 2004 (non-aged) were obtained. The shell was separated from the seeds with a scalpel and ground in a mill to get a flour that was dehydrated in a stove during 24 h at 50°C. Later, 0.5 g of flour was placed in a 125 mL flask and 10 mL of solvent (methanol:acetic acid:water, 10:1:9, v/v/v) was added and the flaks were put in a shaker during 24 h. Samples were centrifuged and the supernadant recovered and filtered with Whatman No. 4 paper disks. The antioxidant activity was determined by the DPPH method using 25 µL of the extracts and 975 µL of 80% methanol and adding to 2 mL of 60 µM DPPH. The absorbance was measured at 515 nm in a spectrophotometer (Perkin-Elmer Lambda 25) at 0, 5, 10, 15, 30, 45, 60 and 120 min. Antioxidant activity was expressed as percent of DPPH reduced according to Soler-Rivas *et al* (2000 The results were subjected to an analysis of variance and Tukey means were calculated ($p \leq 0.05$) using the SAS program (SAS, 1998).

Results and Discusion

Differences between treatments (aged and non-aged) for antioxidant activity was significant ($p \leq 0.05$). The antioxidant activity was higher in the aged than in the non-aged seeds, with 94.4 and 88.8 % of reduced DPPH, respectively. Among the accessions were outstanding Chis-34-A-2 and Chis-268 that, although in non-aged seed had the lowest antioxidant activity, they increased it in about 20 % when stored during a period of 20 years (Table 1). During storage, seeds reduce their metabolism to keep their viability. There exists a lot of information about the changes that occur to proteins, carbohydrates, and lipids during seed storage (Michaels and Stanley, 1991), but little is known about what happen with the polyphenols that are mainly located in seed coat. Among polyphenols, stick out the anthocyanins, proanthocyanins and flavonols which could suffer different chemical transformations that enhance their antioxidant activity. From the total antioxidant activity observed in methanol extracts from seed coat of beans, the high contribution is given by proanthocyanins (Beninger and Hosfield, 2003), but the anthocyanins have also an important participation, particularly delphinidin 3-glucoside, which is abundant in blak (Salinas *et al.* 2005) and purple beans. A better understanding is needed on the metabolism of bean polyphenols during storage, and how these changes can affect the nutritional value of this food.

Table 1. Seed coat antioxidant activity of 13 common bean mexican landraces.

Accession name	Age of seeds (years)	Antioxidant activity (% DPPH)	Seed coat main colour	Seed coat second colour
Mor-30-A	20 2	96.0 a 94.2 a	purple	Absent
Chis-116	20 2	95.5 a 94.2 a	purple	Absent
Zac-13-A	20 2	95.3 a 93.4 a	purple	Absent
A-1355-B	20 2	95.2 a 93.0 b	purple	Absent
Chis-154-A	20 2	94.9 a 92.9 b	black	Absent
Chih-27	20 2	94.7 a 91.4 a	purple	beige
Mich-31	20 2	93.5 a 92.6 a	purple	beige
X-16441	20 2	94.7 a 90.8 a	purple	beige
Jal-29	20 2	93.4 a 90.3 a	pink	Absent
Ags-61	20 2	94.6 a 86.8 b	purple	Absent
VP-44-A	20 2	92.4 a 86.1 b	purple	Absent
Chis-34-A-2	20 2	93.8 a 75.1 b	purple	beige
Chis-268	20 2	93.6 a 73.4 b	pink	Absent

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GENETIC VARIABILITY OF PRE-HARVEST SPROUTING IN BLACK BEANS GENOTYPES

Paulo Campos Granado Zerbinatti[1], Nelson da Silva Fonseca Júnior[2], Milena Pierotti Euzebio[1], Édison Miglioranza[1]. emiglior@uel.br
[1]Universidade Estadual de Londrina; [2]Instituto Agronômico do Paraná nsfjr@iapar.br

Introduction - In Southern Brazil, in the State of Paraná, mainly in the Center-south area, where there is risk of rains during harvest time of dry beans, the farmers prefer to grow black beans. The black beans commercial group have smaller possibility of presenting spots in the seed coat (oxidation), as it happens in the carioca (pinto beans) cultivars, however the pre-harvest sprouting risk of the seeds inside pods persist. The present experiment was aimed to verify the genetic variability among black bean genotypes for seeds sprouting inside pods.

Material and Methods - From the competitive genotypes experiments, plants were picked in each replication from black common beans in the State of Paraná, Brazil. It was conduct in randomized block design with four replications, during spring in Londrina, during fall in Ponta Grossa, and spring and fall in Mauá da Serra. These plants were collected after the physiologic maturation, being stored at open warehouse. Once dried, six to ten pods per plant were separated manually, 10 plants were used per replication. Sprouting inside pods was evaluated through the use of moistened paper rolls that were maintained in germinator at alternated temperatures between 20-30°C, for three days. Each paper roll contained pods of a single plant. The following parameters were evaluated: the number of sprouted pods (% BVG), the percentage of seeds germination in relation to the total of analyzed seeds (% GERM) and the sprout intensity by seed (NOTA), that ranged between zero and three; seeds germination test (% GEMS); electrical conductivity test of the solution containing the whole pod (COND); pod water absorption percentage (% ABS) and cooking test of seeds (COZ) using the Mattson method. To evaluate other variables the factorial design was adopted, grouping data by repetition without other subdivisions (plants, green bean and seed). The variance analyses were conducted using the procedure PROC GLM of the SAS program, because not all of the treatments had the same number of replication, it was unbalanced.

Results and Discussion - For the statistical analysis of the sprouting of seeds inside the pods, with the adopted hierarchical model, it was evident the contribution of the varience components of seeds inside the pods and the pods inside the plants, denoting strong variability inside of each plant, what hinders the selection of genotypes. The combined variance analysis showed significant effect from environments, genotypes, interaction ambient x genotypes, in all traits. Table 1 presents the averages of the different genotypes, in average 24.4% of the seeds germinated inside the respective pods and about 55,9% of the pods allowed sprouting of at least one seed inside the pod. Considering the four environments, ´Diamante Negro` was the most resistant to germination of seeds inside the pods, while FT 120 and FT Nobre were more sensitive. The phenotypic correlations are presented on Table 2, which were positive and highly significant, between: percentage of sprouting of the seeds in the pods, sprouting intensity, and percentage of sprouting pods. The cooking time was correlated negatively with the seed´s germination percentage. The percentage of water absorption per pod and cooking time presented positive correlation, indicating that the less absorbed water per pod, the lower the cooking time of the seeds. Even though there were seeds with easy cooking, these genotypes presented protective pods.

Conclusion -The sprouting of seeds inside the pod on the test in paper roll, was able to discriminate the black bean genotypes, being useful in the selection process of genotypes. The genotype less sensitive to sprouting inside pod test was ´Diamante Negro´ and the most susceptible were ´FT 120´ and `FT Nobre`.

Table 1. Averages of the genotypes for the traits; sprouting in the pods percentage (% Germ), sprouting intensity (Nota), percentage of sprouted pods (% BVG), percentage of seed germination (% GermS), pod water absorption percentage (% Abs), electrical conductivity in the solution containing pods (Cond) and test of cooking of the seeds in minutes (Coz). In four environments in the Paraná state, Brazil.

	Genotypes	*%Germ*	*Nota*	*%BVG*	*%GermS*	*%Abs*	*Cond*	*Coz*
1	*MD 841*	4,0	0,04	18,1	99,0	44,2	258,4	14,5
2	*LP 98-123*	4,0	0,04	13,9	100,0	-	-	-
3	*LP 98-158*	8,4	0,11	25,0	100,0	-	-	-
4	*LP 98-122*	12,8	0,23	29,7	100,0	-	-	-
5	*CNFP-7560 Vale*	13,0	0,17	47,5	98,0	39,0	272,8	9,5
6	*Diamante Negro*	14,0	0,23	43,7	95,4	74,1	187,7	24,4
7	*IPR Grauna*	14,6	0,22	51,5	100,0	-	-	-
8	*FT Soberano*	16,0	0,20	38,7	79,0	47,4	193,1	18,3
9	*Onix*	16,2	0,18	33,2	93,0	31,2	221,1	14,5
10	*LP 98-13*	22,1	0,31	55,1	92,0	53,0	242,8	19,7
11	*IPR Uirapuru*	24,0	0,32	67,0	95,2	76,1	296,3	22,6
12	*FT Tarumã*	27,5	0,33	67,2	92,7	71,1	299,8	21,5
13	*Xamego*	30,8	0,43	79,2	95,4	76,5	221,4	22,7
14	*LP 98-1*	31,2	0,53	64,9	91,7	64,5	244,2	18,7
15	*IAC Una*	33,8	0,42	63,1	94,0	78,9	202,7	21,7
16	*IAPAR 44*	34,8	0,59	79,8	95,8	69,6	242,7	21,9
17	*FT Bionobre*	37,6	0,62	78,2	94,0	92,0	267,7	21,7
18	*FT Nobre*	38,6	0,48	79,2	94,6	73,3	259,1	21,2
19	*RIO Tibagi*	40,2	0,55	85,5	95,3	81,0	221,7	22,2
20	*LP 98-11*	43,3	0,85	72,5	97,5	67,2	234,6	19,0
21	*FT 120*	44,5	0,75	80,9	93,7	74,0	239,0	22,1
	Averages	*24,4*	*0,4*	*55,9*	*95,1*	*65,5*	*241,5*	*19,8*

Table 2. Phenotypic correlations in four environments at Paraná state, Brazil.

	%GERM	*NOTA*	*%BVG*	*%GERMS*	*%ABS*	*COND*	*COZ*
%GERM	1,00	0,94**	0,26**	0,04	0,20**	-0,25**	-0,03
NOTA	0,94**	1,00	0,21**	0,08	0,17*	-0,27**	-0,06
%BVG	0,26**	0,21**	1,00	-0,02	0,09	-0,06	-0,09
%GERMS	0,04	0,08	-0,02	1,00	-0,19*	-0,05	-0,18*
%ABS	0,20**	0,17*	0,09	-0,19*	1,00	0,58**	0,79**
COND	-0,25**	-0,27**	-0,06	-0,05	0,58**	1,00	0,76**
COZ	-0,03	-0,06	-0,09	-0,18*	0,79**	0,76**	1,00

* and ** - Significant differences in the probability levels of 5% and 1%, respectively, teste T.

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GENETIC VARIABILITY OF PRE-HARVEST SPROUTING IN PINTO BEANS GENOTYPES

Paulo Campos Granado Zerbinatti[1], Nelson da Silva Fonseca Júnior[2], Milena Pierotti Euzebio[1], Edison Miglioranza[1].
[1]Universidade Estadual de Londrina (UEL) emiglior@uel.br; [2]Instituto Agronômico do Paraná nsfjr@iapar.br .

Introduction - Pre-harvest sprouting (PHS) in dry beans, caused by moist conditions is a direct effect of weather that has occurred frequently in the State of Paraná, Brazil. PHS effects quality and quantity of harvest, decreasing profits to farmers. In plant breeding programs this characteristic is not systematically evaluated, remaining unknown during the release of a new cultivar, the problem is then only discovered by the growers. This research had the objective of establishing the methodology to study the genetic variability of PHS in pinto beans commercial genotypes.

Material and Methods - Plants of each replication of commercial genotypes experiments of pinto beans were picked in four locations at the State of Paraná, South of Brazil, driven in randomized blocks organized in four replications. These plants were picked after the physiologic maturation, being stored at an open warehouse. Once dry, six to ten pods per plant were separated manually, 10 plants were used per replication. Sprouting inside pods was evaluated through the use moistened paper rolls that were maintained in germinator at alternated temperatures between 20-30°C, for three days. Each paper roll contained pods of a single plant. The following traits were calculated: the number of sprouted pods (% BVG), the percentage of seeds germinated in relation to the total of analyzed seeds (% GERM) and the sprout intensity by seed (NOTA), that ranged between zero and three; seeds germination test (% GEMS); electrical conductivity test of the solution containing the whole pod (COND); pod water absorption percentage (% ABS) and cooking test of seeds using the Mattson (COZ) method.

Results and Discussion -The results of individual and united variance analysis for the hierarchical model, indicated high participation in the variability of data is caused by the discrepancy among seeds from a same pod (55,6%), as well as of pods from a same plant (33,1%). However, the variability detected among the genotypes was low, around 5,7%. The united variance analysis evidenced significant effect for locations, genotypes, interaction ambient x genotypes, in all traits. Table 1 presents the averages of the seeds germination percentages in the pods, of the genotypes on different locations. The genotypes FT-Bonito, LP 96-153, IAC TYBATÃ, Pérola, IPR JURITI, IAPAR 80 and IAC ETÉ, stood out as tolerant, while Rudá and Carioca were the most sensitive to sprouting. The phenotypic correlations are presented in the Table 1, being positive and highly significant, in respect of percentage of sprouting in pods, sprouting intensity, percentage of sprouted pods. The cooking time was correlated negatively with the germination percentage of the seeds. The percentage of pods water absorption and time of cooking presented positive correlation, indicating that as less water absorbed for the pods, minor was the cooking time, suggesting that it was possible to detect genotypes that presented protective pods that contained easy cooking seeds.

Conclusion -The sprouting of seeds inside pods test in paper roll, was capable to discriminate the pinto bean genotypes, being useful in the selection process of genotypes. The genotype less sensitive to sprouting inside pod test was FT-Bonito and the most susceptible was Rudá.

Table 1. Averages of the genotypes for the traits; sprouting in the pods percentage (% Germ), sprouting intensity (Nota), percentage of sprouted pods (% BVG), percentage of seed germination (% GermS), pod water absorption percentage (% ABS), electrical conductivity in the solution containing pods (Cond) and test of cooking of the seeds in minutes (Coz). In four locations in the Paraná state, Brazil.

	Genotypes	*%Germ*	*Nota*	*%BVG*	*%GermS*	*%ABS*	*Cond*	*Coz*
1	Pitoco Arapuã	10,0	0,1	30,0	96,0	-	-	-
2	FT Bonito	10,5	0,2	42,4	94,9	73,4	252,5	18,9
3	LP 96-153	13,3	0,2	43,2	98,2	76,7	232,9	19,9
4	LP 98-76	16,4	0,3	65,0	100	-	-	-
5	IAC Tybatã	17,0	0,2	53,5	92,7	77,0	236,3	21,8
6	Pérola	18,2	0,3	51,5	88,6	72,1	239,9	23,5
7	IAPAR 72	19,3	0,3	37,5	94,0	41,5	219,6	14,0
8	IPR Juriti	20,6	0,3	64,3	94,8	77,1	220,1	23,9
9	LP 98-20	22,0	0,5	66,7	100	-	-	-
10	IAC Eté	22,3	0,3	61,9	92,3	62,3	197,6	21,0
11	IAPAR 80	23,3	0,3	63,7	95,5	70,6	211,6	19,6
12	Pequeno PG	24,1	0,3	57,1	100	-	-	-
13	IAPAR 31	24,3	0,3	63,3	97,4	78,2	234,4	19,2
14	LP 97-28	24,9	0,4	54,3	97,2	42,5	235,8	16,7
15	IAPAR 81	25,2	0,3	63,2	91,7	60,0	223,1	21,4
16	IAPAR 14	28,6	0,5	65,6	96,7	83,0	229,7	18,2
17	Rubi	31,8	0,4	70,8	94,0	42,3	294,5	14,5
18	FT Magnífico	33,6	0,5	71,0	99,0	53,0	273,4	17,7
19	Mallato	35,1	0,5	73,1	99,0	41,0	362,5	13,5
20	Lollatauro	36,5	0,5	92,5	100	0	0	11,0
21	Coroados	40,7	0,9	100	100	-	-	-
22	Carioca	40,8	0,6	80,7	97,4	69,6	249,1	19,0
23	LP 97-4	41,2	0,7	84,6	99,6	53,9	84,0	13,0
24	Rudá	44,0	0,6	87,6	95,3	62,7	221,6	20,8
25	LP 97-13	48,6	0,8	72,1	96,8	51,8	168,7	19,0
	Averages	*26,9*	*0,4*	*64,6*	*96,4*	*59,4*	*219,4*	*18,3*

Table 2. Phenotypic correlations in four locations at Paraná state, Brazil.

	%GERM	*NOTA*	*%BVG*	*%GERMS*	*%ABS*	*COND*	*COZ*
%GERM	1,000	0,923**	0,848**	0,326	-0,417	-0,257	-0,386
NOTA	0,923**	1,000	0,863**	0,446*	-0,363	-0,299	-0,389
%BVG	0,848**	0,863**	1,000	0,416*	-0,410	-0,395	-0,336
%GERMS	0,326	0,446*	0,416*	1,000	-0,361	-0,247	-0,634**
%ABS	-0,417	-0,363	-0,410	-0,361	1,000	0,423	0,780**
COND	-0,257	-0,299	-0,395	-0,247	0,423	1,000	0,291
COZ	-0,386	-0,389	-0,336	-0,634**	0,780**	0,291	1,000

*, ** - Significant differences in the probability levels of 5% e 1%, respectively, teste T.

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GENETIC CONTROL OF SEED SHAPE OF THE COMMON BEAN (*PHASEOLUS VULGARIS* L.)

Dimitar Genchev
Dobroudja Agricultural Institue – General Toshevo 9520
E-mail: genchev@dai-gt.org

In many countries visual classification of varieties is performed through the so called market-classes, for example Navy, Pinto, Alubia, Cranberry, Great Northern, etc. Besides seed shape, size and seed coat color are also important in this type of classification. More rare is the visual classification through comparison to various geometrical shapes: sphere, ellipsis, kidney, Sphaericus (Savi, 1822), Ellipticus (Martens, 1986), Oblongus (Savi, 1822), Subcompressus (Alefeld, 1866) and Compressus (A. De Candolle, 1825). This classification has been applied to some extend in Bulgaria, as well (Gradinaroff, 1939; Hristoforov, 1973). Hristoforov (1973) suggested determining seed shape by a biometric method based on length/width and thickness/width ratios. According to Genchev (1989), bean seed shape is closest to a three-axial ellipsoid. This shape is formed by uneven deformation along the three axes depending on the bean genotype and the environmental conditions. In this study, seed shape of the cross Beslet x Dobroudjanski ran was determined according to Hristoforov (1973), and the heritability coefficients were calculated on the basis of the variation coefficient suggested by Genchev (1989).

Table 1 presents the distribution frequencies by generation and rank of seed shape in the cross Beslet x Dobroudjanski ran. To determine the number and type of genes controlling seed shape, the plants were divided into two groups: seeds with elliptical shape (1-3) and seeds with a shape different from elliptical (4-12). Elliptical seed shape is controlled by three main polymeric genes ($\chi^2 = 3.649$, P>5÷10). Seed shape was inherited intermediary (d/a = 0.015), and H_{BS} was 69.2% and H_{NS} was 61.3%.

The quantitative nature of seed shape has been discussed by Frets (1951), Conti (1985), Genchev (1989), Vallejos & Chase (1991) и Mumba & Galwey (1999); the three dimensions (length, width and thickness) are controlled by polymeric genes. Conti (1985) has established that seed length is affected very strongly by the environmental conditions ($H_{NS} = 0.09$), while width ($H_{NS} = 0.65$) and thickness ($H_{NS} = 0.47$) heritability is evenly distributed between genotype and environmental conditions effects. The full ellipsoidal shape of bean seed can be disturbed by many factors - genes from the plant genome and environmental factors. The following genes can be considered responsible for seed shape: *te* - controls short pod formation, which, on its part, deforms the developing seed due to lack of sufficient space in the pod (Lamprecht, 1961); *miv*: small intervals between funicles in the pod resulting in compulsory compression and their subsequent deformation (Lamprecht, 1952) and *fast*: conical seed shape (Lamprecht, 1934). In case the above genes action is absent, genes *L-1L-2, B-1B-2 и Th-1Th-2* play an equal role for the respective formation of length, width and thickness (Frets, 1951). In the case of cross Beslet x Dobroudjanski ran, the seeds in the pod are not compressed due to a short pod or closely placed funicles and therefore there is a purely additive genetic control of the character. The seed shape heritablity we have determined ($H_{BS} = 69.2$ и $H_{NS} = 61.3$) is in accordance with the heritability established by Mumba & Galway (1999) showing that genotype plays a considerable role, although environmental effect is not insignificant.

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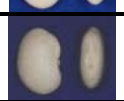
Table 1: *Frequencies of accessions with different seed shape in the cross Beslet x Dobroudjanski ran*

Seed shape		P_1	P_2	F_1	F_2	BCP_1	BCP_2
Sphaericus							
Sphaericu x Ellipticus		17					
Ellipticus		44			3	37	
Ellipticus x Oblongus		13		47	17	44	
Oblongus					28		
Sphaericu x subcompressus							
Ellipticus x subcompressus				23	2		
Oblongus x subcompressus			18		24		28
Subcompressus					1		
Ellipticus x compressus				16	4		
Oblongus x compressus			55		6		49
Subcompressus x compressus					2		
Σ		**74**	**73**	**86**	**87**	**81**	**77**

SEED COAT INTEGRITY: SEED CHARACTERISTICS AND MICRO-STRUCTURE OF SEED COATS RESISTANT TO MECHANICAL DAMAGE OF COMMON BEAN (*PHASEOLUS VULGARIS* L.)

S. J. Park*, and T. Rupert, Agriculture and Agri-Food Canada, Greenhouse & Processing Research Centre, Harrow, Ontario, Canada (* e-mail: parks@agr.gc.ca)

Common beans (*Phaseolus vulgaris* L.) are consumed by humans in various forms of food, mostly processed for baked bean. Therefore, seed coat integrity (high quality wholesome bean) of beans is extremely important. Split beans and cracked seed coats of white beans result in substantial problems in processing. Our first study showed variety differences in response to direct combine harvest (Gillard and Park). The second part of the seed study was carried out during 1999-2004 to determine genetic control of mechanical damage (MD) of navy beans with the F_4 and $F_{4:5}$ RIL of two crosses between resistant and susceptible beans. MD was under quantitative genetic control by multiple minor genes. Heritability of MD estimated by the parent-offspring regression method was 0.55 and 0.65 for the two crosses (Park and Rupert), suggesting that selection for MD tolerance may be moderately effective and breeding is possible. This study aims to investigate a) seed characteristics in relation to MD and b) to examine micro-structure of seed coat of RIL by scanning electron microscopy.

Materials and Methods

RIL were derived from 2 crosses between navy beans susceptible and tolerant to MD; Cross 1, Envoy/OAC Laser, and Cross 2, OAC Speedvale/Vista. 32 $F_{4:6}$ lines from each of the two crosses were grown in field plots at 2 sites in 2003. The lines were selected on the basis of average mechanical damage index (MDI) by selecting the low 10%, mid 10% and high 10% of MDI of the base populations with over 130 RIL.

Seed samples were manually harvested at 18% moisture. 20 average sized whole beans were sampled from each plot, using three replications. For the scanning electron microscope (SEM) study, about 10 seed samples were taken from the same plots and moisture adjusted until they were soft enough to dissect seed to examine micro-structure of seed coat.

Seed characteristics were observed using a digital image analysis system. Digital image was taken on top and side surfaces for length (L)and width (W), and the image was converted to numerical data by Sigma Scan pro@. Then, the ratio of L/W was estimated and also seed shape was visually scored as 1 for round and 5 for oblong seed. Then, correlations between MDI and the seed characteristics were estimated.

Microstructure under SEM: A Hitachi S-510 Scanning Electron Microscope was used for observing the seed coat tissues. Dissection of seed coat tissues observed was palisade, hourglass cell, air space above parenchyma (in between palisade and hourglass cell layers), parenchyma and air space below parenchyma cells (in between parenchyma and endosperm) (Fig. 1). Measurement data taken from the side and centre of seed coat image (300x magnification) were averaged and regressed with MDIs measured by a simulation device and MDIc by direct combine.

Results and Discussion

Seed characteristics: OAC Speedvale has round, large seed, and vista has oblong narrow shaped seed. Association between MDI and seed characteristics was examined by correlation analysis (Table 1). Cross 1 showed closer associations between both MDIc and MDIs and the seed characteristics such as length, width and surface area. The correlation seed between seed surface area and MDI estimated by both methods were significant in cross 1. Some characteristics may be used in selecting for mechanical damage tolerance but this relationship depends on parental variability and appears to be cross specific.

Micro-structure of Seed Coat: Thickness of seed coat cell layers was somewhat different among parental lines. The susceptible cv OAC Laser has thick seed coat layers, 48.5 u of palisade cells and 22 u of parenchyma cells. It also has larger air space, 21.5 u. The resistant cv Envoy has slightly thinner seed coat cell layers (43.5 u of palisade and 13.5 u of parenchyma cells, and 12 u of air space) than OAC Laser. Two parental lines of cross 2, Vista and OAC Speedvale had thinner seed coat cell layers than those of the cross 1 parents. RIL of three selection classes had a wide range of cell layer thickness of palisade and parenchyma, and air space but there were smaller mean differences among the lines than the parental lines.

In correlations analysis, air space below parenchyma cell layer showed significant positive correlations with MDIs from 2003 trials at Harrow (r=0.371) and from 2003 combined MDIs from Harrow and St. Thomas (r=0.366). Other correlation coefficients were not significant but showed similar positive tendency. They are air space below parenchyma cells and MDIc of 2003 trial at Harrow (r=0.336) and MDIc of 2002-2003 trials combined at Harrow (r=0.322). Also, correlation between hourglass cell layer and MDIs of 2003 Harrow was r=0.357. A few negative correlations were also detected between palisade and parenchyma cell layers and MDIc. No conclusive association between micro-structural layers of seed coat and MDI could be obtained with these limited data from SEM observations. . It may be concluded that the air space between seed coat layers and endosperm might have a positive cushioning effect to reduce seed coat damage.

Acknowledgement: D. Pohlman & Ann Fook Young for electron-microscopy work, and C. Hughes for digital work.

Table 1. Correlation coefficients between seed characteristics and MDI of two bean crosses (*, ** Significance at p = 0.05 and 0.01 level).

Seed characteristic	Cross#1 (n=32)		Cross #2 (n=30)	
	MDIc	MDIs	MDIc	MDIs
Length(L)	0.469**	0.490**	0.155	0.400**
Width (W)	0.605**	0.459**	0.04	0.271
Thickness	0.106	0.485**	-0.025	0.096
L/W	0.462**	0.198	0.093	0.135
Shape	0.439*	0.170	0.153	0.077
Area	0.625**	0.596**	0.097	0.400**
Test weight	0.452**	0.523**	0.003	0.258

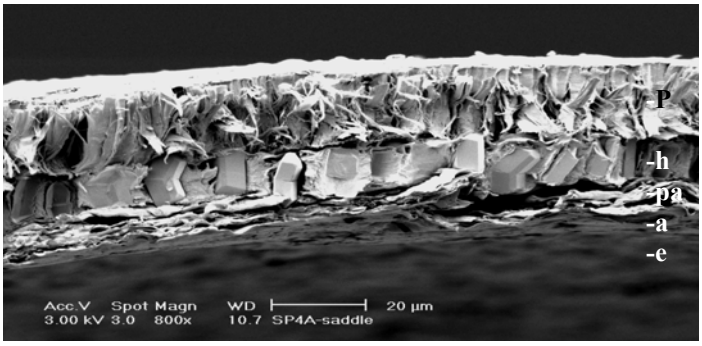


Fig. 1. Electron micrograph of bean seed coat. (P –*pallisade layer*, h - *hourglass cells*, pa -*parenchyma* (*partially crushed*), a – *aleuron*, e – *endosperm)*

EFFECT OF STORAGE ON SEED COAT COLOR CHANGES IN COMMON BEAN

Patricia Vargas Vázquez[1] and Patricia Pérez Herrera[1]

Campo Experimental Valle de México INIFAP-SAGARPA. PO Box 10, Chapingo, State of Mexico, Mexico, 56230.

Introduction. The variability in seed coat colour is one of the traits that define commercial types of common bean in Mexico and the seed coat darkening that occurs in storage can diminish their commercial acceptability, because of that, this phenomena is considered as an indicator of quality decrease and longer cooking time. The seed coat colour changes that occur during storage, even under controlled environmental conditions of temperature and moisture inside the warehouses were seed banks are stored, can be measured to identify germplasm stable in seed coat color when these are submitted to long time storage periods.

Materials and Methods. A group of 53 cultivars of the Mexican Common Bean Core Collection were selected for this study. The number of accessions according with their seed coat color were as follows: 3 white, 20 beige, 8 yellow, 4 brown, 5 pink, 8 purple, and 5 dark grey with beige spots like "rebocero" and "burro" landraces. Samples of 200 g of seed stored for 20 years (1984-2004) in seed bank warehouse under temperatures from 1 to 7°C (treatment 1) and 200 g of seed harvested in 2004 (treatment 2) for each cultivar, were used as test material. The seed coat color of each sample was registered, according to the scale of colour proposed by CIAT, and consisting on a visual comparison of sample colour with those included in the scale (CIAT, 1987). On each sample, the following colour traits were measured with a Minolta Hunter Lab colorimeter (CIE La*b, 10° and D65): lightness (from black=0, to white=100), a (red-purple= positive value and green-bluish= negative value), b (yellow= positive value and blue= negative value), hue angle and chroma that corresponds to the tone and intensity of colour, respectively (McGuire, 1992). The obtained data were submitted to an analysis of variance and means test (Tukey, 0.05), under a complete randomized arrangement, using the SAS program (SAS, 1988). The obtained data for each individual colour were also statistically analyzed separately in order to identify those cultivars with less susceptibility to seed coat darkening.

Results and Discusion. Significant differences between treatments for lightness (L), a, b, chroma and hue were found. Tukey means tests showed that lightness, yellowness and hue values decreased in 69.4 %, 54.8 % and 18.9 degrees, respectively in those seeds stored for 20 years (Table 1). The a values were higher in seeds stored for 20 years than in those stored for only two years, These results are consistent with the visual darkening of seed coat appreciated in older seeds, were seed coats of aged beans tend to present darker and red-brownish colourations. This phenomena has been associated by Oliveira *et al*., (2002) to phenolic oxidation performed by peroxidase and polyphenoloxidase enzymes. Unexpectedly, chroma values were lower in seeds stored for 20 years than in those stored for 2 years. These results indicate that the change in the colour of seed coats is associated properly to changes in colour and not to the increase in the original colour intensity. White coloured beans. Among the three seed coat white coloured common beans (alubia) evaluated, accession 89 (Jal-42) did not show changes in yellowness during storage, while the other two accessions showed reductions in a and b, as a result of the development of brownish colorations on seed coat during long term storage.

Table1. Results of the means test for color traits of 53 bean cultivars.

Treatment	L	a	b	Chroma	Hue
2 years	41.22 **a**	8.44 **b**	16.80 **a**	19.16 **a**	65.26 **a**
20 years	28.59 **b**	8.96 **a**	9.21 **b**	13.33 **b**	46.40 **b**

Means with different letter are statistically different between treatments (Tukey 0.05).

Beige coloured beans. Beige coloured beans tended to reduce it yellowness with storage time, independently of those changes on seed coat redness, thus they show changes in their seed coat towards brownish colorations. Among the accessions studied in this group, a light gray coloured bean, accession 901 (Ags-16-2) named "panza de puerco" was outstanding because of the small changes in seed coat colour during long term storage; as well as accession 889 (Ags-4) a "bayo" bean, since it only showed a small decrease in yellowness, almost maintaining its red value (a).

Yellow coloured beans. In general, those beans included in this group tended to decrease its yellowness and increase it redness, this indicates that their seed coat changed from yellowish towards brownish colorations with storage time. Among them, accession 85 (Jal-40) was less affected by the long time storage, because in spite that it reduced its yellowness, a redness increase was not found.

Brown coloured beans. Brown beans showed a decrease in yellowness, associated to a clear tendency to increase redness with storage time. Among the accessions included in this group, a dark brown bean 957 (Ags-66) known as "frijol grulla", and 907 (Ags-22), known as "bayo rata" showed only light increases in redness and yellowness, thus the color of these cultivars was stable in this group.

Pink coloured beans. The general response of this group was similar to that of brown colored beans, showing a decrease in yellowness, and an increase in redness with storage time, thus color changes in seed coat of pink colored beans tended to the development of red brownish colorations. Accession 72 (Jal-29) was outstanding because no changes in its red color were found.

Purple coloured beans. The purple beans tended to decrease their yellowness, as well as their redness. Into this group, the accession with lower decreases in yellowness and redness was the 547 (Zac-13-A).

Dark gray coloured beans. The dark gray beans, speckled with small beige points, showed a clear tendency to increase redness and decrease yellowness. The five dark gray beans evaluated in this study developed dark brown color during long term storage.

Conclusions. In all commercial classes there were accessions that darkened less with storage than the average.

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SLOW AGING, DARKENING, OR OXIDIZING DRY BEAN

Shree P. Singh

Plant, Soil and Entomological Sciences Department, University of Idaho, 3793 North 3600 East, Kimberly, Idaho. Ph: 208-423-6609; Fax: 208-423-6699; E-mail: singh@kimberly.uidaho.edu

INTRODUCTION

Post-harvest darkening (also commonly referred to as aging or oxidizing) of dry bean (*Phaseolus vulgaris* L.) seed coat color is a widespread phenomenon common to all market classes including black and white. Nonetheless, the trait is more apparent and easily detectable in beige, cream, pink, and red colored dry bean irrespective of color pattern. The presence of secondary and tertiary colors may accelerate post-harvest darkening and facilitate its detection. Thus, darkening is more easily detected in mottled (e.g., Calima, Cargabello, Pinta, and Pompadour), speckled (e.g., Flor de Mayo), spotted (e.g., Barbunya, Cargamanto, Cavalo, Chitti, Coscorron, Cranberry, Frutilla, Pinto, and Speckeled Sugar), and striped (e.g., Carioca and Ojo de Cabra) dry bean. Cultivars with purplish or reddish coloration on hypocotyl, stem, leaf, flower, and/or pod are more likely to exhibit accelerated darkening of seed coat color. Although as time passes, water permeability (or absorption capacity) of dry bean seed coat decreases and soaking and cooking time increases regardless of the degree of post-harvest darkening, darkened dry bean suffers from guilt by association and hence is heavily discounted in the market.

EFFECT OF ENVIRONMENT

Growing environment (especially humidity, temperature, and light) has marked effects not only on post-harvest darkening but also on primary and secondary seed coat color development, as well as on seed shape, size (and thus weight), storability, nutrient concentration, and germination. The problem of seed coat darkening is accentuated when dry bean is grown, harvested, and/or stored in relatively humid and/or warmer environments. Similarly, drought or moisture stress during the reproductive period, soils deficient in or possessing toxic levels of major and minor elements, and other stresses including diseases and insect pests aggravate the problem of post-harvest seed coat color darkening. Thus, non-stressed relatively cooler and dryer environments such as those in the Magic and Treasure Valleys in southern Idaho not only facilitate production of high quality pathogen-free seed, but also favor normal development of dry bean seed characteristics and slow the rate of post-harvest darkening.

VARIABILITY FOR SLOW AGING

Lack of pigmentation in the hypocotyl, stem, pod, and seed coat is recessive to its presence. Similarly, relatively lighter colors and the absence of secondary and tertiary colors imparting mottling, speckling, spotting, and striping are recessive to their darker counterpart seed coat colors. It is likely that Native Americans recognized, appreciated, and selected attractive, stable, seed coat colors throughout the domestication range of dry bean in Mexico, Central America, and Andean South America. Thus, slow darkening is a common feature of most of the highly priced landraces in these regions (e.g., Flor de Mayo, Garbancillo Zarco, Rojo de Ceda, Chingo, Cargamanto, Radical, Frutilla, Coscorron) irrespective of seed size and shape. Often, these landraces have high adaptation specificity and are highly susceptible to bacterial, fungal, and viral diseases and are therefore difficult to grow on a large scale without excessive use of pesticides. I first realized the importance of seed color characteristics of landraces for local

consumers and traders when visiting bean production regions in Brazil (e.g., landraces Cavalo and Sempre Assim), Colombia (e.g., landraces Cargamanto and Liboreño), and Ecuador (e.g., landraces Bola Canario and Bola Roja) in 1977-1978 and subsequently in other countries.

BREEDING FOR SLOW AGING

When highly appreciated dry bean landraces, irrespective of their market class, are crossed with distantly related germplasm to introgress other useful traits, such as erect plant type, early maturity, and disease and insect pest resistance, it is difficult to recover the best of both groups of parents. To this author's knowledge, none of the improved breeding lines and cultivars resistant to insects and diseases developed thus far possesses seed characteristics comparable to that of their respective highly priced dry bean landraces within the same domestication range in Latin America.

In regards to breeding slow darkening pinto bean cultivars for North America, I used landraces from the Mexican highlands in multiple-parent crosses for collaborative projects for Canada, Mexico, and the U.S.A. while at CIAT in Colombia in the 1980's. Slow darkening "Pinto Saltillo" (Sánchez Valdez et al., 2001, 2004) is an example of such collaboration. Among other pinto landraces and breeding lines with slow aging are G17341 and Zacatecano. After moving to Idaho, I have witnessed considerable variation among the U.S. pinto cultivars for slow darkening. For example, under normal room temperature storage in southern Idaho, Bill Z, Montrose, and Topaz are of comparatively lighter color and darken slowly. In contrast, Kodiak and UI 320 darken faster. In addition to slow darkening pinto landraces and cultivars, I have successfully used great northern (e.g., Matterhorn) to develop a wide range of slow- darkening pinto breeding lines, some of which are currently in the Idaho and Western Regional Bean Trials and National Cooperative Dry Bean Nursery. Use of a true white bean broadens the range of seed coat color for slow darkening. But, in crosses with pinto bean, it is useful to keep the genetic contribution of white germplasm <25%. Because slow darkening is a recessive trait also affected by environment and by QTL with small effects, its early generation selection should be avoided. Also, there is need to develop relatively larger populations and use multi-location testing in contrasting environments to select for stable desirable pinto seed coat color. In southern Idaho, we use drought-stressed, low soil fertility, and continual bean cropping environments for selection of stable seed characteristics including slow darkening. Some researchers are using controlled humidity and high temperature environments and UV light as an alternative to or in combination with long-term storage to select for slow darkening. A small quantity of improved slow darkening pinto germplasm for research purposes may be obtained from the author.

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QUANTIFICATION OF CHANGE IN PINTO SEED COAT COLOR AFTER AN ACCELERATED AGING TREATMENT

John Rayapati, Brett Despain, Pat Jennings, Trish DeMark, Roger Hoffman
Archer Daniels Midland Company, Edible Bean Specialties, 1001 N Brush College Rd , Decatur Illinois 61856, USA ; rayapati@admworld.com

Introduction

The market value of pinto beans is determined by visual appearance. Pintos that have darkened due to environmental influences before harvest or during storage lose value. A method is needed that can be used by breeders to screen genotypes for reduced darkening. Processors also need a method for evaluating multiple lots of stored beans in order to identify the best lots for specific applications. This report presents a method that makes use of tools commonly found in seed germination and food technology laboratories to quantify the potential for darkening of specific pinto bean genotypes and lots.

In the 1940s Richard Hunter developed a 3 dimensional color evaluation system. He set the lightness scale (L) to range from 0 to 100, with 0 representing black and 100 representing white. The *a* coordinate represented the location of the color on the red-green axis, with positive *a* values representing red and negative representing green. The *b* coordinate represented the location of the color on the blue-yellow axis, with positive *b* values representing yellow and negative values representing blue.[2] This method of measurement was used to eliminate errors caused by subjective visual evaluation.

Methods

Four varieties were grown in the same field and harvested at the same time near Caldwell Idaho in 2004. A sample was retained as untreated. A sample was placed under accelerated aging treatment of 41^{o}C at 95% relative humidity for 72 hours. Seeds were placed on screens suspended over water in plastic boxes in an incubator with no light. After treatment all seeds were evaluated in a Hunter Color meter. Seeds were not germinated as they are in the accelerated aging test. L, *a* and *b* values from the color meter were recorded. The delta E calculation was used to compare changes [1].

$$\text{delta } E = [(L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2]^{1/2}$$

L = black – white axis
a = red – green axis
b = blue – yellow axis
Subscript 1 = seed from field
Subscript 2 = seed treated with high temperature and humidity

Results

Table 1 Hunter color meter values and delta E calculations for change in pinto color

	L (black-white)		a (red-green)		b (yellow-blue)		delta E
	X	SD	X	SD	X	SD	
Buster AA	**33.78**	**0.24**	**6.62**	**0.05**	**8.15**	**0.08**	**3.89**
Buster	**37.37**	**0.40**	**5.20**	**0.07**	**7.74**	**0.02**	
Canyon AA	**34.97**	**0.12**	**5.69**	**0.14**	**7.77**	**0.14**	**2.80**
Canyon	**37.52**	**1.27**	**4.62**	**0.14**	**7.35**	**0.29**	
Othello AA	**33.93**	**0.10**	**5.68**	**0.05**	**7.62**	**0.02**	**2.05**
Othello	**35.92**	**0.08**	**5.21**	**0.09**	**7.60**	**0.10**	
Maverick AA	**34.15**	**0.44**	**5.96**	**0.11**	**8.04**	**0.14**	**4.03**
Maverick	**38.08**	**0.05**	**5.06**	**0.14**	**7.89**	**0.18**	

X = mean
SD = standard deviation
AA = accelerated aging treatment
n = 4 measurements

Conclusions

1. The Hunter color meter can quantify pinto darkening before it can be seen by the naked eye.

2. The delta E value is a simple way to standardize color changes including darkening.

3. The accelerated aging treatment can enhance darkening in a reasonable time frame for plant breeding and for commercial evaluations.

4. Canyon descends from a cross between Buster and Othello. Its intermediate phenotype is not explained by single gene control of the pinto darkening trait.

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ASSESSING GERMPLASM RESISTANCE TO THE SOYBEAN APHID VIRUS COMPLEX

Michell Sass,[1] Thomas German,[2] and James Nienhuis,[1] UW-Madison, Departments of Horticulture[1] and Entomology[2]

Over the last five years, a vast array of virus-like symptoms has been observed in commercial snap bean fields across the Great Lakes region, particularly in Wisconsin. Some symptoms can be specific to a given year while other symptoms tend to reappear year after year. The most common recurring symptoms include leaf mottling and blistering associated with *Cucumber mosaic virus* (CMV), yellow flecking of the leaves associated with *Alfalfa mosaic virus* (AMV) as well as vein banding, stunting, and flower abortion.

Surveys of Wisconsin's major snap bean production areas identified a high incidence of CMV and AMV in 2002 and 2003 in all of the growing regions (German et al., 2004). Stevenson, Grau, and German (2001-2005) have screened numerous commercial snap bean cultivars and breeding lines and report that currently none of the commercial snap bean germplasm is resistant to the virus complex; however, various levels of tolerance have been observed (Stevenson, et al., 2006). Larsen and Eastwell (2004), detected a strain of *Clover yellow vein virus* in necrotic snap bean pods from Wisconsin in 2000-2004 using RT-PCR. The necrotic pod symptom has also been associated with the CMV/AMV complex. It is also possible that there still may be unidentified viruses, additional strains or other causal agents involved.

The objective of this research was to identify sources of resistance to CMV and AMV by looking at *a)* gene families (e.g. virus resistance genes), *b)* rare alleles, and *c)* inbred lines and backcross populations using visual evaluations and ELISA.

Materials and Methods

To date, we have screened approximately 1000 lines in replicated trials.

1) Because similar genes tend to cluster together in gene families, we evaluated Plant Introduction (PI) accessions from the USDA *Phaseolus* germplasm collection previously identified as resistant to other viruses.
2) We screened the *P. vulgaris* PI core collection (423 accessions) that serves as representative sample with 95% probability of maintaining the rare alleles of the reserve collection (over 12,000 accessions).
3) We evaluated a random sample of *P. vulgaris* PI accessions from the reserve collection.
4) Resistance may already exist in a snap bean background and could be introgressed via breeding into current cultivars. We evaluated heirloom (pre-1950) snap bean varieties.
5) If resistance exists in a highly inbred, mapped population, gene(s) linked to resistance can be identified. We evaluated a recombinant inbred line population (Eagle x Puebla 152) developed by our program. Although we did find repeatable differences for aphid preference, no resistance to CMV or AMV was identified in this population.

Each year, a replicated trial was planted using a replication within block design at Arlington Agricultural Research Station in mid-July. Two weeks prior to planting the trial, mixed spreader rows consisting of a 1:1 seed mix (by weight) of soybean and the snap bean cultivar 'Hystyle' were planted using a 4-row corn planter. The expanding trifoliate leaves of Hystyle in the spreader rows throughout the trial were chosen at random and mechanically inoculated with AMV and CMV. Throughout the growing season plants were evaluated visually for the presence or absence of virus symptoms. At approximately 46 days post-inoculation, a composite sample

of 10 leaves (1 expanding trifoliate leaf per plant) from each plot was harvested and tested by ELISA for CMV and AMV. All symptomless plants inoculated with CMV, and symptomless plants inoculated with AMV that were also ELISA negative were increased in the greenhouse and then included in the multi-location trials in collaboration with Dr. Walter Stevenson, UW-Madison, Dept. of Plant Pathology the following year.

Results

Over locations and years, we identified four accessions (PI 599026, PI 182000 selection S, PI 599014 and selection 2313.9.1000) that were symptomless and although ELISA positive for CMV may possess tolerance to the virus. In addition, we identified six accessions (PI 288016, PI 449412, PI 549853, PI 416468, selection 2313.9.1000, PI 599021) that were both symptomless and ELISA negative for AMV and thus may possess resistance to AMV. All germplasm will be further evaluated using manual inoculations in the greenhouse.

As a result of data obtained in 2002-2004, we initiated the development of two populations:

1) A symptomless x symptomless population (MV185 x 2313.9.1000)
2) A susceptible x symptomless population (Hystyle x 2313.9.1000)

Using these populations, we will determine if we have identified tolerance and if the tolerance is heritable, or if the symptomless phenotype is associated with the dry bean parentage of selection 2313.9.1000. These populations are currently being inbred and will be evaluated as F3 families.

Conclusion

To date, we have not identified resistance to CMV or AMV. However, we will continue to evaluate selections from the PI collection for sources of resistance. Tolerance is likely to be as effective as resistance when there are no other agronomic consequences. The begomovirus (family *Geminivirdae*) *Bean golden mosaic virus* is an example of the successful deployment of virus tolerance in dry beans that has held up for over twenty years in the tropics (Beebe and Pastor-Corrales, 1991).

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DEVELOPMENT OF A SCAR MARKER FOR COMMON BEAN RESISTANCE TO THE BEAN POD WEEVIL *(APION GODMANI* WAGNER).

M.W. Blair[1], C. Cardona[1], C. Quintero[1], R. Garza[2], N. Weeden[3], S.P. Singh[4]

1/ CIAT – International Center for Tropical Agriculture - A. A. 6713, Cali, Colombia, 2/ EVAMEX, INIFAP – Santa Lucía de Prías, Mexico 3/ University of Montana, Missoula, MT, United States 4/ University of Idaho - Kimberly Research and Extension Center - Kimberly ID, United States

Introduction: The bean pod weevil (Apion godmani Wagner) is a serious pest of common bean (Phaseolus vulgaris L.) grown in Mexico and Central America that is best controlled by host-plant resistance available in some landraces from the central highlands of Mexico and Guatemala. Yield loss caused by the insect can be as high as 90% but is variable depending on climatic conditions, insect pressure, and cultivars (Cardona and Kornegay, 1999; In Global Plant Genetic Resources for Insect-Resistant Crops). Chemical control of the bean pod weevil uses organophosphate insecticides, which while effective increase production cost and can lead to environmental contamination and health problems. Mechanisms of resistance to the bean pod weevil in common bean are either antibiosis involving a hypersensitive response that encapsulates the oviposition sites, insect eggs or larvae within necrotic tissue; or antixenosis that affects the preference for oviposition sites (Garza et al., 1996; Theor Appl Genet 92: 357-362). Breeding for resistance is effective but hampered by unreliable insect infestation, making the use of marker assisted selection desirable. In the present study, our goal was to develop a segregating population for Apion resistance, test it in the field and use it to develop SCAR markers for molecular assisted breeding.

Materials and Methods: The segregating population consisted in 50 $F_{5:10}$ recombinant inbred lines derived from the cross Jamapa x J-117, where 'J-117' is a resistance source and 'Jamapa' is a susceptible black-seeded cultivar, both from Mexico. The population was evaluated for Apion resistance over four consecutive seasons (1994-1997) at the Santa Lucía de Prías Experiment Station of the Instituto Nacional de Investigaciones Agropecuarias (INIFAP) near Texcoco, Mexico. All experiments consisted of a randomized complete block design with four replicates. Each test plot consisted of one row, 4 m in length, with spacing of 10 cm between plants within rows and 85 cm between rows. *A. godmani* was the only major insect pest observed causing damage to bean pods and seeds during the experiments and no pesticides were used during the experiments. The number of damaged and total seeds were recorded, and the percentage of damaged seeds was calculated and data were transformed by the arcsine square root proportion and analyzed using the general linear model (GLM) procedure using the software Statistix. Total genomic DNA was extracted for parents and RILs from three young trifoliolates harvested in 1.5 ml Eppendorf tubes by the method of Afanador et al. (1998; BIC 36:10-11) and used for standard RAPD reactions carried out on a PTC-100 thermocycler from MJ Research with decamer primers from Operon. Bulked segregant analysis was carried out with eight resistant and susceptible genotypes. A single RAPD band (OPK16-890R) was isolated from 1% low melting point agarose gels for ligation into the PGEM-T easy vector from Promega using T4 DNA ligase followed by DNA sequencing of the plasmid and SCAR primer design.

Results and Discussion: Significant differences between genotypes were observed for the percentage of damaged seed in the analysis of variance (Table 1). Genotype by season interaction effects were significant indicating differential reactions of the genotypes to *A. godmani* in different seasons however genotype effects were much larger than season effects and Pearson correlations for seed damage between seasons were high (r=0.617 to 0.684, P<0001). Significant differences between the parents were observed in every season with J-117 having an average seed damage of 5.2% and Jamapa having an average seed damage of 49.5%. In all seasons, the mean seed damage of the ten most resistant RILs was significantly different from that of the ten most susceptible RILs (data not shown). Meanwhile, for the marker survey, a single dominant SCAR marker was developed from the OPK16-890R RAPD band. The SCAR marker was named SK16 and had the following 5'to 3' forward and reverse primer sequences: For = GAGCGTCGAACGTGTTG and Rev = GAGCGTCGAAGGAGGAA. Standard PCR reactions were carried out with 25 ng of DNA as template and 25 ul reaction volumes containing 0.2 mM dNTP, 2.5 mM MgCl2, 1X BSA, 1X PCR buffer, 0.5 U Taq DNA polymerase and 0.2 uM each of the corresponding primers. SCAR amplification was best with the following PCR conditions: denaturation at 94°C for 2 minutes followed by 35 cycles of 30 second denaturation at 92°C, 30 second annealing at 65°C and 1 minute extension at 72°C. The SCAR was polymorphic in the Jamapa x J-117 population and in the bulks with clear positive and negative signals in PCR amplification. The dominant allele (band present) was from J-117 and the resistant bulk while in Jamapa and the susceptible bulk the band was absent. Although the SCAR was confirmed to be polymorphic when the bulks were opened, the amount of variance explained by this SCAR using single point regression analysis of the phenotypic data onto the marker genotypes of the entire population was low and only reached 3.6% in the most significant season. Although this locus may not correspond to the important resistance genes (*Agr* and *Agm*) identified by Garza et al. (1996) that provide high and stable levels of resistance, it may be associated to a new locus that provides intermediate resistance which can be affected by seasonal variation and different levels of insect pressure. In summary, we were successful at developing a SCAR marker from bulked segregant analysis but the SCAR was not linked with a high *A. godmani* resistance. Since the Jamapa x J-117 population was reliable both for the detection of polymorphism and for the phenotypic characterization of resistance to *A. godmani*, further study of this cross should be useful for identifying additional markers or resistance genes for this insect pest.

Table 1. Combined analysis of variance for percentage seeds damaged by *Apion godmani* in the recombinant inbred line population 'Jamapa' x J-117 screened over four consecutive seasons (1994-1997) at Santa Lucía de Prías, Mexico.

Source	DF	SS	MS	F Value	Prob.
Season	3	3.71798899	1.23932966	312.27	<.0001
Repetition (Trial)	12	0.08963140	0.00746928	1.88	0.0337
Genotype	51	16.64755351	0.32642262	82.25	<.0001
Season x Genotype	152	4.14146750	0.02724650	6.87	<.0001

FIELD MANAGEMENT OF COMMON BEAN BRUCHIDS BY USING SELECTED PHYTOCHEMICALS IN HARICOT BEAN (*PHASEOLUS VULGARIS*)

Mesele Gemu, Shiferaw Mekonen and Asrat Asfaw
Awassa Agricultural Research Center
P,O.Box 06, Awassa, Ethiopia

Introduction

Bean storage over long periods at small-scale subsistence farming levels in Ethiopia is limited due to two common bean bruchids *(Acanthoscelides obtectus* and *Zabrotes subfasciatus)* infestation that result in heavy losses in terms of quality, weight and nutritional value. To avoid such excessive losses, most farmers are forced to sell off bulk of surplus grain immediately after harvest when the prices in the local market are much low. This scenario negates motivation to increase production and store for longer periods to have uniform supply of food for the household through out the year.

Bean bruchids are field to storage insect pests. They start infestation in the field and this field infestation serves as initial inoculum to start infestation in the store. The available bean bruchids management methods targeted only in storage with no attention to field infestation. These include the use of edible oils and contact insecticides and fumigants. Chemicals are not affordable by most of subsistence and resources poor farmers of the region. On top of this, the approach is not reliable, as it is not environmentally sound and risk to consumers. . In this study an attempt was made to evaluate field management of bean bruchids by selected botanicals, which proven effective in reducing damage by other legumes in the storage or in the field.

Materials and methods

The experiment was carried out at Awassa Agricultura;l Research Center on-station in 2004/2005 cropping season. The common bean variety, Red Woliata susceptible to field infestation was planted in plot of 2mx2m size at spacing of 10 and 40cm within and between rows, respectively. The distance between plots and blocks was 1m and 2m, respectively. The plots were arranged in a randomized complete block design (RCBD) in three replications. The botanicals to consider in the experiment were *Phytolacca dodecandra*, *Tagetes minuta*, *Nicotina tobaccum* and *Milletia frugenia*. Fenithrothion 60% E.C as standard check and the untreated control were included in the experiment for comparison.

Botanical preparation

One kg of green leaf of each botanical was collected from available areas and then grounded by using Iron and pestle. Then, the grounded leaf was soaked in two liters of water and filtered using fine cloth to obtain concentrated extract. Finally, the concentrated extract was further diluted in four liter of water prior to application. Then, 10gm of powdered soap was added before spraying on the crop to increase the adhesive nature of the extract on the crop. The diluted botanical solution was sprayed at the rate of 400lit/ha on each plot.

Treatment application was initiate at pod filling stage after planting, and was continued on weekly bases four more times. The beans were harvested when the pods dry. Four hundred-gram of working samples was placed in 3lit volume polyten bag and incubated at Awassa Agricultural Research Center laboratory under ambient conditions. The samples will be monitored daily for

common bean bruchids emergence. The bruchids that emerged was identified, counted and discarded in daily basis and this was conditued until there was no further emergence. The mean of collected data were analyzed using MSTAT-C statistical package.

Results and discussion

The only bruchid species that emerged in all treatments was *Acanthoscelides obtectus* (Coleoptera: Bruchidae). There was significant differences ($P<0.05$) among botanical treatments in the number of adult *A. obtectus* that emerged (Table 1). The highest number of adult *A. obtectus* was emerged from *Milletia frugenia* treated plot, while the lowest was recorded from *Nicotina tobaccum* treated plot. The number of adult *A. obtecctus* emerged from *Phytolacca Dodecandra* treated plot and *N. tobaccum* was equal where as the number of adult target pest emerged from *M. fruginia* and *Tagetes minuta* was equal and statistically higher compared to both *P. dedocandra* and *N. tobaccum*. The result of this study indicates that *P. dedocandra* and *N. tobaccum* showed potential to suppress field infestation of adult *A. obtectus* compared to other botanicals.

There was significant difference ($P<0.05$) between botanicals treatments and the untreated` control plots in terms of number of adult *A. obtectu* emerged. The highest number of target pest was recorded from *M. fruginia* (13.3) followed by untreated control (12.7), while the lowest was recorded from *N. tobaccum* followed by *Phytolacca dodecandra* treated plot. There was significant differences ($P<0.05$) between botanical treatments and the chemical treated plot interms of number of adult target pest emerged. The lowest number of adult *A. obtectus* was emerged from *N. tobaccum* treated plot followed by fenithrothion 60% E.C treated plot, while the highest was observed in *T. minuta* treated plot. The effect of *N. tobaccum* and *P. dedocandra* in controlling the number of adult *A. obtectus* that emerged was similar to the chemical. In terms of percent seed damage among botanicals applied, tobacco and *P. dedocandra* treated bean plot were the least damaged compared to the other botanicals and the untreated control.

This study has confirmed bruchid bean infestation by *A. obtectus* in the field and its continuation in storage. This was demonstrated by the emergence of F1 generation from the cultured bean seeds in the laboratory.

Table 1. Effect of botanicals on *Acanthoscelides obtectus* mean adult emergence after incubating for one month, Awassa 2004

Treatment	Number of emerged adult bean bruchid	% of damaged seeds
Phytolacca Dodecandra	3.0b	0.2b
Tagetus minuta	12.0a	1.0a
Nicotina tobaccum	2.3b	0.2b
Milletia frugenia	13.3a	1.0a
Fenithrithion 60% E.C.	2.7b	0.2b
Untreated control	12.7a	1.1a
CV% LSD	34.59 4.83	34.66 0.39

ON-FARM EVALUATION OF EDIBLE OILS AGAINST BEAN BRUCHIDS (*ACANTHOSCELIDES OBTECTUS & ZABROTES SUBFASCIATUS)*

Mesele Gemu, Shiferaw Mekonen and Asrat Asfaw
Awassa Agricultural Research Center
P.O.Box 06,Awassa, Ethiopia

Introduction

In Ethiopia common bean (*Phaseolus vulgaris)* is very common crop and covers a considerable acreage each year .It is mainly grown as a food and cash crop and grows best at altitudes between 1700 and 2000 masl (Westphal, 1974). Despite its wider use and high food value, there are many problems, which limit its production and productivity in the field as well as after harvest in the storage. In the field the most production constraint is the wide range of insect pest attacking every parts of the crop. In the store, common bean bruchids, *Acanthoscelides obtectus* and *Zabrotes subfasciatus* are the most important ones. They put a great burden to the crop interms of weight loss, reduction in quality and seeds viability losses. They cause up to 100% seed damage within three months of storage period if sound control measure is not taken immediately after storage section (Awassa Agricultural Research Center Crop Protection Annual report, 2004) The environmentally safely and resource poor farmers affordable technology which have been found effective in controlling these bruchids, edible oils, were verified under farmers condition to facilitate the adoption of technology in the two common bean producing areas of southern Ethiopia.

Material and methods

The verification trial was carried out in two haricot bean bruchid threaten *wereds* of the southern Ethiopia, namely Sodo zuria and Awassa zuria. One PA from each *wereda* was selected with the help of experts from BoA of respective *wereda* based on its potential to haricot bean production and bean bruchid problem. Then, from each PA three model farmers were selected with the help of DAs in each PA. Then, edible oils (Flax and Noug) at the rate of 5ml/kg was being mixed with four kilogram of haricot bean variety, Red Woliata, which is popular to the areas and susceptible to the bruchids. Then, the treated seed was put in the 8k volume of cotton cloth bag. Actellic 2gm/kg and the untreated control were included for comparison. Then, in each treatment 10 pairs of newly emerged bean bruchids was introduced and the treatments were kept in the ambient temperature in the farmers' condition in each PA. Then the observation was made on the treated and the untreated control on number of eggs laid/ sample, number of exit holes and the percentage of damaged seeds were parameters measured for verification of the treatment effects. During treatment application (mixing oils with haricot bean seed, Red Woliata) 10-15 farmers were invited to observe method of mixing oils with seeds in each locations to create awareness about method of mixing oils with seeds. Finally at the end of verification trial the effect of edible oils against bean bruchids was observed by 20-30 farmers in each trial sites. More than 50 farmers were allowed to observe the effect of edible oils against bean bruchids and the effect of edible oils on the taste of boiled haricot bean to check whether it had effect on the taste of boiled haricot bean. In both sites the amount or dosage of edible oils required for different amount of haricot bean was given to the trial farmers and others who invited to observe the effect

Results and discussion

Treatment	Awassa Zuria Wereda		Sodo zuria Wereda	
	Number of eggs/10 seeds	% of damaged seeds	Number of eggs/10 seeds	% of damaged seeds
Niger seed oil Untreated control	1.3b 22.7a	3.3b 80.0a	1.0b 25.3a	2.3b 83.3a
Flax oil Untreated control	0.7b 22.70a	3.0b 80.0a	0.3b 25.30a	1.3b 83.3a
Actellic 2% dust Untreated control	0.3b 22.7a	3.3b 80.0a	0.3b 25.3a	3.0b 83.30a
Niger seed oil Flax oil	1.3 0.7 NS	3.3 3.0 NS	1.0 0.3 NS	2.3 1.3 NS
Niger seed oil Actellic 2% dust	1.3 .0.3 NS	3.3 3.3 NS	1.0 .0.3 NS	2.3 3.0 NS
Flax oil Actellic 2% dust	0.7 0.3 NS	3.0 3.3 NS	0.3 0.3 NS	1.3 3.0 NS

Paired- t-test at P=0.05

Reference: Awassa Agricultural Research Center Crop Protection Annual report, 2004.

PYRAMIDING OF ANTHRACNOSE, ANGULAR LEAF SPOT AND RUST RESISTANCE GENES IN BLACK AND RED BEAN CULTIVARS

[1]COSTA, M.R.; [1]TANURE, J.P.M.; [1]ARRUDA, K.M.A.; [2]CARNEIRO, J.E.S.; [1,3]MOREIRA, M.A.; [1,4,*]BARROS, E.G.

[1]Instituto de Biotecnologia Aplicada à Agropecuária (BIOAGRO), Universidade Federal de Viçosa (UFV), [2]Departamento de Fitotecnia (UFV), [3]Departamento de Bioquímica e Biologia Molecular (UFV); [4]Departamento de Biologia Geral (UFV), Viçosa, Minas Gerais, Brazil

*Corresponding author: *barros@ufv.br*

Brazil is the largest common bean consumer country in the world, and the second bean producer (FAO, 2006). Common bean varieties with "carioca-type" grains are the most popular ones and are grown all over the country. However, black and red beans are also consumed in specific regions of the country. Black beans are highly consumed in the states of Santa Catarina, São Paulo, Espírito Santo and Rio de Janeiro. As for red beans, the concentration of consumers and farmers is in the Zona da Mata Mineira (Minas Gerais state). These grains are commercialized in specific periods of the year, and their market price can reach up to three times that of the "carioca-type" beans.

'Diamante Negro' is a black bean cultivar derived from a crossbreeding process conducted at CIAT, in Colombia, and further selection carried out by Embrapa Rice & Beans (Brazil). This cultivar is highly productive, resistance to common bacterial blight and to common mosaic virus. In addition it presents remarkable commercial and culinary qualities (Embrapa, 1991). Nevertheless, it is susceptible to several races of anthracnose (*Colletotrichum lindemthianum*), angular leaf spot (*Phaeoisariopsis griseola*) and rust (*Uromyces appendiculatus*). 'Vermelhinho' is a landrace cultivar with red grains which is highly consumed and planted in the Zona da Mata Mineira region. However, it is also highly susceptible to the diseases previously mentioned.

In previous works by the BIOAGRO/UFV Bean Improvement Program, a line derived from cultivar Rudá, designated Rudá "R", with "carioca-type" grains harboring resistance genes for anthracnose (*Co-6* and *Co-4*), angular leaf spot (*Phg-1*) and rust (*Ur-ON*), was developed through a process assisted by molecular markers (Ragagnin et al., 2005). The main goal of this work was to transfer these genes to cultivars Diamante Negro and Vermelhinho in a process monitored by molecular markers.

The molecular markers used for the indirect selection of the resistance alleles were: SCAR $Y20_{830a}$, SCAR $AZ20_{940a}$, SCAR $H13_{490a}$ and SCAR $F10_{1050a}$ which are linked to the genes *Co-4*, *Co-6*, *Phg-1* and *Ur-ON*, respectively (Miklas, 2005). Cultivar Diamante Negro was crossed with Rudá "R" and the F_1 plants obtained were backcrossed with 'Diamante Negro'. The backcrossing process was repeated until seeds BC_3F_1 were obtained. During each backcrossing cycle, the presence of the appropriate molecular markers was checked in the plants.

Due to the low number of plants which simultaneously harbored the four markers mentioned, plants possessing at least three of the markers were also selected. Thirty-two BC_3F_1 plants with at least three markers were obtained; six of them carried the four markers. All 32 plants were selfed and 16 BC_3F_2 plants with at least three markers, including one with the four markers were obtained. All these plants were selfed and 39 BC_3F_3 plants were generated with at least three markers, including eight with the four markers. These plants are now being multiplied in the field for further evaluation of their yield potential and reaction to several races of *C. lindemthianum*, *P. griseola*, and *U. appendiculatus*.

As for the Vermelhinho cultivar, F_1 seeds were obtained from crosses with Rudá "R". F_2 and F_3 plants were selected based on the presence of the appropriate molecular markers. Among 68 F_3 plants, eight presented the four markers. These plants were backcrossed with cultivar Vermelhinho in order to recover the red color of the grains. Six BC_1F_1 plants were selected with at least three markers. These plants are now being backcrossed once more because one backcrossing cycle was not enough for the recovery of the red color. By the end of the breeding process we shall have black and red bean cultivars to be released for specific growing areas in Brazil.

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POTENTIAL USE OF TRAP MARKERS FOR MAPPING TELOMERES IN COMMON BEAN

Phillip N. Miklas[1] and Jinguo Hu [2]

[1]USDA-ARS, Vegetable and Forage Crop Research Unit, Prosser, WA 99350;
[2]USDA-ARS, Northern Crop Science Laboratory, Fargo, ND 58105

A characteristic of genetic linkage maps is genomic length measured in centiMorgan (cM) which can be influenced by polymorphism between the parents, number of markers, and recombination frequency. The length of the *Phaseolus* genome is estimated to be 1250 cM (Gepts et al., 1993). The length of corresponding individual linkage groups varies greatly among maps, and the actual genetic lengths are unknown because each linkage group starts and ends with the polymorphic markers at its outermost positions. Each linkage group corresponds to one chromosome, and each chromosome has two terminal ends called telomeres. Our objective was to pursue the possibility of employing TRAP (Target Region Amplified Polymorphisms; Hu and Vick, 2003; Miklas et al., 2006) markers to map bean telomeres in order to provide a better estimate of the actual length of linkage groups (chromosomes) and to develop anchor markers to facilitate map integration and alignment of linkage group ends.

The telomeric DNA of higher plants (*Arabidopsis*) was determined to consist of TTTAGGG repeats (Richards and Ausubel, 1988). Fixed primers were based on this repeat: CCCTAAACCCTAAACCCTAAAA (TeloRA) and CCCTAAACCCTAAACCCTAAAG (TeloRG). Arbitrary primers used with TeloRA were TTCTAGGTAATCCAACAACA (Sa12-700) and GGAACCAAACACATGAAGA (Ga5-800), and with TeloRG were CTATCTCTCGGGACCAAAC (Odd26-700) and CCAAAACCTAAAACCAGGA (Odd3-800). The TRAP protocol standardized by (Hu and Vick, 2003) was followed except for the following modifications: 1) molar ratio of fixed primer to arbitrary primer was changed from 30:1 to 10:1, and 2) the annealing temperature of the first five cycles was changed from 35º C to 40º C and for the last 30 cycles it was changed from 50 to 53º C.

There were 45 TRAP markers generated by the four primer combinations in the BAT 93/Jalo EEP558 (BJ) RIL mapping population, ranging from 6 to 14 TRAP per combination. Ten TRAPs were codominant. Thirty-nine TRAPs where located on the BJ linkage map and six were unlinked. Each linkage group had at least one TRAP marker, except linkage group B7 which had none. Linkage group B3 had ten TRAPs with five of them clustered near RFLP marker D1377. Only two TRAPs mapped to the very end of a linkage group (B3 and B10), and three TRAPs mapped near the end but internal to terminal markers for linkage groups B2, B8, and B9. Note that location of a TRAP marker at the terminal end of a linkage group will be dependent upon the marker dataset used. The BJ lines (70 RILs) and dataset for this study consisted of ~400 markers, and were kindly provided by Dr. P. Gepts (UC-Davis). Probes based on the *Arabidopsis*-type telomeric repeat TTTAGGG were similarly dispersed in chickpea with a cluster on chromosome B suggesting ancient chromosomal fusion or rearrangement occurred with satellite DNA (Gortner et al., 1998). Telomeric repeats were also found in interstitial, centromeric, and subterminal locations in other plants (Gardiner et

al., 1996; Presting et al., 1996), and based on the preliminary results herein, may be similarly dispersed in common bean.

These preliminary results suggest that the TRAP technique using fixed primers derived from the *Arabidopsis*-type telomeric repeat may not be useful for mapping telomeres in common bean. However, trying different fixed primers and additional primer combinations is warranted before abandoning the technique altogether given its success in sunflower. In sunflower, 18 primer combinations generated 226 TRAP markers of which 183 were located on 17 linkage groups (Hu, 2006). Although most of the TRAPs were dispersed across the genome, 32 markers were mapped to the outermost positions of the linkage groups, defining 21 of the 34 linkage group ends of the sunflower linkage map. The telomeric origin of a few of those markers was confirmed by sequence analyses.

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PROTOCOL FOR VISUALIZING SEQUENCE RELATED AMPLIFIED POLYMORPHISM (SRAP) AND TARGET REGION AMPLIFIED POLYMORPHISM (TRAP) MARKERS ON AGAROSE GELS

Karolyn A. Terpstra, Evan M. Wright, and James D. Kelly
Department of Crop and Soil Sciences, Michigan State University, East Lansing, MI

Introduction

The choices of molecular marker systems available to bean breeders and geneticists have expanded greatly in recent years. However, many newer marker systems have been developed for use on acrylamide gel systems. Sequence related amplified polymorphisms (SRAP) markers were originally developed in *Brassica* to be run on polyacrylamide gels (Li and Quiros, 2001). Target region amplified polymorphisms (TRAP) markers were originally developed in sunflower and run on denaturing acrylamide gels (Hu and Vick, 2003). Recently, TRAP markers have shown potential applications for mapping disease resistance in common bean (Miklas et al., 2006). The ability to screen these markers on agarose gel systems would allow for their wider adaptation and utilization within the bean breeding community. Utilizing agarose gels with ethidium bromide staining is less costly and faster to use than acrylamide alternatives for marker screening as well as for potential marker assisted selection applications. Both SRAP and TRAP markers have been successfully screened and genotyped in our laboratory for multiple populations utilized in QTL mapping studies on denaturing polyacrylamide gels using published SRAP and TRAP primer combinations. We were interested in evaluating these marker types on agarose gels in order to determine if agarose gels would provide a more efficient method to screen these markers.

Materials and Methods

DNA was extracted and quantified according to a modified CTAB extraction protocol. PCR was conducted using the same amplification protocol for both the SRAP and TRAP markers as optimized in our lab. The PCR recipe includes 1X PCR buffer, 1.5mM $MgCl_2$, 0.2 mM dNTP mix, 0.1 mM forward primer, 0.1 mM reverse primer and 1 unit Taq polymerase. The PCR was performed by initially denaturing the sample at 94ºC for 2 min; followed by five cycles of 94ºC for 45 sec, 35ºC for 45 sec, and 72ºC for 1 min; followed by 35 cycles of 94ºC for 45 sec, 50ºC for 45 sec, and 72ºC for 1 min; followed by 7 min at 72ºC. The PCR products were run on 2% agarose gels containing ethidium bromide (0.2 µL EtBr / mL gel) for 1.5 to 3 hours at 95V in TBE buffer. The bands were then visualized under UV light.

Results and Discussion

We have been able to successfully resolve the PCR products from both the SRAP and TRAP marker systems on 2% agarose gels in several bean cultivars (Figures 1 & 2). Multiple bands were resolved per primer combination and multiple polymorphisms per primer combination have been observed in the mapping populations we have evaluated to date. Interestingly, in figure 1, the four large seeded cultivars representative of the Andean gene pool (lanes 4-7) each have two bands present that are absent in the four small seeded cultivars from the Middle American gene pool (lanes 2-3, 8-9). Similarly, in figure 2, there is one band present in the large seeded cultivars that is absent in all small seeded cultivars. Figure 2 also shows a clear polymorphism

between the two black bean cultivars ‘Tacana’ and ‘Jaguar’. These polymorphisms represent differences within a market class and within the Middle American gene pool.

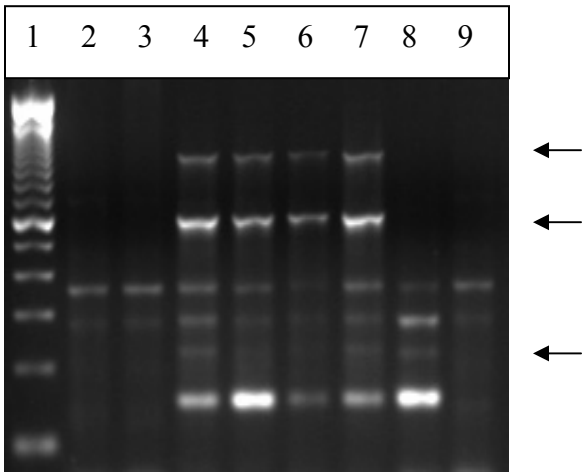

Figure 1. SRAP ME7/EM2. Lane 1: 100bp Ladder, Lanes 2-9: Bean Cultivars: Tacana, Jaguar, Red Hawk, Chinook 2000, Beluga, Capri, Merlot, Bunsi.

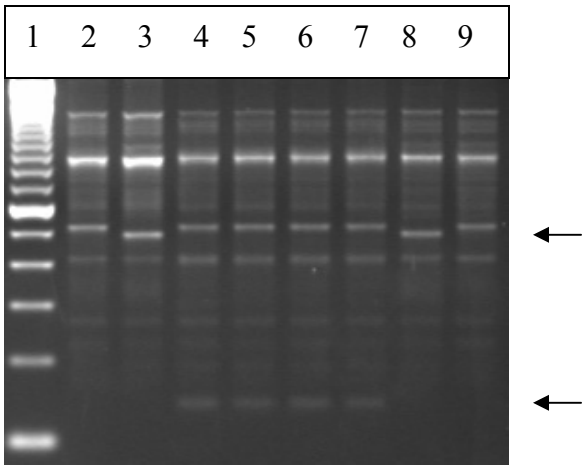

Figure 2. TRAP F7/R10. Lane 1: 100bp Ladder, Lanes 2-9: Bean Cultivars: Tacana, Jaguar, Red Hawk, Chinook 2000, Beluga, Capri, Merlot, Bunsi.

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VALIDATION AND USE FOR MARKER-ASSISTED SELECTION OF SCAR MARKER LINKED TO COMMON BEAN RUST RESISTANCE GENE *UR-5*

Thiago Lívio P. O. de Souza[1], Suelen N. Dessaune[1], Ana L. Alzate-Marin[2], Endson S. Nunes[1], Vagner T. de Queiroz[1], Maurilio A. Moreira[1,3] & Everaldo G. de Barros[1,4]*

[1]Instituto de Biotecnologia Aplicada à Agropecuária (BIOAGRO), Universidade Federal de Viçosa (UFV), 36570-000 Viçosa , Minas Gerais, Brazil. [2]Laboratório de Genética Vegetal, Departamento de Genética, Universidade de São Paulo (USP), 14049-900 Ribeirão Preto, São Paulo, Brazil. [3]Departamento de Bioquímica e Biologia Molecular, BIOAGRO/UFV. [4]Departamento de Biologia Geral, BIOAGRO/UFV.
*Corresponding author: ebarros@ufv.br

Conventional breeding methods have been widely used to develop new common bean (*Phaseolus vulgaris* L.) cultivars resistant to rust disease. However, this resistance can be easily overcome due to the extensive virulence diversity of the rust fungus *Uromyces appendiculatus* (Pers.: Pers) Unger. Resistance gene pyramiding assisted by molecular markers has been proposed as an alternative strategy to overcome this problem. DNA markers tightly linked to the resistance genes may be used for the indirect selection of resistant plants in segregating populations, without the need for multiple inoculations.

In Brazil, Faleiro *et al*. (1999) demonstrated that the Middle American differential cultivar Mexico 309 (gene *Ur-5*) is immune to nine and moderately resistant to two of 13 *U. appendiculatus* pathotypes identified in the state of Minas Gerais. The rust resistance gene *Ur-5* is also effective against 11 *U. appendiculatus* pathotypes from Goiás state (Santos & Rios, 2000). These data indicate that 'Mexico 309' is an important rust resistant source for Central Brazil. Haley *et al*. (1993) reported the identification of $OPI19_{460}$ RAPD (Random Amplified Polymorphic DNA) molecular marker as linked in coupling phase and without recombinants to *Ur-5*. This RAPD marker was converted to the SCAR (Sequence Characterized Amplified Region) marker $SI19_{460}$ by Melotto & Kelly (1998).

Data reported by Alzate-Marin *et al*. (2004) indicate that *U. appendiculatus* resistance of 'Mexico 309' is controlled by a single dominant gene which is distinct from genes *Ur-11* (cv. PI 181996 and line Belmidak RR-3) and *Ur-ON* (cv. Ouro Negro). The main goal of this work was to evaluate if the molecular marker $SI19_{460}$ would be useful in BIOAGRO/UFV breeding program, which involves crosses between "carioca-type" cultivar Rudá, a Brazilian commercial cultivar susceptible to rust, and 'Mexico 309'. In this program the rust resistance genes *Ur-5*, *Ur-11* and *Ur-ON* are being used for gene pyramiding in "carioca-type" backgrounds.

One of the primary leaves from 61 BC_3F_2 plants derivates from crosses between cultivars Mexico 309 and Rudá were collected and kept at -80°C for DNA extraction, which was based on Doyle & Doyle (1990). The remaining primary leaves were inoculated with *U. appendiculatus* pathotype 10 (race 29-3). The leaves were scored visually for the rust disease symptoms using a 1-to-6 scale proposed by Stavely *et al*. (1983). Five resistance and susceptible BC_3F_2 individuals were used to construct two contrasting bulks, which were tested with marker $SI19_{460}$. The PCR reactions (25 μL) contained 30 ng of genomic DNA, 0.2 mM of each SCAR primer (F: 5' – AAT GCG GGA GTT CAA TAG AAA AAC C – 3' and R: 5' – AAT GCG GGA GAT ATT AAA AGG AAA G – 3'), 10mM/50mM Tris/KCl (pH 8.0), 2 mM $MgCl_2$, 0.48 mM of total dNTP, and 1 U of *Taq* DNA polymerase. The amplification program included an initial step of 3 min at 94°C; 34 cycles of 94°C/1 min, 50°C/1 min and 30 s, and 72°C/1 min and 30 s; and one final step at 72°C for 7 min.

The bulk analysis (Figure 1) indicated that SCAR marker was indeed linked to the *Ur-5* resistance gene. The analysis of all BC_3F_2 plants confirmed that $SI19_{460}$ marker is linked in coupling phase at 3.31 cM of the gene *Ur-5* in this population (Table 1). The calculated LOD score was 11.0, and the estimated selection efficiency was 91.32%. In addition, $SI19_{460}$ was polymorphic between 'Mexico 309' and the rust resistance sources 'Belmidak RR-3' (gene *Ur-11*) and 'Ouro Negro' (gene *Ur-ON*) (Figure 1).

This SCAR marker is now being used in the molecular marker-assisted pyramiding program at the BIOAGRO/UFV to aid the development of new common bean cultivars with wide and durable resistant to rust and adapted to Central Brazil.

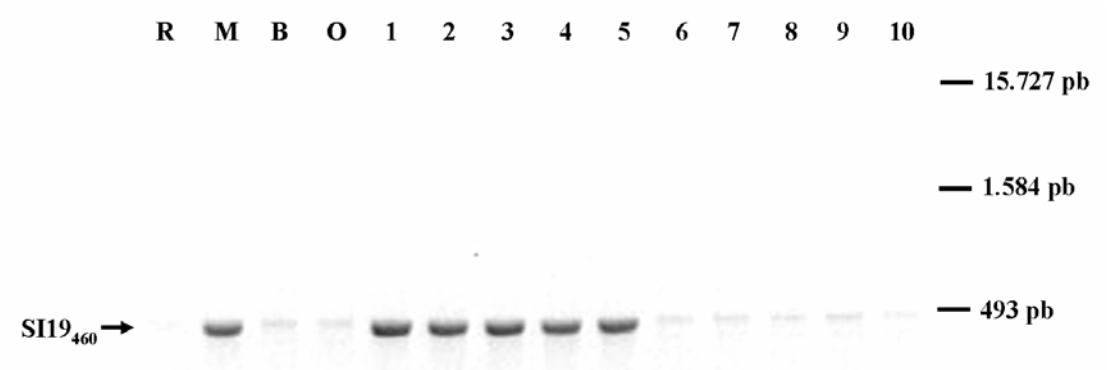

Figure 1. Electrophoretic analysis of amplification products obtained with $SI19_{460}$ SCAR molecular marker. Lanes are as follows: R, 'Rudá' (susceptible); M, 'Mexico 309' (*Ur-5*); B, 'Belmidak RR-3' (*Ur-11*); O, 'Ouro Negro' (*Ur-ON*); 1-5, BC_3F_2 Rudá x Mexico 309 resistant plants; and 6-10, BC_3F_2 susceptible plants to *U. appendiculatus* pathotype 10 (race 29-3). The arrow indicates marker $SI19_{460}$, a DNA band with 460 bp linked in coupling phase to the rust resistance gene *Ur-5* present in common bean cultivar Mexico 309.

Table 1. Segregation for resistance and linkage analysis of molecular marker $SI19_{460}$ and rust resistance gene *Ur-5* in BC_3F_2 population derived from a crosses between the cultivars Rudá and Mexico 309 (*Ur-5*)

Locus tested	Expected ratio	Observed ratio[a]	χ^2	P(%)[b]	cM[c]
Ur-5	3:1	44(R):17(S)	0.2677	60.48	-
$SI19_{460}$	3:1	46(+):15 (-)	0.0054	94.11	-
Ur-5/$SI19_{460}$	9:3:3:1	44(R/+):0(R/-):2(S/+):15(S/-)	54.7887	0.00	3.31

[a]Resistant plants (R), susceptible plants (S), presence of marker (+), absence of marker (-).
[b]Probability in percentage.
[c]Genetic distance in centimorgans (cM) of the marker $SI19_{460}$ in relation to resistance gene *Ur-5* with a LOD score of 11.0.

Acknowledgement: This work was supported by grants from CNPq and FAPEMIG (Brazilian Government). T. L. P. O. de Souza was supported by an M.S. scholarship from CNPq.

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DEVELOPMENT OF STS MARKERS TIGHTLY LINKED TO THE MAJOR QTL FOR COMMON BACTERIAL BLIGHT RESISTANCE IN COMMON BEAN

Shuyu Liu, Kangfu Yu, Soon J. Park
Greenhouse and Processing Crops Research Center, Agriculture and Agri-Food Canada, 2585 County Rd. 20, Harrow, Ontario N0R 1G0 Canada. Lius@agr.gc.ca

Common bacterial blight (CBB) is one of the major diseases to decrease bean production in the world. One major CBB resistance quantitative trait loci (QTL) in line 'XAN159' was derived from the tepary bean (*P. acutifolius*) PI 319443 and transferred to 'HR67' from the cross Centralia/3/HR13-621//XAN159/OAC Rico at Harrow, Ontario. Previous studies identified two markers linked to this QTL on one side. The main objective of this study was to develop high-throughput STS markers tightly linked to this QTL. Using bulked segregant analysis (BSA), 58 AFLP markers were mapped to an F_8 recombinant inbred line (RIL) population from the cross HR67/OAC95-4. The major QTL was mapped to a 14.5 cM genomic region covered by nine molecular markers. Seven of them are newly developed AFLP markers and were converted into eight STS markers. However, only three STSs were polymorphic and mapped back to the QTL region. Since the STS330 marker was mapped to B_6 of the bean core map, these are the first set of AFLP-derived STS markers, which are tightly linked to this major CBB resistance QTL, being developed and mapped to the B_6 or chromosome 1.

Introduction

Common bean (*Phaseolus vulgaris* L.) is a major plant protein source in the world, especially in Asia, Latin America and Africa. Common bacterial blight (CBB) is one of the most serious diseases affecting its yield and quality. It was reported that CBB resistance is a quantitative trait but controlled by a few major genes or QTL. Mapping QTL of interest to a genetic map is an efficient way to study the quantitative traits. The 11 major linkage groups of the bean core map were assigned to the 11 bean chromosomes (Pedrosa et al. 2003). The major sources of CBB resistance were from tepary bean. Among the molecular markers linked to CBB QTL reported so far, UBC420.900 is one of the most efficient markers for MAS across different genetic backgrounds (Yu et al. 2000). The problem is that UBC420 is linked to the *V* gene, which often confers the QTL carrying plants with unfavorable seed coat color in pinto bean. The newly developed STS markers along with the two previously identified high throughput markers should be very useful for marker-assisted selection in diverse bean breeding backgrounds and market classes.

Materials and Methods

DNA samples from both HR67 and OAC95-4 were amplified using the AFLP primer pairs of interest with *Eco*RI labeled using florescent IRDyes™ and run on an 8% polyacrylamide gel. The target band was cut out and eluted from the gel. After purification, the target bands were ligated into pGEM®-T vector and then transformed into DH5α competent cells. Transformed cells were screened on LB/Ampicillin/IPTG/X-gal medium. White colonies with the right insert were extracted for DNA from at least three colonies and then sequenced. Sequence tagged site (STS) primers were designed. The optimized annealing temperatures for each STS marker were determined using a Px2 gradient thermal cycler. Polymorphic STS markers were mapped back to RILs from this study and the bean core map.

Results and Discussion

The seven AFLP bands tightly linked to the QTL on chromosome 1 (Liu et al. 2005) were cloned and sequenced. Only E33M49.333 had two unique sequences while others had one sequence. Eight STS markers were designed based on the sequences of the target AFLP bands (Table 1). However, only three of them were polymorphic between HR67 and OAC95-4. They were mapped back to the CBB resistance QTL region with STS330 and STS333a at the peak LOD position. All three STS markers are co-segregated with their corresponding AFLP markers. Furthermore, STS330 was mapped on B_6 which was assigned to chromosome 1 (Pedrosa et al. 2003). Yu et al. (2000) found that MAS for CBB resistance using UBC420.900 is very reliable. In this study, we found that marker OD12S linked to the *V* gene did not show in HR67. Furthermore, the STS330 and STS333a markers were at the peak LOD position and 2.5 cM further away from the *V* gene than UBC420. Therefore, we expected that the probability of identifying plants with the CBB resistance QTL but without the *V* gene would be higher if STS330 and/or STS333a markers were used in MAS. The new STS markers tightly linked to this major CBB resistance QTL can provide additional opportunities in MAS for segregating populations with wider genetic backgrounds. We used these markers to screen the bean genomic BAC library (Yu et al. 2005) and several target BAC clones were identified. Therefore, these tightly linked markers should improve the efficiency of MAS for CBB resistance and will be very useful for physical mapping and positional cloning of this major CBB resistance QTL.

Table 1. STS primers and their sequences, band sizes, annealing temperatures.

STS markers	Forward (F)/Reverse(R) primer sequences (from 5'-end to3'-end)	Band size (bp)	Annealing temperature	Polymorphism	
				HR67/ OAC95-4	BAT93/Jalo EEP558
STS100	F: CACTTGCCACAGCTTCAAGA R: ACACCACTGAGGAACTTTGG	71	63	+/+	-/-
STS123	F: GACAGTTAGTCAACCAAGTC R: CACAACATAATCGATTG	79	48	+/+	-/-
STS183	F: CCTATGTACTTCTTGAGGGAGAC R: AGAAGCCCAGGGACTTGGAT	142	67	+/-	-/-
STS293	F: GCTGGCAGAAAGGGCAAACT R: GTCAATATCATTTCCGTGAAGG	239	70	+/+	+/+
STS305	F: CCGATCGCCAGGATGTCATA R: CGAGATACCTGATGTTGGCAGT	265	63	+/+	+/+
STS330	F: AAAACTTGAAATTGGCCGCG R: CACAGGCAATCAGTGGGAAGG	293	69	-/+	+/-
STS333a	F: CATAAGATGAATGGTTCTTGAC R: CCATTTGGTGAGATTCACTT	274	60	+/-	-/-
STS333b	F: GACGTGGGTGCTGGGGTGAT R: AGCCCCACCAACCGCTCTAC	255	63	+/+	+/+

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PYRAMIDING ANGULAR LEAF SPOT RESISTANCE GENES IN A "CARIOCA-TYPE" COMMON BEAN

Demerson A. Sanglard[1], Jeziel D. Damasceno[1], Thiago Lívio P. O. de Souza[1], Bruno P. Balbi[1], Maria A. C. Medonça[1], Everaldo G. de Barros[1,2] and Maurilio A. Moreira[1,3] *

[1]Instituto de Biotecnologia Aplicada à Agricultura (BIOAGRO), Universidade Federal de Viçosa (UFV), 36571-000, Viçosa, MG, Brasil. [2]Departamento de Biologia Geral. [3]Departamento de Bioquímica e Biologia Molecular.
*Corresponding author: *moreira@ufv.br*

Angular leaf spot incited by the fungus *Phaeoisariopsis griseola* is a major common bean disease, and may cause important yield losses. An effective, practical and economic way to control this disease is the use of resistant cultivars. However, the high pathogenic variability of *P. griseola* makes the development of such resistant cultivars a difficult task. In this case, pyramiding of resistance genes may be used if a longer lasting resistance is to be sought.

The introgression of disease resistance genes in elite-cultivars has been one of the targets in the bean improvement programs. The use of molecular markers has shown to be an increasingly efficient tool, mainly in pyramiding of these genes. The objective of this work was to pyramid resistance genes to angular leaf spot in "carioca-type" bean cultivars.

Three lines were obtained in three separated backcross programs. The individual lines contained the following resistance genes: lines BC_2F_5 (Rudá x MAR-2) - *Phg-4* and/or *Phg-5*[2], lines BC_3F_4 (Rudá x BAT 332) – *Phg-6*[2], lines BC_3F_5 (Pérola x Ouro Negro) - *Co-10*, *Ur-ON* and *Phg-ON*. In generation BC_2F_2 of the Rudá x MAR-2 cross resistant plants were selected based on their reaction to *P. griseola* race 63.39 and also on the presence of RAPD marker $OPE04_{500}$ (table 1). These plants gave rise to 19 $BC_2F_{2:3}$ families with "carioca-type" grains, which were used in progeny tests. Two families were identified as resistant and homozygous. Determination of genetic distances based on RAPD markers, allowed the selection of four BC_2F_3 plants which were closer to the recurrent parent Rudá (8.38 to 11.26%). In the Rudá x BAT332 cross, resistant plants were selected using the RAPD marker $OPAO12_{950}$ (table 1). These plants gave rise to 17 $BC_2F_{2:3}$ families with "carioca-type" grains, which were used in the progeny tests. Five families were identified as resistant homozygous. Six BC_2F_3 individuals were selected through the RAPD analysis, with relative genetic distances, ranging from 13.94 to 14.14% in relation to Rudá. In generation BC_3F_2 of Pérola x Ouro Negro cross, resistant plants were selected using $SCAR\text{-}F10_{1150}$, $SCAR\text{-}BA08_{560}$, $SCAR\text{-}BA16_{669}$ and $OPX11_{550}$ markers (table 1). Forty BC_3F_2 plants were selected for progeny tests, and 9 families were identified as resistant homozygous. Six BC_3F_3 individuals were selected through the RAPD analysis, with relative distances, ranging from 2 to 4% to Pérola. After selection for homozygosity, molecular characteristics, and resistance to angular leaf spot, the individual lines were intercrossed according to the following scheme: [(Rudá x MAR-2) x (Rudá x BAT 332)] x (Pérola x Ouro Negro). The F_1 seeds produced by these plants were sown, and the corresponding F_1 plants were selected with molecular markers linked to the resistance genes (figure 1) and through inoculation of the pathogens. Nine plants were identified that harbored all the markers of interest. The next step will be to multiply the seeds for evaluation of the agronomic traits under field conditions.

Table 1. **RAPD and SCAR markers used for selection of plants with resistance alleles from cultivars MAR-2, BAT 332 and Ouro Negro.**

Markers	Distance to the gene	*Resistance Genes*	*Diseases*	Reference
OPX-11_{550}	5.80 cM	*Ur-ON, Co-10*	Rust and Anthracnose	FALEIRO et al. (2000)
OPE-04_{500}	5.80 cM	*Phg-4* , *Phg-5*[2]	Angular leaf spot	FERREIRA et al. (2000)
OPAO-12_{950}	5.83 cM	*Phg6*[2]	Angular leaf spot	CAIXETA et al. (2003)
SCAR-$F10_{1050}$	6.90 cM	*Ur-ON, Co-10*	Rust and Anthracnose	CORRÊA et al. (2000)
SCAR-$BA16_{669}$	9.70 cM	*Phg-ON*	Angular Leaf Spot	QUEIROZ et al. (2004)
SCAR-$BA08_{560}$	6.00 cM	*Ur-ON, Co-10*	Rust and Anthracnose	CORRÊA et al. (1999)

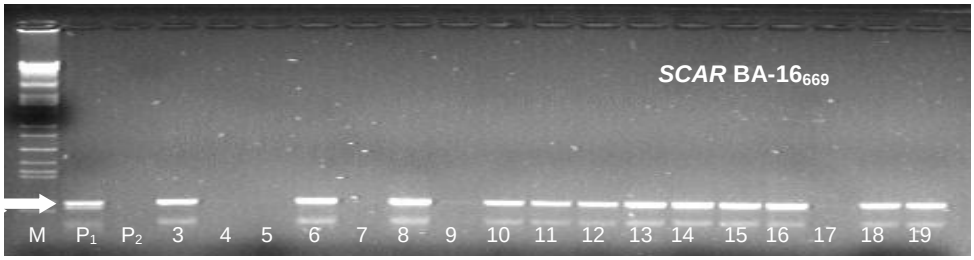

Figure 1. Electrophoretic analyses of DNA amplification products produced with SCAR-$BA16_{669}$. Lanes are as follows: P_1, Ouro Negro; P_2, Rudá; 3-19, plants derived from the intercrosses. M refers to lambda phage DNA digested with *Eco*RI, *Bam*HI and *Hin*dIII (size markers). The arrow indicates the SCAR marker.

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THE IMPACT OF CMV IN REDUCING YIELD OF SELECTED SNAP BEAN CULTIVARS AND LINES

A. G. Taylor and J. W. Shail

Department of Horticultural Sciences, New York State Agricultural Experiment Station, Cornell University, Geneva, New York, USA 14456

Yield reductions were attributed to viruses in snap bean production in western New York and the mid-west since 2001. The major virus present was cucumber mosaic virus (CMV), though alfalfa mosaic virus (AMV) and potyviruses have also been found. Aphids are the major vectors of viruses in snap beans, and the newly introduced soybean aphid is the major vector of CMV. In addition, soybean aphid is a late season pest and would have its greatest impact on late sown snap beans (plantings after July 1st).

Unfortunately, there are few practical methods to effectively control the spread of viruses by aphids with non-persistent feeding habits. One possible strategy is to examine plant tolerance to viruses or to reduce the impact of viruses on yield. Commercial varieties do not have resistance to CMV; however, varietal tolerance to CMV may be an important tool in scheduling commercial plantings. In this manner, varieties that are tolerant to CMV (or exhibit little reduction in yield after inoculation with CMV) could be planted late in the season when the soybean aphid pressure is high.

Visual symptoms are commonly used to determine if a bean plant is infected with cucumber mosaic virus (CMV); however, symptoms vary by variety. Selected snap bean varieties and lines were inoculated in the greenhouse at the first true leaf stage. In this manner, all plants were exposed to the same inoculum dosage and same environmental conditions. Symptoms were rated according to their severity from 1 – 5, with 5 being the most severe.

Severity rating and symptoms	Varieties and Lines
1 –very mild	Dandy, Alicante
2 – mild chlorosis, slight twisting	Igloo, Masai, Tyro, 864
3 – twisted leaves, epinasty	Caprice, Hayden, Labrador, Summit, HS906
4 – blisters, severe epinasty on young leaves	Hercules, Hystyle, Titan, Zeus, Ex 0771, SB 4282, HMX2950, XP 633
5 – severe distortion, blisters, necrosis	Goldrush, BBL 156, XP 670

Field studies were conducted at the Vegetable Crop Research Farm, Geneva in 2004 and 2005. The growing season was wet in 2004 and warm and dry during 2005. Selected varieties or lines were sown in replicated plots in late June, and a portion of each plot was inoculated with CMV (legume strain) at the first trifoliate. Plots were then covered with a row cover (Reemay) to exclude aphid transmission of viruses. Yield, and grade was determined, and the percentage reduction in yield due to CMV inoculation was calculated. Yield reduction was ranked from low to high within each snap bean pod type. Varietal differences were measured, and Zeus did not show a reduction in yield in either 2004 or 2005. Yield reductions were dissimilar between years for Hystyle, Summitt and Dandy, which was attributed to different growing season (wet and cool vs. hot and dry). Yield reduction was not related to foliage symptoms.

The effect of CMV inoculation on yield reduction on snap beans in 2004 and 2005.

			% Reduction in Yield	
Variety	Type	Source	2004	2005
Zeus	large	Seminis	-1	-24
864	large	Brotherton		10
Hystyle	large	Harris Moran	31	12
SB4282	large	Syngenta		18
Ex 0771	large	Seminis		23
Orion	large	Brotherton		26
XP 670	large	Seminis		33
XP 633	large	Seminis		36
BBL 156	large	Syngenta		38
Hercules	large	Seminis		40
Tryo	large	Brotherton		41
Titan	large	Seminis		45
HS 906	large	Brotherton		48
HMX 2950	large	Harris Moran		52
Summitt	large	Syngenta	22	55
Hayden	large	Syngenta		56
Alicante	medium	Seminis	-7	
Caprice	medium	Harris Moran	9	
Labrador	medium	Seminis		19
Slender Pak	small	Seminis		-28
Dandy	small	Syngenta	-7	32
Masai	small	Seminis	17	41
PIX	small	Seminis		60
Goldrush	wax	Seminis	6	5
Goldmine	wax	Seminis		33
Tapia	Romano	Seminis		32

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EVALUATION FOR RESISTANCE TO ANTHRACNOSE OF A CORE COLLECTION ESTABLISHED FROM THE CRF-INIA COMMON BEAN COLLECTION

E. Pérez Vega[1] ; Campa A.[1]; L. de la Rosa[2] , R. Giraldez[3], J.J. Ferreira[1]

[1] Área de Cultivos Hortofrutícolas, S.E.R.I.D.A., Villaviciosa, Asturias, Spain; [2] Centro Recursos Fitogenéticos, CRF-INIA Madrid, Spain; [3] Área de Genética, Dpto. Biología Funcional, University of Oviedo, Spain

Introduction

The CRF-INIA (Centro Recursos Fitogenéticos) preserves the largest bean collection in Spain, with accessions mainly collected in the Iberian Peninsula. Currently, this collection includes 2842 common bean accessions (*Phaseolus vulgaris* L.). A core collection was established by De la Rosa *et al*. (2000) including a total of 211 accessions. This core collection is being subjected to different characterizations and evaluations to assess the representation of genetic diversity and the lack of repetitions.

Bean anthracnose, caused by fungus *Colletotrichum lindemuthianum* (Sacc.&Magn.) Scrib., is a common disease in Northern Spain. Numerous pathogenic variants or races have been described in this pathogen (Mahuku & Riascos, 2004). Up to now, six races have been reported in the Iberian Peninsula (races 3, 6, 9, 19, 38 and 102). In this summary, we present the results of the anthracnose evaluation of this core collection (races 3, 6, 19, 38 and 102) in order to contribute to its utilization, validation and improvement.

Material and Methods

A total of 202 accessions included in the core collection were evaluated. Races 3, 6, 19, 38 and 102 were used in these evaluations. These races were isolated from local cultivars grown in Northern Spain (Ferreira *et al*., 1998). The evaluations were carried out according to standard methods (Pastor Corrales *et al*., 1994), inoculating at least 16 seedlings of each accession with each race. The reaction was evaluated 7-9 days after inoculation using a scale from 1 to 9 (van Schoonhoven & Pastor Corrales, 1987) and considering three main types of reactions: resistant accessions (R), susceptible accessions (S), and accessions showing an intermediate reaction (I).

The accessions were grouped according to the seed phenotypes and market classes described by Santalla *et al*. (2001). The accessions having a seed phenotype different from these market classes, were included in the group "others".

Results and Discussion

In the evaluation, the three types of reaction were identified in the five races, the susceptible reaction being the most common. The virulence of individual races (% of susceptible accessions), fluctuated from 84 % of race 19 to 57 % of race 102. Accessions with good resistance against all five races were not found.

Table 1 presents the different resistance spectra to the five races, considering the intermediate reaction as resistant. These results show that accessions included in the same market class can have different resistance spectra. Also, several accessions included into the same market class can have the same spectrum, suggesting the possibility of some repeated accessions being present. Additional work in characterization an evaluation, including molecular markers, will be necessary in order to test for redundancy in this set of accessions.

It is worth of mention that in the 196 accessions analyzed, not all possible resistance spectra were found. The possibility of independence between the reactions against the different races was considered. Table 2 shows the values of the contingency chi squares for the different reactions to pairs of races, considering the intermediate reaction (I) as resistant. In five cases (pairs of races 3-6, 3-19, 3-38, 6-38 and 19-38) a significant excess of accessions showing either resistance or susceptibility to the two races was found. Races 38 and 102 showed the opposite situation: an excess of accessions showing resistance to

only one of these two races was found. A possible reason for these deviations could be the presence of a relatively low number of loci involved in the resistances shown by this core collection.

Table 1. Number of accessions showing the different possibilities of resistance/susceptibility to anthracnose races 3, 6, 19, 38 and 102. R = resistant; S = susceptible. Intermediate reaction (I) was considered as resistant.

Anthracnose races						
3	**6**	**19**	**38**	**102**	**Accesions**	**Bean market classes (number of accessions)**
R	R	R	R	R	2	Other (2)
S	R	R	R	R	1	Negro brillante (1)
R	S	R	R	R	-	
R	R	S	R	R	3	L. great nothern (1); Other (1); Small yellow (1)
R	R	R	S	R	4	Azufrado (1); D. Red Kidney (1); Rosada (1); Other (1)
R	R	R	R	S	6	Black turtle (2); Canario bola (1); Great northern (1); L. great nothern (1); Small white(1)
S	S	R	R	R	-	
S	R	S	R	R	3	Large Cramberry (1); Manteca (1); Other(1)
S	R	R	S	R	-	
S	R	R	R	S	1	Rosada (1)
R	S	S	R	R	-	
R	S	R	S	R	12	Bayo gordo (2); Brown garbanzo (1); Fabada (2); L. great nothern (1); Negro brillante (1); Sangre de toro (1); Small red (1); Rounded caparron (1); White kidney (2)
R	S	R	R	S	3	Great northern (1); Other (1); Rosada (1)
R	R	S	S	R	1	Other (1)
R	R	S	R	S	7	Brown marrow (1); Brown mottled (1); Great northern (1); Other (4)
R	R	R	S	S	-	
S	S	S	R	R	-	
S	S	R	S	R	5	Bayo gordo (1); Canela (1); Fabada (1); Ojo de cabra (1); Small yellow (1)
S	S	R	R	S	-	
S	R	S	S	R	14	Cramberry (2); Great northern (1); Large Cramberry (1); L. great nothern (1); Rosada (4); Canela (1); Negro brillante (1); Sangre de toro (1); Other (2)
S	R	S	R	S	7	Brown garbanzo (1); Brown mottled (1); Great northern (2); Marrow (2); White kidney (1)
S	R	R	S	S	-	
R	S	S	S	R	3	Other (2); Sangre de toro (1)
R	S	S	R	S	3	Other (2); White kidney (1)
R	S	R	S	S	3	Great northern (1); Negro brillante (1); Rosada (1)
R	R	S	S	S	-	
S	S	S	S	R	36	Azufrado (1); Bayo gordo (1); Black Canellini (1); Brown mottled (1); Canela (2); Canellini (1); Dark garbanzo (2); D. red kidney (1); Fabada pinto (1); Great northern (1); Large Cramberry (1); L. red mottled (1); Light r. kidney (1); Marrow (4); Negro brillante (5); Ojo de cabra (1);Other (6); Rosada (3);Sangre de toro (1); White kidney (1)
S	S	S	R	S	9	Brown mottled (2); Great northern (2); Small white (1); Small yellow(1); Rosada(1); Other (2)
S	S	R	S	S	1	Other (1)
S	R	S	S	S	19	Azufrado (1); Black Canellini (1); Black motled (1); Brown garbanzo (1); Carioca (1); Marrow (3); Other (7); Red pinto (1); Rounded caparron (1); Small yellow (1); White kidney (1)
R	S	S	S	S	2	Canela (1); Sangre de Toro (1)
S	S	S	S	S	51	Azufrado (1); Bayo gordo (1); Black Canellini (3); Brown garbanzo (2); Brown marrow (2); Brown mottled (1); Canellini (1); Cramberry (1); Dark garbanzo (1); Fabada pinto (1); Marrow (2); Mulatinho (2); Negro brillante (1); Other (12); Red caparron (2); Red pinto (3); Rosada (7); Rounded caparron (5); Sangre de toro (1); Small yellow (2)

Table 2. Contingency Chi-square values for the reactions against the different pairs of anthracnose races.

	Race 3	Race 6	Race 19	Race 38
Race 6	4.32*			
Race 19	73.17**	0.09 n/s		
Race 38	25.01**	26.35**	3.37 n/s	
Race 102	1.78 n/s	0.12 n/s	7.93**	12.46**

*= 0.05>p>0.01; **= 0.01>p; n/s= not significant.

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DISSECTION OF THE ANTHRACNOSE RESISTANCE IN THE DIFFERENTIAL CULTIVARS TU AND MDRK

Campa A.[1]; E. Pérez. Vega[1] C. Rodríguez-Suarez[2], R. Giraldez[2], J.J. Ferreira[1]
[1] Área de Cultivos Hortofrutícolas, S.E.R.I.D.A., Villaviciosa, Asturias, Spain [2] Área de Genética, Dpto. Biología Funcional, University of Oviedo, Spain

Anthracnose, caused by fungus *Colletotrichum lindemuthianum* (Sacc.&Magn.) Scrib., is a serious disease in common bean (*Phaseolus vulgaris* L.). This fungus shows numerous pathogenic variants or races (Mahuku & Riascos, 2004). At least nine independent genes involved in the genetic control of the resistance to this pathogen have been described in common bean (see review by Kelly & Vallejos, 2004). The majority of these genes are present in the twelve differential cultivars used for the identification of the pathogenic variants of the fungus. It has been considered that most differential cultivars have only one gene controlling the resistance reaction against different races. Up to now, it has been assumed that differential cultivars MDRK and TU carry only the anthracnose resistance genes Co-1 and Co-5, respectively (Kelly & Vallejos, 2004). In the present work, we show evidences indicating the presence of at least two independent and dominant resistance genes in each one of the bean differential cultivars TU and MDRK. The aim of this work is to consider the possibility of the presence of more than one resistance gene in these two differential cultivars, through the combined analysis of the resistance to different anthracnose races in segregations proceeding from the cross TUxMDRK.

The analysis was carried out in a F_2 population (and the corresponding F_3 families) obtained from the cross between the differential cultivars TU and MDRK. The anthracnose evaluation for races 31, 38, and 1545 was carried out according to standard methods (Pastor Corrales *et al*., 1994) on a total of 86 F_3 families. The resistance to each race was independently evaluated in at least 16 plants per F_3 family.

The segregation for resistance to specific anthracnose races was also analyzed in four F_3 families. In these cases, the genotypes of the F3 plants were determined after the anthracnose evaluation of the corresponding F4 families.

The segregations of different SCAR or RAPD markers linked to anthracnose resistance genes (Kelly & Vallejos, 2004) and SSR markers previously included in the genetic map of common bean (Blair *et al*., 2003), were analyzed in order to know the genes or chromosome regions involved. The different DNA markers were analyzed according to the instructions of the respective authors. The genetic distances between loci were determined with the aid of JOINMAP V3.0 (van Ooijen & Voorrips, 2001).

Table 1. Segregations for the reaction against three different anthracnose races in the F_2 population derived from the cross TUxMDRK. R = F3 families with all plants resistant; R/S = F3 families showing resistant and susceptible plants; S= F3 families with all plants susceptible.

	Parents		F_2 segregation (F_3 families)			Exp. ratio		
Race	Tu	MDRK	R	R/S	S	R : R/S : S	χ^2	p
31	R	S	28	33	8	7 : 8 :1	3.28	0.18
38	R	S	22	38	19	1 : 2 : 1	0.34	0.84
1545	S	R	32	36	2	7 : 8 :1	1.38	0.50

Table 1 shows the observed segregations for the resistance to three anthracnose races in the F_2 population derived from the cross TUxMDRK. For race 38, the observed segregation fitted a 1:2:1 ratio, indicating that a single dominant gene is involved in this resistance. This gene was previously linked to markers SAB3 and Phs, located on B7 linkage group, and identified as Co-5 (Campa *et al*., 2005). However, the observed segregation for resistance to race 31 suggests that two dominant resistance genes are involved in this resistance specificity. In order to confirm this hypothesis, a F3 family (2900.1-9) was analyzed in more detail. In 36 plants of this family, the genotype for resistance to races 31 and 38 was determined through the evaluation of the corresponding F4 families. All plants of this F3 family were

homozygous susceptible to race 38 and had the genotype for markers SAB3 and Phs (linked to Co-5) corresponding to the parental MDRK (susceptible). Concerning race 31, this F3 family showed a segregation of 14 plants homozygous resistant, 17 heterozygous, and 5 homozygous susceptible, ($\chi^2_{1:2:1}$= 4,61; p>0.05). These results indicate that TU carries a gene, conferring resistance to race 31, different from Co-5.

With respect to race 1545, the observed F_2 segregation (table 1) suggests the presence of two independent dominant resistance genes in MDRK. In this case, three F3 families (2900.1-24, 2900.1-29 and 2900.1-74) showing a 3:1 segregation for the resistance were analyzed in more detail. In these families, the genotype for resistance to race 1545 was determined through the evaluation of the corresponding F4 families. In F3 families 2900.1-24 and 2900.1-29, RAPD $OF10_{530}$ (linked to Co-1) was also segregating. The results concerning the joint segregation of the resistance to race 1545 and RAPD $OF10_{530}$ are shown in table 2.

Table 2. Segregation for the reaction against anthracnose race 1545 and RAPD $OF10_{530}$ in F_3 families 2900.1-24 and 2900.1-29 (added). R = F4 families with all plants resistant; R/S = F4 families showing resistant and susceptible plants; S= F4 families with all plants susceptible. += amplification of RAPD $OF10_{530}$ positive; -= amplification of RAPD $OF10_{530}$ negative.

	Segregation of F3 families 2900.1-24 and 2900.1-29 (added)								
	R	R	R/S	R/S	S	S			
Marker	+	-	+	-	+	-	Total	RF	LOD
$OF10_{530}$	4	5	24	0	13	0	46	0.09	3.75

In F3 family 2900.1-74, markers $OF10_{1000}$, SW12, SBA8 and PHVPVPK (all located in linkage group B4) were also segregating. Table 3 shows the results concerning the joint segregation of the resistance to race 1545 and these markers.

Table 3. Segregation for the reaction against anthracnose race 1545 and four molecular markers in F_3 family 2900.1-74. R = F4 families with all plants resistant; R/S = F4 families showing resistant and susceptible plants; S= F4 families with all plants susceptible. += amplification of marker positive; -= amplification of marker negative. PHVPVPK is a microsatellite showing codominant segregation.

	Segregation of F3 family 2900.1-74											
	R	R	R	R/S	R/S	R/S	S	S	S			
Marker	+	+/-	-	+	+/-	-	+	+/-	-	Total	RF	LOD
SW12	4	-	6	9	-	1	6	-	1	27	0.24	0.92
$OF10_{1000}$	4	-	5	9	-	1	6	-	0	25	0.2	1.25
SBA8	6	-	4	9	-	1	7	-	0	27	0.28	0.68
PHVPVPK	0	3	7	0	10	0	7	0	0	27	0.06	7.73

In summary, the results obtained agree with the existence of more than one dominant resistance gene in each of the bean differential cultivars TU and MDRK. The TU cultivar has a resistance gene in B7 linkage group (*Co-5*) involving in the resistance to races 38 and 31. This cultivar has a second gene conferring resistance to race 31 in an unknown location. MDRK carries two dominant and independent resistance genes implicated in the genetic control of the resistance to race 1545. One of these genes is probably Co-1 (linkage group B1), and the other is located in linkage group B4 (it could be allelic of Co-3/Co-9 or Co-10).

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REACTION OF BLACK BEAN LINES TO ANTHRACNOSE, ANGULAR LEAF SPOT AND RUST PATHOGENS

José Eduardo V. Cintra[1]; Pedro Crescêncio S. Carneiro[2]; José Eustáquio S. Carneiro[3], Carlos Lásaro P. Melo[1]; Alisson C. Pereira[1] & Everaldo G. Barros[2*]

[1]Instituto de Biotecnologia Aplicada à Agropecuária (BIOAGRO), [2] Departamento de Biologia Geral; [3]Departamento de Fitotecnia. Universidade Federal de Viçosa (UFV), 36570-000, Viçosa, Minas Gerais, Brazil; *Corresponding author: *ebarros@ufv.br*

INTRODUCTION: Black beans are grown in the state of Minas Gerais, Brazil, predominantly in Zona da Mata and Vale do Rio Doce regions. One of the major factors leading to the low bean productivity is the incidence of diseases. The foliar diseases incited by *Colletotrichum lindemuthianum* (anthracnose), *Uromyces appendiculatus* (rust) and *Phaeoisariopsis griseola* (angular leaf spot) are among the most relevant ones. The use of resistant cultivars is a low cost input, easy to be used and adopted by farmers. However, the great variability of pathogens makes it difficult the development of bean cultivars with a durable and wide resistance spectrum. Considering the importance of recommending new bean cultivars for the state of Minas Gerais, an agreement was signed by the Universidade Federal de Viçosa (UFV), Universidade Federal de Lavras (UFLA), Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG) and Embrapa Rice and Bean (CNPAF) to evaluate the lines developed by the breeding programs of these research institutions in several growing regions of the country. In this work, the black common bean lines of the 2005/2006 trials were tested for their reactions to the anthracnose, angular leaf spot and rust pathogens.

MATERIAL AND METHODS: Nineteen lines and three controls were evaluated under controlled conditions. For anthracnose and rust inoculation, 12 seeds of each line were sown in plastic trays. For angular leaf spot, 12 seeds of each line were sown in 3 vases. For inoculation with *U. appendiculatus*, a mixture of pathotypes (53.19, 61.3, 63.3 and 63.19) was used. Inoculation was performed in the primary leaves and the plants were incubated in a mist chamber and later transferred to a greenhouse, where they were kept until evaluation according to a scale described by Stavely et al. (1983). For evaluation of resistance to *C. lindemuthianum*, the pathotypes 65, 81, 89 and 2047 were used, with inoculation being performed in the primary leaves 7 days after germination of the seedlings, which were incubated and maintained in a mist chamber until evaluation according to scale described by Pastor-Corrales (1992). For inoculation of *P. griseola*, pathotypes 63.23, 31.23 and 31.15 were used, with inoculation being performed in the first trifoliolated leaves and the plants incubated in a mist chamber, and later transferred to a greenhouse. Evaluation was performed twenty-one days later, according to scale described by Schoonhoven et al. (1987).

RESULTS AND DISCUSION: Lines CNFP 10180, CNFP 10217, VP 16, VP 17, VP 18 and VP 19 were resistant to at least one of the pathotypes of *C. lindemuthianum* inoculated (Table 1). All lines were susceptible to pathotype 2047. It should be observed that this pathotype does not occur in Brazil but it is of great relevance for common bean growing in the USA. The results obtained for inoculation with the pathotypes *P. griseola* showed that lines CNFP 8108, CNFP 7677, VP 14, VP 15 and VP 19 were resistant to all the pathotypes used in this evaluation. Besides, the lines VP-16 and VP-17 were resistant to the pathotypes 31.15 and 31.23. Some of

the lines were superior to cultivar Ouro Negro, displaying a greater level of resistance against anthracnose and angular leaf spot. Lines MN 38-44 and VP 15 besides cultivar Ouro Negro were resistant to the mixture of *U. appendiculatus* pathotyped used in this study. On the other hand, lines CNFP 7726, CNFP 10047, MN 37-2 and MN 34-20 were susceptible to all pathotypes of the three different pathogens.

CONCLUSIONS: The most outstanding lines were: CNFP 10180, CNFP 10217 and VP 16 for *C. lindemuthianum* resistance; CNFP 8108, CNFP 7677, VP 14, VP 15 and VP 19 for *P. griseola* and MN 38-44 and VP 15 for *U. appendiculatus*.

Table 1. Reaction of the lines of the black bean group, included in the national assays (2005/2006 cycle) to *C. lindemuthianum, P. griseola,* and *U. appendiculatus*.

Lines	Anthracnose				Angular leaf spot			Rust
	65	81	89	2047	63.23	31.15	31.23	Mixture[1]
CNFP 10180	R[2]	R	R	S	S	S	S	S
CNFP 8108	S	S	S	S	R	R	R	S
CNFP 10217	R	R	R	S	S	S	S	S
CNFP 7726	S	S	S	S	S	S	S	S
CNFP 7677	S	S	S	S	R	R	R	S
CNFP 10047	S	S	S	S	S	S	S	S
MN 37-2	S	S	S	S	S	S	S	S
MN 34-20	S	S	S	S	S	S	S	S
MN 34-66	S	S	S	S	S	R	S	S
MN 34-53	S	S	R	S	S	R	S	S
MN 34-46	S	S	R	S	S	R	S	S
MN 38-44	S	S	R	S	S	S	S	R
VP 14	S	S	S	S	R	R	R	S
VP 15	S	S	S	S	R	R	R	R
VP 16	R	R	R	S	S	R	R	S
VP 17	S	R	R	S	S	R	R	S
VP 18	S	S	R	S	S	R	S	S
VP 19	S	S	R	S	R	R	R	S
OURO NEGRO*	S	R	R	S	R	S	R	R
BRS-VALENTE*	R	R	R	S	S	S	R	S
BRS-SUPREMO*	S	R	R	S	S	S	S	S

[1]Mixture of pathotypes 53.19, 61.3 and 63.3; [2]R: resistant plants, S: susceptible plants; *Controls (Ouro Negro, BRS-Valente and BRS-Supremo)

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REACTION OF "CARIOCA-TYPE" BEAN LINES TO ANTHRACNOSE, ANGULAR LEAF SPOT AND RUST PATHOGENS

José Eduardo V. Cintra[1]; Pedro Crescêncio S. Carneiro[2]; José Eustáquio S. Carneiro[3]; Carlos Lásaro P. Melo[1]; Márcia R. Costa[1]; Maurílio A. Moreira[4] & Everaldo G. Barros[2*]

[1]Instituto de Biotecnologia Aplicada à Agropecuária (BIOAGRO); [2]Departamento de Biologia Geral; [3]Departamento de Fitotecnia; [4]Departamento de Bioquímica e Biologia Molecular. Universidade Federal de Viçosa (UFV), 36570-000, Viçosa, Minas Gerais, Brazil;
*Corresponding author: *ebarros@ufv.br*

INTRODUCTION: "Carioca-type" beans are the most popular common bean (*Phaseolus vulgaris* L.) cultivars grown in Brazil. Many of these cultivars have excellent traits, but are susceptible to several pathogens. Foliar diseases caused by *C. lindemuthianum*, *P. griseola* and *U. appendiculatus,* have wide occurrence, and represent a constraint for the culture. The use of resistant cultivars is the most economical form of disease control. However, these pathogens present high variability, which makes it difficult to obtain cultivars with broad and durable resistance. Given the importance to recommend new bean cultivars for the state of Minas Gerais, Brazil, an agreement was signed by the Universidade Federal de Viçosa (UFV), Universidade Federal de Lavras (UFLA), Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG) and Embrapa Rice and Beans (CNPAF) aiming to evaluate in national assay trials the elite bean lines developed by the breeding programs of these institutions in various growing regions of the country. The objective of this work was to evaluate the "Carioca" common bean elite lines of the 2005/2006 trials in relation to their reactions to anthracnose, angular leaf spot and rust pathogens.

MATERIAL AND METHODS: A total of 19 lines and 3 controls were evaluated under controlled conditions. For anthracnose and rust inoculation, 12 seeds of each line were sown in plastic trays. For angular leaf spot, 12 seeds of each line were sown in 3 vases. For inoculation with *U. appendiculatus*, a mixture of pathotypes (53.19, 61.3, 63.3 and 63.19) was used. Inoculation was performed in the primary leaves and the plants were incubated in a mist chamber and later transferred to a greenhouse, where they were kept until evaluation according to a scale described by Stavely et al. (1983). For evaluation of resistance to *C. lindemuthianum*, the pathotypes 65, 81, 89 and 2047 were used, with inoculation being performed in the primary leaves 7 days after germination of the seedlings, which were incubated and maintained in a mist chamber until evaluation according to scale described by Pastor-Corrales (1992). For inoculation of *P. griseola*, pathotypes 63.23, 31.23 and 31.15 were used, with inoculation being performed in the first trifoliolate leaves and the plants incubated in a mist chamber, and later transferred to a greenhouse. Evaluation was performed twenty-one days later, according to scale described by Schoonhoven et al. (1987).

RESULTS AND DISCUSSION: Most lines presented resistance to at least one of the pathotypes of *C. lindemuthianum*. The most resistant lines were CV-55, CNFC 10453, VC 7 and VC 12, presenting resistance to three pathotypes, to which cultivar Pérola is susceptible (Table 1). It should be observed that line CNFC 10453 was also resistant to pathotype 2047, which does not occur in Brazil, but has caused significant losses in the USA. Thus, this line may be used as an alternative source of resistance against anthracnose. With regard to angular leaf spot, a greater susceptibility of the lines was observed. For pathotype 63.23, no resistant line was detected while for 31.23, ten resistant lines were identified. As for rust evaluation, only two lines, VC 6 and OP-NS-331, were found to be resistant to the mixture of *U. appendiculatus* pathotypes.

CONCLUSIONS: Some lines showed more resistance to anthracnose than the cultivar Pérola, which was taken as reference. Line OP-NS-331 presented the best behavior regarding resistance to the three pathogens. It should be emphasized that this line is being recommended for the state of Minas Gerais, Brazil.

Table 1. Reaction of "carioca-type" lines included in the national assays (2005/2006), to *C. lindemuthianum, P. griseola,* and *U. appendiculatus*.

Line	Anthracnose				Angular leaf spot		Rust
	65	81	89	2047	63-23	31-23	Mixture[1]
RC-1-8	S[2]	S	S	S	S	R	S
Z-22	S	R	R	S	S	S	S
MAI-2-5	R/S	S	R/S	S	S	R	S
CV-46	S	S	S	S	S	R	S
MAI-18-13	R/S	R	R	S	S	R	S
MAI-8-9	S	R	R	S	S	R	S
CV-55	R	R	R	S	S	S	S
CNFC 10443	S	R	R	S	S	S	S
CNFC 8065	R	S	R	S	S	S	S
CNFC 8059	S	S	S	S	S	S	S
CNFC 10476	S	R	R	S	S	S	S
CNFC 10453	R/S	R	R	R	S	S	S
CNFC 8075	S	S	S	S	S	-	R/S
VC 6	S	R	R	S	S	S	R
VC 7	R	R	R	S	S	S	S
VC 8	S	R/S	R/S	S	S	R	S
VC 9	S	R	R	S	S	R	S
VC 10	S	R	R	S	S	R	S
VC 11	S	R	R	S	S	R	R/S
VC 12	R/S	R	R	S	S	R/S	R/S
OP-NS-331	S	R	R	S	S	R	R
VC 3	R	S	R/S	S	S	R	S
BRS-HORIZONTE*	S	R/S	R/S	S	S	S	S
TALISMÃ*	S	R	R	S	S	S	S
PÉROLA*	S	S	S	S	S	S	S

[1]Mixture of pathotypes (53.19, 61.3, 63.3 and 63.19); [2]S: Susceptibility reactions; R: Resistance reactions; and R/S: Resistant and susceptible plants; - missing datum; *Controls (BRS-Horizonte, Talismã and Pérola)

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PHAEOISARIOPSIS GRISEOLA VIRULENCE PATTERN AND RAPD DIVERSITY

R. A. V Garcia,.[1], M. S Carneiro,.[1], A. Sartorato, [2]
[1]Universidade Federal de Goiás, C. P. 131, 74001 - 970, Goiânia, GO, Brazil, [2]Embrapa Arroz e Feijão, C. P. 179, 75375 - 000, Santo Antônio de Goiás, GO, Brazil.
E-mail: sartorat@cnpaf.embrapa.br

Angular leaf spot (ALS) caused by the fungus *Phaeoisariopsis griseola* (Sacc.) Ferraris, is one of the most damaging diseases of common bean around the world. The fungus infects most plant aerial parts, especially leaves and pods, causing premature defoliation that culminate in poorly filled pods and reduced seed quality. In Brazil, the world's largest common bean producer and consumer, ALS is both prevalent and economically important. Without adequate disease control, yield reductions of up to 80% have been reported. In addition to yield losses, bean market quality may be affected. In this country, a major part of bean production is by medium/small or subsistence farmers who do not apply fungicides to their crops due to its high cost. As a result, the development of resistant cultivars would provide a more environmentally and friendly control alternative that could be used in integrated crop protection strategies. Breeding beans for ALS resistance is, however, complicated by the wide pathogenic variability that *P. griseola* presents, rendering a resistant variety to be susceptible in a different year or locality. In Brazil, all isolates that have been identified belong to the Mesoamerican gene pool which are capable of inducing disease in cultivars of both bean gene pools. This work presented the objectives (i) to study the pathogenic variability of *P. griseola* using the international differential cultivars set, (ii) to study the genetic diversity of this fungus by the RAPD technique and (iii) to investigate the hypothesis of the presence of multiple infections in angular leaf spot lesions, in leaves and pods, caused by this pathogen.

The 96 *P. griseola* isolates used in the present study, were obtained from naturally-infected bean leaves and pods of the genotypes FEB 209, FEB 200, FEB 170 and A 805, collected in the Embrapa Rice and Beans Experimental Station, in the county of Santo Antonio de Goias, Goias, Brazil. To verify the multiple infection hypothesis, one leaf and one pod were harvested from each of the above mentioned genotype. From each of these leaves and pods it was selected four lesions and from each lesion it was made three isolates, totaling 24 isolates (12 from leaf and 12 from pod) per genotype. For virulence analysis, the international set of 12 common bean differential cultivars were sown in aluminum pots containing 2,0 kg of soil at the rate of five seeds per pot. Conidia suspensions, for all inoculations, were obtained by culturing the fungus in bean-leaf-dextrose-agar medium. Inoculum was adjusted to 2×10^4 conidia mL^{-1}. The bean plants were then inoculated at the V_3 development stage by spraying the conidial suspension onto the upper and lower leaves surfaces. The inoculated plants were incubated in a moist chamber (> 95% RH) for 36 h. After this period of time, plants were transferred to greenhouse benches for another 14-18 days and evaluated for symptoms according to the 1-9 descriptive scale developed at CIAT. Plants rating from 1 to 3 were considered as resistant and from 4 to 9 as susceptible**.** For the molecular study *P. griseola* was grown in liquid medium (200 g potato and 10 g glucose/L of water) for 12-14 days. The RAPD reactions were carried out with primers OPA18, OPD06, OPD07, OPE09, OPJ10, OPK09, OPL12, OPL14 and OPL17 and performed in a final volume of 25 μL containing 25 ng of template DNA, 0.1 mM of each dNTP, 2.0 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 0.4 μM of one primer decamer, and 1 unit of *Taq* DNA polymerase. Amplification reactions were performed in a thermocycler model

PTC – 100 as follows: a preliminary denaturation step (3 min at 94°C), followed by 45 cycles of denaturation (1 min at 94°C), primer annealing (1 min at 35°C) and prime extension (2 min at 72°C), and a final extension step of 10 min at 72°C. Amplified fragments were separated by electrophoresis on 1.5% agarose gel immersed in TBE (89 mM Tris-borate, 2 mM EDTA(pH8.0)) at 3 V cm^{-1}. DNA bands were visualized under UV light after staining the gels with ethidium bromide and photographed with the Eagle Eye photosystem.

Up to five different pathotypes were identified in either leaves or pods of each cultivar. In the present study, from the same lesion more than one pathotype was identified. The existence of more than one pathotype of the fungus in a single lesion is recognized as a multiple infection process. Pathotypes 63-63, 63-55, 63-31, 31-55, 63-47, 63-39 and 63-23 were identified (Table 1). All this variability had previously been described by other authors in Brazil. The fact that most of these pathotypes caused disease in all Andean cultivars suggest that only the incorporation of resistance genes from this gene pool may not be effective to control angular leaf spot under Brazilian conditions. As a result, the best way of controlling bean angular leaf spot could be pyramiding resistance genes from both Andean and Mesoamerican genes pools what could lead to effective and durable disease management.

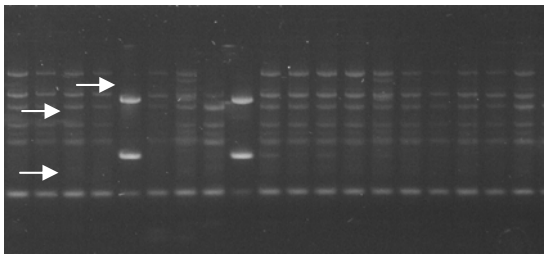
Table 1. Pathotypes identification based on the reaction of 12 differential cultivars inoculated with 96 isolates of *Phaeoisariopsis griseola* collected in the S[to] Antonio de Goiás, GO

Pathotype	Differential Cultivars												Number of isolates
	Andean						Mesoamerican						
	1[a]	2	3	4	5	6	7	8	9	10	11	12	
31-55	+[b]	+	+	+	+	-[b]	+	+	+	-	+	+	7
63-23	+	+	+	+	+	+	+	+	+	-	+	-	5
63-31	+	+	+	+	+	+	+	+	+	+	+	-	9
63-39	+	+	+	+	+	+	+	+	+	-	-	+	5
63-47	+	+	+	+	+	+	+	+	+	+	-	+	6
63-55	+	+	+	+	+	+	+	+	+	-	+	+	25
63-63	+	+	+	+	+	+	+	+	+	+	+	+	39

[a]: 1-Don Timóteo; 2-G 11796; 3-Bolón Bayo; 4-Montcalm; 5-Amendoin; 6-G 5686; 7-PAN 72; 8-G 2858; 9-Flor de Mayo; 10-México 54; 11-BAT 332; 12-Cornell 49-242. [b]: +/- = Compatible/Incompatible reaction.

Based on nine RAPD primers, a total of 57 fragments were generated. Out of these 35 were polymorphic in at least one of the 96 *P. griseola* isolates under study. As a mean 6.33 bands/primer and 3.88 polymorphic bands/primer were produced. RAPD analysis performed on the 96 isolates revealed great genetic variability clustering them into six groups at a distance of 64%. No association was observed between results from the molecular analysis and the inoculation of the isolates in the international differentials.

Figure 1. Electrophoretic analysis of amplification products obtained with primer OP L14. Arrows indicate polymorphic bands.



Evaluation of part of CIAT´s *Phaseolus vulgaris* core collection for resistance to

ANGULAR LEAF SPOT.

R. A. V. Garcia[1], M. S.Carneiro[1], A. Sartorato[2]

[1]Universidade Federal de Goiás, C 131, 74001 - 970, Goiânia, GO, Brazil, [2]Embrapa Arroz e Feijão, C. P. 179, 75375 - 000, Santo Antônio de Goiás, GO, Brazil
E-mail: sartorat@cnpaf.embrapa.br

Common bean angular leaf spot (ALS), caused by *Phaeoisariopsis griseola* (Sacc.) Ferr., is widely distributed in tropical and subtropical regions worldwide, being responsible for great economic losses, especially in Latin America and the great lakes region in Africa In recent years, this disease became one of the most important bean productions constraints in Brazil. In this country, seed yield loss can be as high as 80% depending on the susceptibility of the cultivars and the time of the disease symptoms appearance In addition to yield losses, the quality, market value and suitability of seed for transport and use across bean-producing regions and national borders may be severely affected. In Brazil, a major part of bean production is by medium/small or subsistence farmers who do not apply fungicides to their crops due to its high cost. Although ALS can be controlled by fungicides, the development of resistance cultivars would constitute a more environmentally and friendly alternative of the disease control that could be used in integrated crop protection strategies to reduce pesticide inputs. This paper reports results of the evaluation for bean angular leaf spot resistance to Mesoamerican races of *P. griseola* aiming the development and deployment of durable resistance do this disease.

Plant material for the screening consisted of part of the CIAT´s *P. vulgaris* core collection, including 357 accessions, representing the available range of crop types and ecogeographical location within the species. Out of the 357 *P. vulgaris* accessions evaluated, 281 belonged to the Middle American and 76 to the Andean gene pool. The pathotypes 63-15, 63-39, 63-23 and 31-31 of *P. griseola* were obtained from naturally infected common bean cultivars collected in different places in Brazil. Seeds of each cultivar were sown in aluminum pots containing 2,0 kg of soil at the rate of five seeds per pot. Conidia suspensions, for all inoculations, were obtained by culturing the fungus in bean-leaf-dextrose-agar medium. Inoculum was adjusted to 2 x 10^4 conidia mL^{-1}. Bean plants were inoculated 14-16 days after planting by spraying the conidial suspension onto the upper and lower leaves surfaces The inoculated plants were incubated in a moist chamber (> 95% RH) for 36-40 h. After this period of time, plants were transferred to greenhouse benches for another 14-18 days and evaluated for disease symptoms by determining the percentage of leaf area affected by the disease. Plants up to 5% of leaf area affected by the disease were considered as resistant.

As expected a great variability of reactions to Mesoamerican races of *P. griseola* was found between accessions of the core collection, ranging from complete resistance to full susceptibility. Fourteen (Table 1) of the 357 accessions were resistant to the four pathotypes, 44 to three, 35 to two and 70 to only one pathotype. Pathotype 31-31 were the most pathogenic followed by pathotypes 63-51, 63-39 and 63-23.

Table 1. Characteristics of the 14 most resistant genotypes to four pathotypes of *Phaeoisariopsis griseola*.

Genotype	Gene pool	Seed color	Country of origin
G 23804 B	Andean	White	Peru
G 23565	Andean	Yellow	Peru
G 6861	Mesoamerican	Others	Honduras
G 21130	Andean	Pink	Mexico
G 10909	Mesoamerican	Purple	Guatemala
G 19048	Andean	Pink	Mexico
G 18780 A	Mesoamerican	Black	Mexico
G 18451	Mesoamerican	Yellow	Nicaragua
G 21178	Andean	Beige	Mexico
G 22651	Mesoamerican	Purple	Zaire
G 10436	Andean	Red	Portugal
G 22623	Mesoamerican	Purple	Zaire
MUNHOND 002	Mesoamerican	-	-
Leche Blanco	Mesoamerican	White	-

Most of the resistant genotypes were originated from three countries, Peru (14.3%), Mexico (28.6%) and Zaire (14.3%), that together, represented 57.2 % of the resistant germplasm. However, when considered as a percentage of the total accessions, Honduras (14.3%), Nicaragua (14.3%), Zaire (18.1 %) and Portugal (20%) were the countries with highest proportion of resistant accessions to Mesoamerican *P.* griseola pathotypes. It is important to emphasize, the performance of the Leche Blanco (G3936) accession, that when inoculated with four of *P.* griseola pathotypes, did not present any symptom of the disease. Although the grain color of this genotype is not of commercial interest in Brazil, this accession showed a great potential to be explored in breeding programs for the improvement for resistance to the common bean angular leaf spot. The accessions G 21130, G 10909, G 19048, G 18780, G 22651 and G 22623 are also good sources of genetic resistance.

A PROPOSAL FOR A UNIFORM SCREENING PROCEDURE FOR THE REENHOUSE EVALUATION OF VARIABILITY OF *XANTHOMONAS AXONOPODIS* PV. *PHASEOLI* AND RESISTANCE ON LEAVES OF *PHASEOLUS VULGARIS*

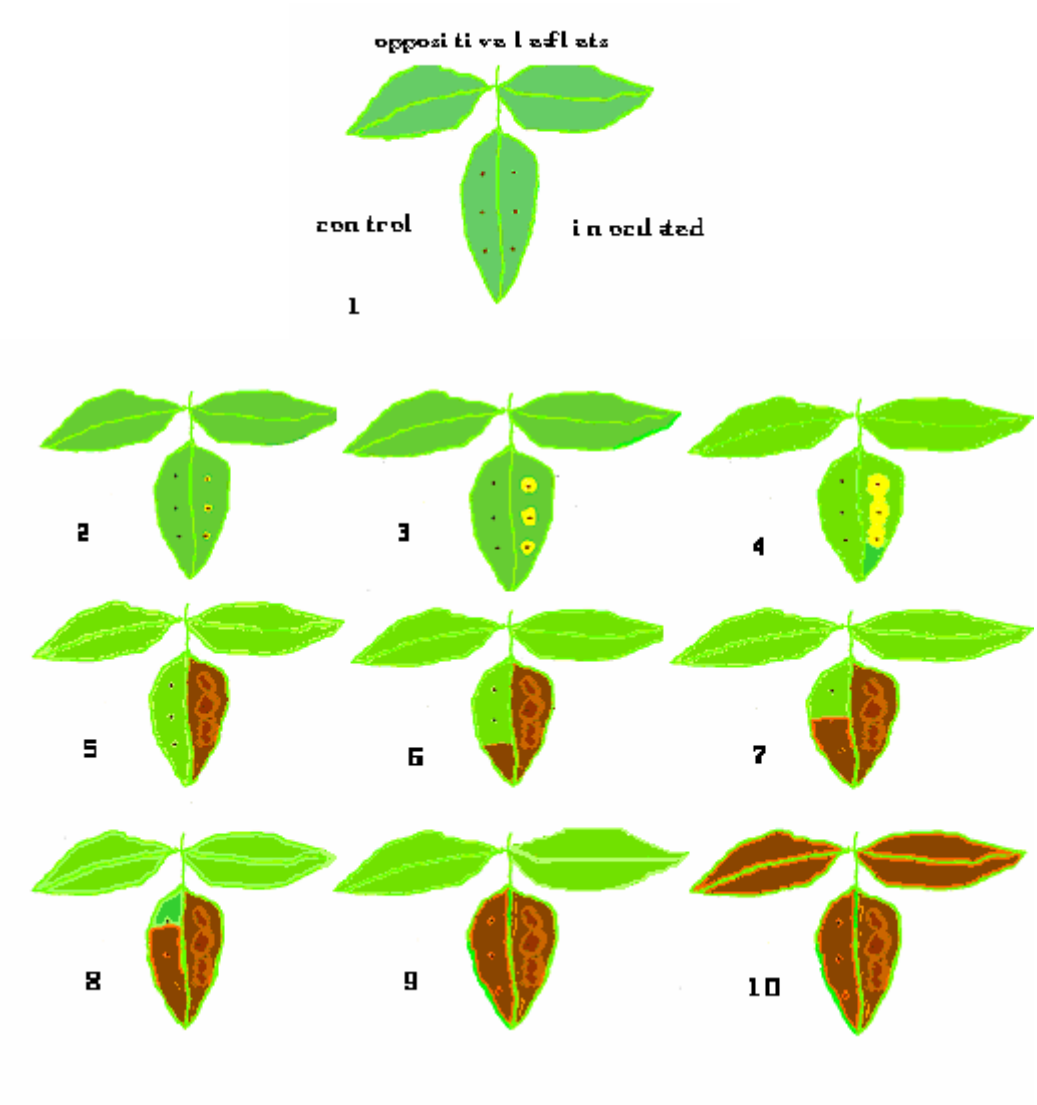
MILDRED ZAPATA

Crop Protection Department, P.O. Box 9030, University of Puerto Rico, Mayagüez campus, Mayagüez, Puerto Rico 00681-9030. mzapata@uprm.edu

Common bacterial blight of bean caused by *Xanthomonas axonopodis* pv. *phaseoli* (Xap) is a limiting factor in many common bean (*Phaseolus vulgaris* L.) production regions. Consequently, evaluation and screening for resistance have been conducted by different researchers in different countries using different techniques and scoring systems. There is a need for uniform and reproducible inoculation procedures to avoid duplications, and to produce reliable results. Leaves have been inoculated using spraying, multiple needles, razor blades, and pin pricking with flat needles or pipette tips. Results from studies using bacterial strains from Central América and the Caribbean have indicated variability of the pathogen and specificity of the host reaction of some bean genotypes. The methodology employed by Zapata to discriminate between strains of the pathogen and differential host reactions is based on a simple technique developed and employed successfully on leaves with coefficients of variability of 9.0 and 7.1 at 14 and 21 days, after inoculation, respectively. It also discriminates between Xap pathogenic races and host reactions with high level of resistance, moderate susceptibility and a high degree of susceptibility expressed by systemic infection. In susceptible genotypes, 5% and 42% of systemic infection have been detected at 14 and 21 days after inoculation, respectively. Only one strain is recommended to be inoculated per trifoliolate leaf per plant using three replicates because some genotypes may have high susceptibility and get systemic infection. The following screening and scaling are proposed to utilize uniform methods for the determination of pathogenic variability and the evaluation of resistance of common bean germplasm to Xap under greenhouse conditions. The method is also recommended for *P. acutifolius* and *P. coccineus*.

The bacterium is activated in nutrient agar for 48 hours. The estimate of the inoculum concentration is 10^7 CFU per site. The youngest but fully expanded trifoliolate leaf is inoculated 14 days after planting. Three sites on the left side of the center leaflet are used as control and the right side is inoculated with the pathogen by pricking with pipette tips impregnated with the bacterium. A dilution effect is generated since the first site is inoculated with the first inoculum and the others with the remaining amount left in the tip. A laceration is produced by pressing the tip without tissue penetration. Symptoms are evaluated at 7, 14 and 21 days after inoculation using a **1-10 scale:** A score of **1** indicates resistant reaction with no symptoms and **10** indicates high susceptibility with a systemic infection that invades the non inoculated opposite leaflets of the trifoliolate leaf tested. It has been observed that the disease progresses down to the terminal tip and then invades the control side up to the petiole of the central leaf. A score of **2** = 1-2 mm chlorotic margins surrounding the inoculation site; **3**= 3-5 mm chlorotic or translucent lesions; **4**= lesions are coalescing, flaccid; **5**= all inoculated side is flaccid or necrotic; **6**= inoculated side is necrotic and is progressing to the non inoculated side by 1/3; **7**= inoculated side is necrotic and is progressing to the non inoculated side by 2/3; **8**= inoculated side is necrotic as well as non inoculated by more than 2/3; **9**= both sides, inoculated and non inoculated, are necrotic and the central leaflet becomes necrotic but no infection of the opposite leaflets is observed (they maintain healthy looking tissue and no bacteria having been reisolated); **10=** indicative of systemic infection: central leaflet is necrotic, the right leaflet is the first opposite leaflet to

develop the necrosis followed by the left leaflet. Xap has been reisolated from these non inoculated tissues, thus confirming the systemic infection of the opposite leaflets.



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REACTION OF 36 COMMON BEAN CULTIVARS INOCULATED WITH FOUR *XANTHOMONAS CAMPESTRIS* PV. *PHASEOLI* ISOLATES.

R. Navarrete-Maya[1], J. A. Acosta-Gallegos[2], F. J. Ibarra-Pérez[2], J. Navarrete-Maya[1], F. M. Mendoza Hernández and J. D. Kelly[3]. [1]UNAM, FES-Cuautitlán, UNIGRAS. Apdo. Postal 25. C. Izcalli, Edo. de México, CP 54700, rnavarre@servidor.uanm.mx, [2]INIFAP, Apdo. Postal 112, Celaya, Gto. 38000, México, [3]CSSD Michigan State University, E. Lansing, MI. 488824

INTRODUCTION

Bean common blight (CBB) [*Xanthomonas campestris* pv. *phaseoli* (*Xcp*)] is an important damaging disease in the semiarid highlands of Mexico (SAHM), where the use of certified seed is nil and the seed exchange among farmers is a widespread practice. Furthermore, the causal agent is seed transmitted (6). On the other hand, there is evidence of pathogenic variation of *Xcp* isolates from different countries (2, 4, 5, 6). The aim of this research was to evaluate the reaction of thirty-six bean cultivars to the inoculation with four *Xcp* isolates from the SAHM, under greenhouse conditions.

MATERIALS AND METHODS

The experiment was conducted under greenhouse conditions at El Bajio Experimental Station of INIFAP. Thirty-six bean cultivars (cv.) were sown on March 23, 2005 in 5-l pots, four seeds per pot and six pots per cv. Four Xcp isolates proceeding from different bean cultivars and producing areas of Mexico were tested (Table 1). Aqueous suspensions of Xcp ($3X10^6$ cfu/ml) were inoculated by twin razor blade on trifoliate leaves of the plants at the V_3 stage, three-replicates/isolate. Inoculated plants were kept at 26 ºC, >80% R.H., and 12/12 h day/night duration. Disease severity was scored 11 days after inoculation using a visual scale (1-9), where 1: no symptoms and 9: maximum severity (7). The same experiment was done in a second set of thirty-six cv. at the R_5 stage. The cv. were chosen on the basis of their previous reaction to Xcp under natural infection during three crop cycles at Texcoco, State of Mexico, half of the cv. were considered susceptible (S) and the rest as tolerant (T).

RESULTS AND DISCUSSION

None cv. showed resistance to all four isolates. The reactions induced by the isolates ranged from 1.7 to 9.0 (data no shown) and there were differences in the reaction among isolates (Table 1). Results indicated the existence of highly virulent isolates, such as 96 which badly damaged all cv., while other strains damaged few cv. Isolates 96 and 78 from the same location, but from different cultivars, caused different reaction on the tested cv. Average from all cv. isolate 130 was more aggressive at the V_3 stage than at R_5, while the others caused similar damage in both stages. Some cv. were tolerant to the inoculation with isolates 6 and 78 (Table 2), but this reaction only occurred at the V_3 stage, those cv. were included in both, the susceptible or tolerant groups previously defined, and its response was independent of the commercial class of bean. Afn, a French snap bean cv., was tolerant to isolates 78 and 130 at both phenological stages. Thus, variation was observed in the aggressiveness of Xcp on common bean from the SAHM region, similar to what has been mentioned by other authors in different countries (2, 4, 5, 6). Since there is large genetic variation in the bean crop in the region and since the use of certified seed is low, thus the use of seed that is probably infected is a widespread practice in the region

and may be the cause of the large variation observed in the Xcp pathogen. The pathogenic variation found can hamper the development of resistant cultivars.

Table 1. Reaction of leaves of thirty-six bean cultivars inoculated with four isolates of *X. campestris* pv. *phaseoli* at two phenological stages under greenhouse conditions.

Isolate	Origin		V_3		R_5	
	Cultivar	Location	Score	Average	Score	Average
6	Flor de Mayo Media Oreja	F. I. Madero, Dgo	2.0 - 9.0	5.3	3.0 - 9.0	5.3
78	Negro Altiplano	Ocampo, Gto	2.3 - 9.0	5.2	1.7 - 9.0	5.0
96	Negro 99039	Ocampo, Gto	4.0 - 9.0	7.4	5.7 - 9.0	7.4
130	Pinto Mestizo	Bachiniva, Chih	3.3 - 9.0	6.3	3.3 - 9.0	5.3

Table 2. Outstanding tolerant cultivars inoculated with four isolates of *X. campestris* pv. *phaseoli.*

	Cultivars	Isolates							
		6		78		96		130	
		V_3	R_5	V_3	R_5	V_3	R_5	V_3	R_5
T[1]	PS 99	2.7	-	3.0	-	7.7	-	4.3	-
T	Afn	2.7	3.7	3.0	2.7	5.7	9.0	3.7	3.7
T	A 285	2.3	5.0	2.3	7.7	9.0	8.3	6.7	6.0
S	Negro Durango	2.0	4.3	2.3	4.0	4.7	6.7	4.0	4.7
S	Black Jack	3.0	3.7	2.3	5.3	9.0	9.0	4.7	7.7
S	Pinto Bayacora	2.0	6.3	2.3	6.3	4.3	6.7	3.3	8.3
S	Flor de Mayo Sol	2.3	4.7	2.7	8.7	8.0	8.3	7.0	5.0

[1] T and S = From the previously classified as tolerant and susceptible groups, respectively, - not evaluated.

ACKNOWLEDMENTS

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A NEW METHOD FOR SCREENING RESISTANCE OF COMMON BEANS (*PHASEOLUS VULGARIS* L.) TO *FUSARIUM* ROOT ROT

S. Chaudhary, T.R. Anderson, S. J. Park, K. Yu. Greenhouse and Processing Crops Research Centre, Agriculture and Agri-Food Canada, 2585 County Road 20, Harrow, Ontario N0R 1G0, Canada yuk@agr.gc.ca

Introduction:

Root rot of common beans, is a soil-borne disease that may be incited by several fungal pathogens including *Fusarium solani, F. oxysporum, Pythium ultimum*, and *Rhizoctonia solani*. It occurs in all bean-growing areas of the world. Root rot caused by *F. solani* f. sp. *phaseoli* is a major concern for common bean producers in Ontario, Canada. Different greenhouse screening methods have been used for screening common beans for resistance to *Fusarium* root rot. In this study, we modified a method developed for soybeans by Schmitthenner and Bhat (1994) to screen for tolerance to *Phytophthora* root rot and compared it to the screening procedure developed by Schneider and Kelly, (2000) with common bean cultivars and breeding lines.

Materials and Methods

Plant materials: A recombinant inbred line (RIL) population, derived from a cross between AC Compass (susceptible to root rot) and NY2114-12 (*P. coccineius*-derived line tolerant to root rot), was developed and advanced to the F_3 by single seed descent. The F_3 seeds were bulked and advanced to the F_7 to produce 80 F_3 derived RILs. In preliminary trials, 30 RILs including parents were used to evaluate the appropriate distance above the root to section hypocotyls to observe vascular discolouration using the inoculum layer method (ILM). In additional experiments, 80 $F_{3:7}$ RILs of the population including parents were evaluated for resistance to root rot by both root rot rating and the vascular discolouration method using the ILM. These RILs were also used in conjuction with root rot rating to evaluate the consistency of the liquid inoculum method (LIM). This procedure was essentially the same as that developed by Schneider and Kelly (2000). We adapted and modified the ILM developed by Schmitthenner and Bhat (1994) to screen for soybean tolerance to *Phytophthora* rot. This method has not been evaluated for effectiveness with common beans and *Fusarium* root rot. The modified method is described by Chaudhary et al. 2006.

Pathogen strain: The isolate of *F. solani* f. sp. *phaseoli***,** Huron 2a, used in the current study was obtained from Dr. J. Kelly, Michigan State University, MI and was isolated from common beans in Huron county in Michigan (Schneider and Kelly, 2000).

Data analysis: The arithmetic average of the ratings of 5 to10 single plants per line was calculated. Pearson correlation coefficients between trials in all experiments were calculated using the PROC CORR procedure of SAS System (SAS Institute, 1994). Analysis of variance (ANOVA) for cross section experiments was performed using AGROBASE (Agronomix Software Inc., Winnipeg, MB, Canada).

Results and discussion:

With the ILM, disease severity was rated with two methods: (1) root discoloration and hypocotyl lesion, and (2) the number of discoloured vascular bundles in hypocotyl cross sections (Fig.1). The vascular bundle method of scoring in ILM gave us the highest reproducibility among experiments (Tables1 and 2). The only limitation of this method is that it is a destructive method in that we have to sacrifice the plant in order to rate it.

Table 1. Analysis of the number of discoloured vascular bundles in common bean hypocotyls at 4 distances above the root 10 days after inoculation with *Fusarium solani* f.sp. *phaseoli* using the inoculum layer technique.

Distance from root (cm)	Trial I. Mean number of discoloured bundles	CV (%)	Trial II. Mean number of discoloured bundles	CV (%)	Correlation coefficient I vs II
0.5	4.7	32.83	2.3	66.70	-0.0155
1.0	3.7	45.60	2.6	50.60	0.5038***
2.0	2.3	51.77	2.3	54.64	0.5714***
3.0	1.2	78.12	1.2	86.94	0.2430

*** = significant at P< 0.001 level of probability. cv = coefficient of variation.

Table 2. Pearson's correlation coefficients (r_p) between trials and methods for evaluating $F_{3:7}$ RILs for resistance to *Fusarium solani* f.sp.*phaseoli*.

			Method					
			Inoculum layer (ILM)				Liquid inoculum (LIM)	
			VB		RD		RD	
			T_1	T_2	T_1	T_2	T_1	T_2
ILM	VB	T_1	1					
		T_2	0.7113***	1				
	RD	T_1	0.7723***	0.5166***	1			
		T_2	0.5742***	0.6854***	0.5555***	1		
LIM	RD	T_1	-0.0293^{ns}	0.2156^{ns}	-0.0534^{ns}	0.0704^{ns}	1	
		T_2	0.1239^{ns}	0.2165^{ns}	0.1083^{ns}	-0.099^{ns}	0.1084^{ns}	1

VB = Vascular bundle, RD = Root discolouration, T = trial
*** = significant at P = <0.001, ns = not significant at P = <0.05 level of probability.

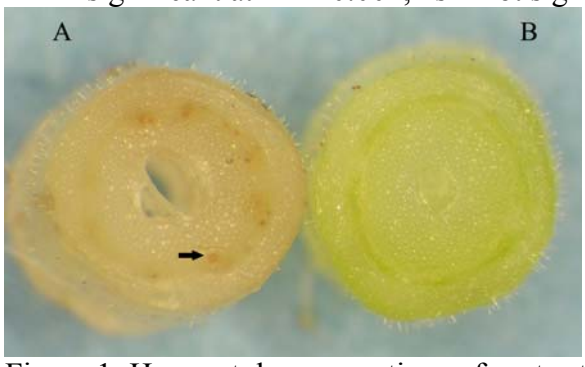

Figure 1: Hypocotyl cross sections of root rot infected (A) and healthy control (B) common bean plants. Brown discolouration can be seen in the major vascular bundles within the vascular cylinder in the infected plant. A plant normally has 8-10 large vascular bundles at this stage of development, 1 cm above the root.

References: 1. Schmitthenner, A.F., R.G. Bhat (1994. OARDC special circular 143. The Ohio State University, Wooster, OH.; 2. Schneider, K.A., J. D. Kelly. 2000. HortScience **35**:1095–1098. 3. Chaudhary et al.2006. Journal of Phytopathology (in press).

SOIL MOISTURE AFFECTING ACTIVITIES OF *RHIZOCTONIA SOLANI* AND *TRICHODERMA HARZIANUM*

Paula Jr., T.J. [1] **and Hau, B.**[2]

EPAMIG, Vila Giannetti 47, 36570-000 Vicosa (MG), Brazil (trazilbo@epamig.ufv.br); Institute of Plant Diseases and Plant Protection, Univ. of Hannover, Herrenhäuser Str. 2, 30419 Hannover, Germany

Rhizoctonia solani can survive for a long period in soil. Cultural practices including water management and biological control can reduce the survival of the pathogen and diminish the damage of root rot on beans. The purpose of this study was to investigate how soil moisture can influence the activities and dynamics of *R. solani* and *T. harzianum* when soil was infested with both microorganisms. In addition, the development of root rot on beans and the biological control with *T. harzianum* were evaluated under different water regimes for a period of one year.

MATERIALS AND METHODS

The experiments were conducted under greenhouse conditions. *R. solani* AG-4 was grown on rice grains and *T. harzianum* on wheat bran. The content of each pot (300 ml of sterilized soil-sand) was poured on a tray and carefully mixed with inoculum of both fungi. Ten seeds of the bean cultivar Dufrix were sown per pot. Treatments not infested with *R. solani* or *T. harzianum* received non-inoculated rice grains and wheat bran, respectively. Soil moisture was periodically monitored and kept at four levels varying from -0.0007 to -1.03 MPa. The pots were weighted to monitor water loss and irrigated once a day. Disease severity according to a 1 to 9 scale adapted from Van Schoonhoven and Pastor-Corrales (1987), percentage of emerged plants, plant height and dry weight were evaluated three weeks after planting. The following combinations were tested: no *R. solani*/with *T. harzianum* (rT), with *R. solani*/no *T. harzianum* (Rt), with both fungi (RT) and without both fungi (rt). For a long-term experiment, planting was carried out immediately after inoculation and at 20, 60, 180 and 360 days after inoculation (DAI). For a complementary short-term experiment, planting was done immediately after inoculation and at 3, 6, 12 and 18 DAI.

RESULTS AND DISCUSSION

The pathogen effectively survived in the soil in absence of host tissue at least one year after the soil infestation. However, severity of root rot and damage to plants were lower in the test with sowing done at 360 DAI than at the other tests (Figure 1). Soil moisture did not affect the severity of root rot. The pathogen could easily be recovered even from dryer soil, but in the presence of *T. harzianum* this was hardly possible (Table 1).

The antagonist improved the emergence of seedlings and led to higher weights of plants grown in *R. solani*-infested soil. However, when the pathogen was well established in the soil, antagonistic protection was lower. Consistent antagonistic effects were observed until 180 DAI, but at 360 DAI they were hardly detectable. The antagonist improved plant growth until 60 DAI even on plants not infected by *R. solani*. The antagonistic ability and activities of *T. harzianum*

were greater in soils held at intermediate soil moisture levels than in wet or dry soils, but were also influenced by the inoculum potential of both fungi in the soil.

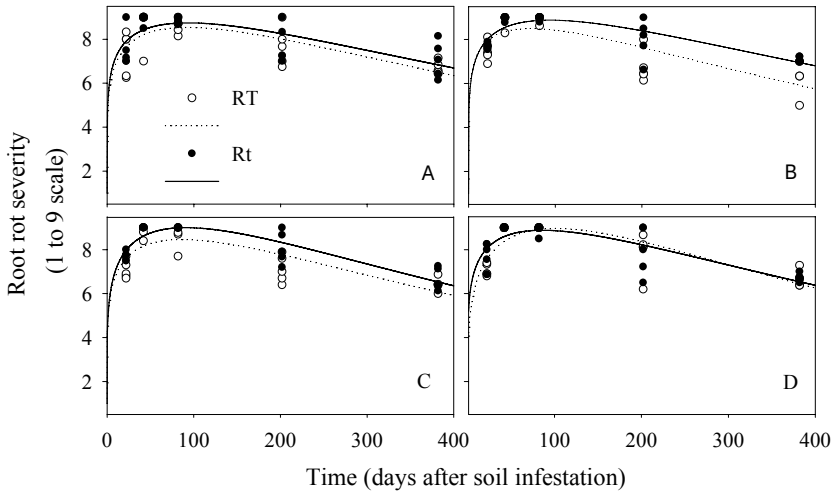

Figure 1. Root rot severity in five activity tests over time (DAI) in the treatments RT and Rt at four soil moisture levels (A = -0.0007 MPa, B = -0.005 MPa, C = -0.034 MPa, and D = -0,274 MPa). The disease severities are assigned to the evaluation day, which in each test was done at 22 DAS

Table 1. Population density of *R. solani* and *T. harzianum*, expressed as cfu/g of soil, determined at the end of the long-term experiment

Treatments	Moisture levels (MPa)	Population density of *R. solani* (cfu/g of soil)	Population density of *T. harzianum* (cfu/g of soil)
rT	-0.0007	-	3.60×10^5 a
	-0.005	-	4.46×10^5 a
	-0.034	-	3.82×10^5 a
	-0.274	-	1.28×10^5 b
RT	-0.0007	1.50 c*	6.00×10^5 a
	-0.005	2.37 b	10.40×10^5 a
	-0.034	2.13 bc	9.57×10^5 a
	-0.274	3.88 a	8.80×10^5 a
Rt	-0.0007	1.70 c	-
	-0.005	3.26 b	-
	-0.034	3.22 b	-
	-0.274	3.88 a	-

***Values are means of 10 replicates for *R. solani* and 5 replicates for *T. harzianum*. For each fungi combination, means followed by the same letter are not significantly different (*P* = 0.05)**

Reference: Van Schoonhoven, A.; Pastor-Corrales, M.A. Standard system for the evaluation of bean germplasm. Cali: CIAT, 1987.

Acknowledgments: This work was supported by CNPq, Brasilia, Brazil.

WHITE MOLD ON COMMON BEAN RELATED TO PLANT DENSITY, FUNGICIDE, IRRIGATION AND APPLICATION OF *TRICHODERMA* SPP.

Paula Jr., T.J.[1]; Vieira, R.F.[1]; Bernardes, A.[2]; Ribeiro do Vale, F.X.[2]

[1]*EPAMIG, Vila Giannetti 47, 36570-000 Viçosa (MG), Brazil (trazilbo@epamig.ufv.br);*
[2]*Universidade Federal de Viçosa, Dep. de Fitopatologia, 36570-000, Viçosa (MG), Brazil*

Common beans are staple food in Brazil. Bean diseases caused by soilborne pathogens, like white mold by *Sclerotinia sclerotiorum*, have increased in the last years, especially during the "winter season", in the irrigated areas, because the growers do not properly manage the irrigation, leading to high relative humidity and soil moisture conditions. No single treatment provides a satisfactory disease management, but some practices used simultaneously may be effective to control white mold. Low temperature, high humidity and plant canopy and/or soil surface wetting favour disease progress. Therefore, wider row and/or plant spacing may provide less favourable environmental conditions due to better light penetration into plant canopy and soil, and more ventilation.

The objective of this work was to study the association of control strategies of bean white mold in an irrigated area, including biological control, plant density adjustments, water management and fungicide application.

Materials and Methods

Two field experiments were carried out in an experimental field in Viçosa (MG), Brazil. This field is naturally infested with sclerotia of *S. sclerotiorum*. The trials were conducted as a 2 x 2 x 3 x 4 factorial in the randomized complete-block design: two irrigation frequencies (seven und 14 days), two plant densities (rows spaced 0.5 m apart with 6 or 12 plants/m), three disease control treatments (no control, application of fluazinam, application of *Trichoderma* spp.) and four replications. Seeds of the bean cultivar Talismã were sown on May (end of fall) of 2004. The trials were sprinkler irrigated. The fungicide fluazinam and conidial suspensions of *T. harzianum* and *T. stromaticum* (0.5 L a.i./ha) was applied at 45 (early bloom) and 55 days after emergence (DAE). An area of 1.2 m² of each plot was separately harvested for disease evaluation at 90 DAE. Incidence of white mould was evaluated considering % of plants with symptoms on stem or branches. Plants were rated for severity with a scale from 0 to 4 (Hall and Phillips, 1996). Yield, number of pods/plant and seeds/pod and 100-seeds weight were also evaluated.

Results and Discussion

Main results are presented in Table 1. No significantly effect of the irrigation frequencies was observed either on the disease development or on the bean yield, probably due the untypical rain occurred during the experiments. Higher disease severity was observed in the plant density 12 plants/m, compared to 6 plants/m. Some yield components were higher in the plant density 6 plants/m, compared to 12 plants/m, but no differences on yield were observed for both plant densities.

These results confirm the viability of using lower plant density in sclerotia infested fields to control bean white mold. The fungicide fluazinam was efficient to control the disease and to

improve the bean yield, compared to the treatments with no application of fungicide and application of *Trichoderma* spp. New tests will be carried out with *Trichoderma* isolates more adapted to temperatures under 20°C.

Table 1 - Sclerotia weight, white mold incidence and severity (Mckinney index) for different irrigation regimes, plant densities and application of fungicide and *Trichoderma* spp.

Treatments	Sclerotia weight[1] (g)	Incidence (%)	Mckinney index[2] (%)	Yield (kg/ha)
Experiment 1				
Irrigation 14 days	3.80	95.80	64.06	1297.00
Irrigation 7 days	3.70	95.80	57.00	1471.00
Difference	0.10 ns	0.00 ns	7.06 ns	242.00 ns
6 plants/m	3.44	94.97	55.31	1382.00
12 plants/m	4.07	96.63	65.74	1.385.00
Difference	0.63 ns	1.66 ns	10.43 **	3.00 ns
No *T. harzianum* and fluazinam	4.59 A	98.34 A	67.59 A	1230.00 B
T. harzianum	5.25 A	96.96 AB	64.86 A	1116.00 B
Fluazinam	1.41 B	92.10 B	49.14 B	1805.00 A
CV (%)	45.05	6.63	17.04	23.40
Experiment 2				
Irrigation 14 days	3.98	92.75	55.60	1272.00
Irrigation 7 days	4.33	92.29	60.02	1143.00
Difference	0.35 ns	0.46 ns	4.42 ns	129.00 ns
6 plants/m	4.66	93.58	57.77	1021.00
12 plants/m	3.64	94.46	57.85	1396.00
Difference	1.02 ns	0.88 ns	0.08 ns	375.00 ns
No *T. stromaticum* and fluazinam	4.47 AB	95.63 A	62.47 A	997.00 B
T. stromaticum	5.76 A	93.25 A	63.06 A	943.00 B
Fluazinam	2.24 B	93.19 A	47.89 B	1684.00 A
CV (%)	64.60	7.55	19.26	33.78

[1] Sclerotia bigger than 2 mm attached to bean pods and mixed to the seeds;

[2] ID (disease index, %) = $\frac{\sum (\text{disease rate x number of plants with this rate})}{(\text{total number of plants x maximum value of disease scale})} \times 100$

Irrigation regimes and plant densities were compared by Test F; means for application of fungicide and *Trichoderma* spp. and treatment control were compared by Tukey (5%); ns e ** = not significant and significant (1%), respectively.

Acknowledgements

This work was supported by International Foundation for Science (IFS, Sweden) and CNPq (Brasília, Brazil).

Reference

Hall, R.; Phillips, L.G. 1996. Evaluation of parameters to assess resistance of white bean to white mold. Ann. Rep. Bean Improv. Coop. 39, 306-307.

IDENTIFICATION OF PARTIAL RESISTANCE TO *SCLEROTINIA SCLEROTIORUM* IN COMMON BEAN AT MULTIPLE LOCATIONS IN 2005

J.R. Steadman, coordinator and collaborator, L.K. Otto-Hanson, co-coordinator, and J. Breathnach, statistical analysis (University of Nebraska-Lincoln). Data from C. Kurowski (California), R. Mainz (Minnesota), J. Kelly (Michigan), P. Griffiths (New York), J. Myers (Oregon), P. Miklas (Washington), H. Schwartz (Colorado), S. Singh (Idaho), and A. Oppelaar (France).

There is no complete resistance to *Sclerotinia sclerotiorum*, cause of white mold, in common bean. The development of bean cultivars with partial physiological resistance and architectural avoidance to white mold 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 line or variety, resulting in a three-row plot 4.6 m (15 ft.) long replicated three times in a randomized complete block design. Thirteen separate screening tests, seven field and six greenhouse (straw test), were rated on a scale ranging from most resistant (1) to most susceptible (12). Spearman and Pearson correlations were used to compare entry rankings in each test. The field test included 12 lines, and the greenhouse tests included the same 12 field lines, plus an additional 14 for a total of 26 lines.

The highest positive field correlations were WA and the outlier field France (r=0.63269, p=0.0272), NE and MI (r=0.66720, p=0.0178), and MN and WA (r=0.64691, p=0.0230). The highest positive greenhouse screening correlations were CO and ID (r=0.43557, p=0.0261), NY and OR (r=0.57080, p=0.0023), NY and WA (r=0.57580, p=0.0021), OR and WA (r=0.53184, p=0.0052), and ID and OR (r=0.59313, p=0.0014).

When an ANOVA was used on ranking with each test as a block and bean line (entry) as a treatment, there were significant differences (p=0.0001) among lines. When greenhouse and field tests were analyzed separately, WM 55, Cornell 601, Cornell 501, Cornell 604, Cornell 606, Ex Rico, and G122 were most resistant (lowest ranking)(Table 1) .The greenhouse screening test also separated G122, Cornell 606, and Cornell 604 as resistant lines. Other lines with resistance for the greenhouse test were B05002, VA-19, Dwarf Bees, Cornell 602, Cornell 603, Cornell 605, and B05003, which were only tested in the greenhouse. WM 55 and Ex Rico looked resistant in the field, but were the most susceptible in greenhouse tests. WM 55 and Ex Rico exhibit field avoidance to white mold. Twelve lines were tested in both the field and greenhouse over ten locations in 2005 (Table 2). The highest positive correlations for the twelve lines in both tests over the ten locations were WA and MI (r=0.55372, p=0.0050); WA and OR (r=0.51774, p=0.0096); MI and OR (r=0.40535, p=0.0494); NE and MI (r=0.66720, p=0.0178); France and WA (r=0.6329, p=0.0272); NY and OR (r=0075295, p=0.0047); ID and OR (r=0.57267, p=0.0516); and MN and France (r=0.64691, p=0.0230). There were no significant differences between the means of the lines in each location, or between the means of the lines in the field and greenhouse/lab screening tests. Cornell 604, G122, Cornell 606, and Cornell 501 were the

most resistant of the twelve lines tested in both tests at ten locations. Rankings of 26 lines tested in greenhouse as well as severity data on entries is posted at www.sclerotinia.com.

Table 1. Mean ranking of 12 bean lines (1=most resistant) for white mold reaction in the field.

	Rankings at field locations							LSD=3.2427	Alpha=0.05
Entry	WA	NE	MI	OR	CA	MN	FR	Mean Ranking	t Grouping
Beryl	12	10	12	12	12	12	12	11.714	A
PS02-006C-30	11	12	9	8	.	4	11	9.167	B A
AN 37	7	8	9	4	10	8	10	8.000	B C
B05001	10	6	4	3	9	11	8	7.286	B C D
IO1892-115M	4	11	11	10	2	8	5	7.286	B C D
WM 55	6	9	7	9	8	1	1	5.857	E C D
Cornell 601	7	4	4	4	7	8	7	5.857	E C D
Cornell 501	4	5	1	4	4	6	9	4.714	E D
Cornell 604	9	3	4	7	1	1	4	4.143	E D
Cornell 606	2	1	1	11	2	5	6	4.000	E
Ex Rico	3	1	7	2	4	7	3	3.857	E
G122	1	7	1	1	11	1	2	3.429	E

Table 2. Mean ranking of 12 bean lines (1=most resistant) for white mold reaction in both field and greenhouse/laboratory tests at 10 locations. LSD=2.2834, Alpha=0.05

Entry	Mean Ranking	t Grouping	Seed Class	Source
Beryl	11.000	A	GN	*Check-Susceptible*
PS02-006C-30	8.667	B	PTO	P. Miklas
B05001	7.385	C B	BLK	J. Kelly
WM 55	7.385	C B	BLK	S. Singh
AN 37	6.692	C B	PTO	P. Miklas
IO1892-115M	6.385	C B	BLK	J. Kelly
Ex Rico	6.385	C B	NAVY	*Check-Intermediate*
Cornell 601	5.538	C D	RK	P. Griffiths
Cornell 501	5.154	C D E	SNAP	P. Griffiths
Cornell 606	4.077	D E	BLK	P. Griffiths
G122	4.000	D E	CRAN	*Check-Resistant*
Cornell 604	3.000	E	BLK	P. Griffiths

PATHOGENIC VARIABILITY OF POPULATIONS OF *UROMYCES APPENDICULATUS*, CAUSE OF BEAN RUST IN INDIVIDUAL BEAN FIELDS AND DEVELOPMENT OF BEAN RUST SAMPLING PLANS BASED ON COSTS OF SAMPLING

C.N. Jochua[1], J.R. Steadman[2], X. Xue[3], K.M. Eskridge[3] and M.I V. Amane[1]

[1] Instituto National de Investigação Agronómica de Moçambique, P.O. Box 3658, Maputo, Mozambique. [2] Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583-0722. [3] Department of Biometry, University of Nebraska, Lincoln, NE 68583-0712.

There is a dearth of information on pathogen variation, either genotypic or phenotypic, within an individual field or defined ecological site. In this study, pathogenic variability of *Uromyces appendiculatus*, cause of bean rust, within and between individual fields was investigated. Six bean fields from tropical and temperate regions, within and outside of the two centers of common bean domestication, Andes and Middle America, were sampled. Ten bean leaf samples with rust uredinia were collected in different sites of each field. The standard set of 12 bean differential lines/cultivars containing Andean and Middle American derived rust resistance genes was used to differentiate 65 bean rust pathotypes from 380 isolates (Steadman, et al. 2002). Pathotype diversity was detected among bean rust pathogen isolates collected in different sites of a field and among isolates from different geographic regions. The number of pathotypes within individual fields varied from 2 to 25 and the number of pathotypes at individual sites within a field varied from 1 to 8 (Table 1). Pathotype variation among bean rust isolates from different geographic regions was found, and pathotypes found in fields from tropical and subtropical regions were more virulent and diverse than those found in fields from temperate regions. The Middle American differentials were resistant to more of the 380 isolates compared to the Andean differentials. Each of the 12 bean differentials was able to differentiate pathotypes of *U. appendiculatus*, and therefore are useful for phenotypic evaluation of pathogen populations. The variance components between fields was greater than the variance within field based on mean disease score on the 12 differentials, but when the number of pathotypes was considered, the variance components within field were greater than the variance between fields. This is the first report that multiple site samples are needed to represent the fungal pathotype variation in a diseased field. Thus, more than one site in a field needs to be evaluated for virulence phenotype. In developing sampling plans, the cost of sampling one field is higher than the cost of one sample, therefore, we recommend selecting fewer fields and collecting more samples per field. In this way, the cost of sampling can be minimized while obtaining a better estimate of pathogen variation.

Reference:

Steadman, J.R., M.A. Pastor-Corrales and J.S. Beaver. 2002. An Overview of the 3rd Bean and 2nd Bean Common Bacterial Blight International Workshops. Ann. Rep. Bean Improv. Coop. 45:120-124.

Table 1. Number of bean rust pathotypes in each sample and total number of unique pathotypes and isolates per field.

	No. of pathotypes per sample					
Sample number	*NE	MI	YH	TH	DR	SA
1	3	2	2	4	4	5
2	3	1	2	3	4	6
3	3	2	5	4	4	4
4	2	2	5	3	5	5
5	2	2	4	2	6	4
6	2	2	8	4	4	5
7	4	2	4	3	5	4
8	3	2	5	3	4	5
9	1	2	6	4	4	6
10	4	1	6	4	4	5
Unique pathotypes per field	7	2	25	19	20	23
Total # of isolates per field	56	70	67	44	65	78

* Fields where the samples were collected: NE-Nebraska, USA; MI-Michigan, USA; YH-Yuscaran, Honduras; TH-Tatumbla, Honduras; DR-Dominican Republic; SA-South Africa.

Table 2. Variance, components used to calculate the number of fields and samples per field based on the cost of sampling

Variable	**Model**	σ_1^2	σ_2^2	**Number of Fields**	**Number of Samples**
Mean Disease Score	All locations	**0.05229**	0.02453	9	5
Mean Disease Score	Temperate	**0.06308**	0.01054	9	2
Mean Disease Score	Tropical/subtropic	**0.06164**	0.03152	9	6
Mean Disease Score	Field (Region)	**0.06218**	0.02453	9	4
Number of Pathotypes	All locations	0.13960	**0.25190**	8	19
Number of Pathotypes	Temperate	0.07211	**0.21800**	7	31
Number of Pathotypes	Tropical/subtropic	0.01897	**0.27190**	4	144
Number of Pathotypes	Field (Region)	0.02986	**0.25490**	5	86

σ_1^2 = variance component between fields; σ_2^2 = variance component within field

VIRULENCE DIVERSITY OF *UROMYCES APPENDICULATUS* IN MINAS GERAIS STATE, BRAZIL

Thiago Lívio P. O. de Souza[1], Vilmar A. Ragagnin[1], Demerson A. Sanglard[1], Maurilio A. Moreira[1,2] & Everaldo G. de Barros[1,3] *

[1]Instituto de Biotecnologia Aplicada à Agropecuária (BIOAGRO), [2]Departamento de Bioquímica e Biologia Molecular, [3]Departamento de Biologia Geral; Universidade Federal de Viçosa (UFV), 36570-000 Viçosa, Minas Gerais, Brazil. *Corresponding author: ebarros@ufv.br

Introduction: Until recently determination of virulence diversity of *Uromyces appendiculatus* (Pers.: Pers) Unger, the causal agent of rust disease in common bean (*Phaseolus vulgaris* L.), was based on a set of 19 differential cultivars and a symptom evaluation scale with 37 reaction degrees (Stavely *et al*., 1983; Stavely, 1984). To facilitate this process, in 2002, during the "3rd Bean Rust International Workshop", a new differential series with six Andean and six Mesoamerican cultivars (Table 1), a new symptom scale, and a binary nomenclature system to designate the physiological races were defined. The new symptom scale presents only two classes, resistant and susceptible, and the binary designation of the race is based on the reaction of each differential cultivar (Steadman *et al*., 2002). If this new system is internationally used it will aid the standardization of the determination of physiological races of *U. appendiculatus*, facilitating the exchange of information and the cooperative use of the results obtained by different research groups. The main goal of the present work was to use the new differential series and the international binary system to characterize the virulence diversity of 12 *U. appendiculatus* isolates from state of Minas Gerais, Brazil.

Material and Methods: The inoculations were according to Carrijo *et al*. (1980). To obtain single pustule isolates, cultivar US Pinto 111 (susceptible control) was inoculated using a diluted spore suspension (1.0×10^4 spores/mL). One pustule of each pathotype was collected to generate the single pustule isolates. The isolates obtained were multiplied in US Pinto 111 through successive inoculations, using a standard concentration of 2.0×10^4 spores/mL. To characterize the virulence of isolates in relation to the differential cultivars, twelve different assays were conducted. In each assay, ten plants of each cultivar were evaluated. This experiment was performed during the Winter and repeated during the Summer season. Symptom evaluation was done 14 days after inoculation. The physiological races were designated by the international binary system nomenclature (Steadman *et al*., 2002).

Results and Discussion: The twelve isolates were classified into seven different virulence groups, into seven distinct physiological races (Table 1). Races 61-3 and 63-3 were the most frequent. They were represented by five and two isolates, respectively. The others races were represented by just one isolate. The differential cultivars Redlands Pioneer, PC-50, PI 260418 and CNC were the most important ones for the virulence differentiation of the isolates evaluated in the present study. The reactions of the other differential cultivars did not contribute for the distinction among the isolates. The cultivars Mexico 309, Mexico 235 and PI 181996 showed incompatibility with all the isolates characterized (Table 1). It is suggested that these cultivars should be preferentially used as sources of resistance to rust in breeding programs in the state of Minas Gerais, Brazil. Another important contribution of this study was the identification of races with potential use for the resistance gene pyramiding process. Races were identified that

are able to discriminate specific resistance genes. For instance, considering the pyramiding of the resistance genes found in the cultivars Redlands Pioneer (*Ur-13*) and CNC (*Ur-?*), which provide a wide spectrum in Brazil (Souza *et al*., 2005), races 53-19 and 63-3 could be respectively used for the identification of genotypes that contain simultaneously these two genes (Table 1). In the present study, for the first time the reactions of the new differentials cultivars Montcalm, PC-50, PI 260418, GN 1140, and PI 181996 to Brazilian *U. appendiculatus* isolates were demonstrated (Tables 1). These data will be useful in studies concerning the geographical distribution of the pathogen. This is the first report using the new international classification procedure to classify *U. appendiculatus* physiological races in Brazil. It represents an effort toward the standardization of the evaluation of *U. appendiculatus* virulence diversity.

Table 1. Virulence diversity characterization of *Uromyces appendiculatus* isolates collected in the state of Minas Gerais, Brazil, using the differential series and the international binary system reported by Steadman *et al*. (2002)

Binary Value	Differential cultivars (Resistance gene)	*Uromyces appendiculatus* single pustule isolates*											
		C	1	2	4	6	7	8	9	10	11	12	13
1	Early Gallatin (*Ur-4*)	+	+	+	+	+	+	+	+	+	+	+	+
2	Redlands Pioneer (*Ur-13*)	-	-	+	-	-	-	-	+	-	-	-	+
4	Montcalm (*Ur-?*)	+	+	+	+	+	+	+	+	+	+	+	+
8	PC-50 (*Ur-9*, *Ur-12*)	-	+	+	+	+	-	-	+	+	+	+	+
16	Golden Gate Wax (*Ur-6*)	+	+	+	+	+	+	+	+	+	+	+	+
32	PI 260418 (*Ur-?*)	-	+	+	+	+	+	+	+	-	+	+	+
1	Great Northern 1140 (*Ur-7*)	+	+	+	+	+	+	+	+	+	+	+	+
2	Aurora (*Ur-3*)	+	+	+	+	+	+	+	+	+	+	+	+
4	Mexico 309 (*Ur-5*)	-	-	-	-	-	-	-	-	-	-	-	-
8	Mexico 235 (*Ur-3*$^{+}$)	-	-	-	-	-	-	-	-	-	-	-	-
16	CNC (*Ur-?*)	-	-	+	-	-	+	-	-	-	-	-	-
32	PI 181996 (*Ur-11*)	-	-	-	-	-	-	-	-	-	-	-	-
Nomenclature of the races		**21-3**	**61-3**	**63-19**	**61-3**	**61-3**	**53-19**	**53-3**	**63-3**	**29-3**	**61-3**	**61-3**	**63-3**

*Reaction to *U. appendiculatus* single pustule isolates: compatible (+) and incompatible (-). The isolates were obtained from Fungal Collection of the BIOAGRO/UFV common bean breeding program.

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RESISTANCE SOURCES FOR RUST, ANGULAR LEAF SPOT, AND COMMON BACTERIAL BLIGHT IN COMMON BEAN FOR ECUADOR.

Á. Murillo I., E. Falconí, N. Mazón, and E. Peralta I.
Programa Nacional de Leguminosas y Granos Andinos (PRONALEG-GA). INIAP. Casilla 340.
Quito-Ecuador. legumin@pi.pro.ec

Introduction

Common bean is the most important legume in the northern region in Ecuador. Almost 20,000 ha of bush beans are planted in monocrop while 50,000 ha of climbing beans are planted in association with maize every year in Carchi, Imbabura, and Pichincha provinces (III Censo Agropecuario, 2002). Crop production is affected mainly by bean rust, caused by *Uromyces appendiculatus*. However, in the recent years, angular leaf spot (ALS) caused by *Phaeoisariopsis griseola* and common bacterial blight (CBB) caused by *Xanthomonas campestris* pv. *phaseoli* have been recognized as an increasing problem in this region causing around 50% of yield losses. It was our objective to evaluate a bean nursery reported by the International Center for Tropical Agriculture CIAT as valuable sources of disease resistance to identify the best genotypes to incorporate in the bean breeding program.

Materials and methods.

Twelve bean genotypes with three controls (Table 1) were evaluated in Ecuador in five bean production locations during two cycles of production in 2005. The selected bean production locations (Santa Lucía, La Concepción, San Clemente, Tumbaco, and El Tambo) at altitudes between 1,400 and 2,400 masl provide ideal climatic conditions for the development of the diseases. The trials were naturally infected with the pathogens under study. Evaluations at flowering and pod fill were performed after the symptoms of the diseases appeared. The evaluations were conducted with the scale of evaluation of germplasm developed by CIAT (1991). Plants rated 1-3 were classified as resistant, 4-6 intermediate resistant, and 7-9 susceptible. Plants rated 1 did not show symptoms (immune) and those rated 9 were destroyed by the pathogen. The modal value instead of the mean was employed to avoid the effect of the outliers.

Results

The environmental conditions and the presence of the pathogens allowed the fast and homogeneous development of the diseases in the trials under evaluation. Disease development rates in controls `Bola Pallatanga´, for all the diseases, and the rates in `Cocacho´, for ALS and CBB, showed that there was high disease pressure to conduct reliable evaluations. The cultivar `Red Small Garden´ was extremely susceptible for rust in all the locations evaluated so all the plants died and no evaluations for other diseases were performed. Genotype VAX 2 showed the best levels of resistance to all the diseases in all the locations. CAL 143 and G916 were resistant to rust and ALS and intermediate resistant to CBB. Je.Ma was highly resistant to rust. VAX 6, VAX 4, MAR 2, and G2333 were resistant to ALS and CBB in all the locations but showed intermediate to susceptible reaction to rust. POA 10, BAT 477, and `Mexico 54´ were resistant to

ALS and showed intermediate reaction to rust and CBB. AND 277 showed good source of resistance to ALS, but was susceptible to rust and CBB (Table 1).

Table 1. Rust, Angular Leaf Spot (ALS), and Common Bacterial Blight (CBB) rates of 15 bean genotypes in Northern Valleys of Ecuador, 2005.

	Rust					ALS			CBB		
Genotype	Concepción	Santa Lucía	San Clemente	El Tambo	Tumbaco	Santa Lucía	Concepción	El Tambo	Santa Lucía	Concepción	Tambo
JE.MA	*1**	*1*	*1*	*1*	*1*	4	4	5	4	4	4
CAL 143	*2*	*2*	*2*	*3*	*3*	*2*	*2*	*2*	5	4	4
G 916	*2*	*2*	*2*	*2*	*2*	*3*	*1*	*2*	5	4	4
VAX 2	*1*	*2*	*1*	*2*	*2*	*2*	*2*	*2*	*2*	*3*	*2*
VAX 6	6	3	2	5	6	*2*	*2*	*1*	*3*	*3*	*1*
VAX 4	5	3	1	4	6	*3*	*3*	*2*	*2*	*3*	*2*
MAR-2	*5*	*3*	*3*	*2*	*2*	*2*	*3*	*2*	*3*	*3*	*2*
G2333	5	5	1	3	3	*3*	*3*	*2*	*3*	*3*	*3*
POA 10	3	3	4	5	4	*3*	*3*	*3*	6	4	2
BAT 477	6	4	1	5	2	*3*	*3*	*2*	4	3	5
AND 277	4	3	7	7	6	*3*	*2*	*2*	7	5	5
Mexico 54	3	4	2	3	2	*3*	*2*	*3*	5	4	4
Red Small Garden (control)	7	8	8	9	7	....	.....	.....	.....	.....	.....
Bola Pallatanga (control)	7	7	7	7	7	6	7	7	4	5	3
Cocacho (control)	*1*	*3*	*2*	*2*	*2*	7	7	8	7	7	8

* 1 – 9 scale evaluation.- 1 = resistance (Immune), 9 = death plant caused by the disease.

Conclusions

There were important sources of resistance for rust, ALS and CBB in the nursery evaluated. The genotypes with high and wide scope of resistance might be crossed with local cultivars to transfer the resistance. The genotype VAX 2 was the best source of resistance for all diseases, so this genotype is strongly recommended to be part of the breeding crossing program to develop genotypes with multiple resistance. Several backcrosses must be considered as well since the seed quality differs widely from the Andean seed type. Other resistant genotypes with Andean seed type such us Je.Ma, CAL 143, G 916, and AND 277 might be intercrossed to generate multiple resistant lines with commercial characteristics.

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PATHOGENIC VARIABILITY OF *UROMYCES APPENDICULATUS* IN BEAN PRODUCTION AREAS IN NORTHERN ECUADOR.

Betty Paucar[2], Angel Murillo I.[1], Esteban Falconí[1], Eduardo Peralta I.[1]
[1]Programa Nacional de Leguminosas y Granos Andinos (PRONALEG-GA). INIAP. Casilla 340. Quito-Ecuador. legumin@pi.pro.ec, [2]Departamento Nacional de Protección Vegetal. INIAP.

Introduction

Rust, caused by *Uromyces appendiculatus*, is one of the most important diseases of common bean that occurs in the major bean production areas of Ecuador. The northern region in Ecuador, involving Carchi, Imbabura, and Pichincha provinces, represents one of the most important regions of the country where bush bean are planted on 20,000 ha every year (III Censo Agropecuario, 2002). The disease is particularly serious as many local cultivars are highly susceptible and farmers have sequential plantings that favors the spread of the disease. The bean breeding program in Ecuador has focused on the development of rust resistance cultivars for this important production area, but in order to generate cultivars with durable resistance it is important to study the variability of rust races present in the area to know which genes could be effective throughout the region.

Materials and methods

Bean tissue samples infected with rust were collected in production areas during 2004. The samples were processed in the laboratory of the National Institute of Agricultural Research (INIAP) at Santa Catalina Research Station. Monosporic isolates obtained from single uredinia were increased in the susceptible cultivar `Red Small Garden´ (RSG). Eleven isolates were inoculated on the 12 new standard bean rust differentials. The inoculum consisted of a suspension of 30,000 spores/ml. Primary leaves of bean seedlings from the differential set were uniformly inoculated with each isolate using a hand spryer. The plants were placed in a mist chamber at 100% RH for 18 h. Evaluations were conducted twice using the scale described by Stavely (1984) after the signs of the disease appeared. Plants rated 1-3 were classified as an incompatible reaction and 4-6 as a compatible reaction. To name the races the binary system proposed in the 3rd International Rust Workshop (Steadman et al., 2002) was employed.

Results

Eight different physiological races were identified from the 11 isolates evaluated. The eight races identified were 0:20, 0:24, 2:24, 0:28, 2:28, 0:29, 0:30, 0:61 (Table 1). The isolates were highly virulent on genotypes with Andean origin. The race 0:61 was the most virulent race in this group infecting five of 12 differentials. The five differentials belong to the Andean gene pool. The races 2:24 and 2:28 were able to infect not only Andean differential genotypes but cultivar `Aurora´ from the Mesoamerican gene pool. Race 0:30 was the only race able to infect cultivar Redlands Pioneer from the Andean gene pool. Cultivar `Golden Gate Wax´ was susceptible to all isolates. PC-50 and Montcalm showed a compatible reaction with almost all isolates. The time to

express the symptoms was variable with different isolates so for that reason two evaluations were conducted.

Table 1. Races of *Uromyces appendiculatus* identified with the standard bean differential set for rust. Santa Catalina Research Station. 2005.

	Mesoamerican Gene Pool							Andean Gene Pool						
Isolate code	GN 1140	Aurora	Mex 309	Mex 235	CNC	PI 181996	Binary number	Early Gallatin	Redlands Pioneer	Montcalm	PC-50	GGW	PI 260418	Binary number
1	-[1]	-	-	-	-	-	0	+	-	+	+	+	+	61
2	-	-	-	-	-	-	0	+	-	+	+	+	-	29
3	-	-	-	-	-	-	0	-	-	+	+	+	-	28
4	-	-	-	-	-	-	0	-	-	-	+	+	-	24
5	-	-	-	-	-	-	0	+	-	+	+	+	-	29
8	-	-	-	-	-	-	0	-	-	+	+	+	-	28
9	-	-	-	-	-	-	0	-	-	+	+	+	-	28
13	-	-	-	-	-	-	0	-	-	+	-	+	-	20
16	-	+	-	-	-	-	2	-	-	+	+	+	-	28
20	-	+	-	-	-	-	2	-	-	+	+	+	-	24
21	-	-	-	-	-	-	0	-	+	+	+	+	-	30

[1] - = Incompatible reaction, + = Compatible reaction

Conclusions

Large variability was observed among the isolates evaluated. The small sample size showed that more depth studies on the pathogenic variability must be conducted since eight races out of 11 isolates were identified. The susceptibility displayed by the majority of Andean genotypes in the differential set suggested that the breeding strategy must focus on the incorporation of Mesoamerican resistance genes in local cultivars. Only the differential cultivar `Aurora´ (*Ur-3* gene) among the Mesoamerican group of differential showed compatibility reaction with isolates

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CONTENTS OF CARBOHYDRATES IN SALT-STRESSED *PHASEOLUS* SPECIES

Leobardo Bahena-Betancourt[1], Lourdes Macías-Rodríguez[2] and Jeannette S. Bayuelo-Jiménez[1*]

[1] Instituto de Investigaciones Agropecuarias y Forestales, Universidad Michoacana de San Nicolás de Hidalgo, Mexico. Km. 9.5 Carr. Morelia-Zinapécuaro Tarímbaro, Michoacán, 58880 México jsbayuelo2002@aol.com *Corresponding author.

[2] Instituto de Investigaciones Agropecuarias y Forestales, Universidad Michoacana de San Nicolás de Hidalgo, Ciudad Universitaria, Av. Francisco J. Mújica s/n Morelia, Michoacán, C.P. 58030 México.

Many studies have dealt with osmotic potential decrease in common bean because of water deficit in the leaf tissue, but none quantitatively differentiates between the various solutes contributing to osmotic adjustment (Lazcano-Ferrat et al., 1997). Thus the contribution of various osmolytes to osmotic adjustment in bean leaves and roots remains poorly understood although several *Phaseolus* species are considered to be one of the annual species best adapted to saline environments (Bayuelo-Jiménez, et al., 2003). There is also no information about carbohydrate status as determinant of the level of osmolyte accumulation and the active role in plant stress responses in *Phaseolus* species. The study reported here represents a contribution to this approach.

MATERIALS AND METHODS

Two wild genotypes representing two species (*P. acutifolius*, G40169 and *P. filiformis* PI535309) and two cultivated species (*P. acutifolius* G40142 and *P. vulgaris* G04017) were used. Plants were grown in nutrient solution under greenhouse conditions at Universidad Michoacana de San Nicolás de Hidalgo, Mexico between April and July 2005. Seedlings were allowed to grow with no salinity stress until the emergence of the first trifoliate leaf, when several NaCl treatments were added to the solutions (0, 30, 60 and 90 mM). A randomized complete block design with a split-plot arrangement of salt treatments and four replications was used. Soluble carbohydrates were extracted in ethanol/water (85:15). Extracts were purified and concentrated with a rotary evaporator at 40^0 C. All neutral fractions were derivatized as described by Macías-Rodríguez et al. (2002). Hydroxilamine pyridine derivatives were separated and analyzed in a Pelkin Elmer gas chromatography, equipped with a FID detector, and a hp-5 column. Data were analyzed using the GLM procedure (SAS Institute, Cary, NC, 1985). Four replicates per salinity treatment per species at 20 days after the initiation of salt treatment were used for carbohydrate analyses.

RESULTS AND DISCUSSION

Glucose, inositol and xilose contents (mg/g fw) increased significantly in response to salt treatments (Table 1). Saline-induced changes in the soluble carbohydrate concentrations were also highly dependent upon the species and plan organ. Glucose content increased (119 % – 429 %) during salt stress, to reach high levels (3.31 - 23.3 mg/g fw) in leaves of all the species except wild *P. acutifolius* G40169 in which its contents was already relatively high only in control

leaves 15.9 mg/g fw) (data not shown). Inositol contents were already high (1.2 – 21.9 mg/g fw) in salt-stressed leaves of all *Phaseolus* species. Xilose contents were moderately high in leaves of only cultivated *P. acutifolius* G40142 (1.1 - 2.7 mg/g fw) and roots of wild *P. acutifolius* G40169 (2.5 – 3.3 mg/g fw). Glucose and inositol were clearly the major protectant sugar accumulation in the highest salt stressed *Phaseolus* plants. The increase of these soluble sugars in stressed leaves was previously described in other species such as *Lupinus albus* and *Helianthus annus* (Quick et al. 1992). A higher amount of glucose was also found in grasses (Marques da Silva and Arrabaca, 2004). This should also contribute to the osmotic potential decrease because a much lower osmotic potential was found in salt stressed leaves than in control leaves. The contribution of soluble sugars to osmotic adjustment of *Phaseolus* species remains, however, uncertain and it has to be estimated.

ACKNOWLEDGEMENTS
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Table 1. Total concentration of glucose, inositol and xilose of *Phaseolus* species following a 20-day salinization period.

Species/NaCl mM		Glusoce	Inositol	Xilose
		(mg g $^{-1}$ FW)		
G40169				
	0	9.70 a	5.77 b	3.25 a
	60	10.21 a	11.93 a	3.06 a
	90	6.82 b	9.97 a	2.46 a
PI535309				
	0	5.64 b	0.32 b	0.50 a
	60	13.25 a	2.35 a	1.38 a
	90	12.78 a	2.06 a	0.94 a
G40142				
	0	3.70 b	2.02 b	0.36 b
	60	4.41 b	3.39 a	1.08 a
	90	10.23 a	4.29 a	1.95 a
G04017				
	0	3.45 b	2.34 b	-
	60	4.31 b	4.11 a	-
	90	6.06 a	4.23 a	-
F values from ANOVA				
NaCl		4.90***	2.35 *	2.32 *
Species		8.85***	31.21**	8.70***
NaCl * Species		3.30***	2.04 *	1.19 ^{ns}

*, **, *** Significant at P≤ 0.05, 0.01 and 0.001, respectively, ns= no significant.
(-) carbohydrate no detectable.

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COMPARATIVE WATER RELATIONS OF WILD AND CULTIVATED *PHASEOLUS* SPECIES GROWN UNDER SALINE CONDITIONS

Lebardo Bahena-Betancourt[1] and Jeannette S. Bayuelo-Jiménez[1*]

[1] Instituto de Investigaciones Agropecuarias y Forestales, Universidad Michoacana de San Nicolás de Hidalgo, Mexico. Km. 9.5 Carr. Morelia-Zinapécuaro Tarímbaro, Michoacán, 58880 México jsbayuelo2002@aol.com *Corresponding autor.

Tepary bean, *Phaseolus acutifolius* A. Gray and *P. filiformis* Bentham, are adapted to hot arid and saline conditions and might be a valuable source of genes to improve the drought and salinity tolerance of *P. vulgaris* L. We examined the effects of salinity on water relations of two wild (*P. acutifolius* and *P. filiformis*) and two cultivated (*P. acutifolius* and *P. vulgaris*) species.

MATERIALS AND METHODS

The experiment was conducted with accessions of different salt tolerance: two wild accessions representing two species (*P. acutifolius*, G40169, sensitive and *P. filiformis* PI535309, tolerant) and two cultivated accessions (*P. acutifolius* G40142, tolerant and *P. vulgaris* G04017, sensitive) were used. Plants were grown in nutrient solution under greenhouse conditions at Universidad Michoacana de San Nicolás de Hidalgo, Mexico between May and August 2005. Seedlings were allowed to grow with no salinity stress until the emergence of the first trifoliate leaf, when several NaCl treatments were added to the solutions (0, 30, 60 and 90 mM). A randomized complete block design with a split-plot arrangement of salt treatments and four replications was used. Predawn water potential (Ψ_w) at 9, 14 and 19 days after transplanting was measured with a pressure chamber. Leaf solute potential (Ψ_π) was measured with a Wescor-5500 vapor pressure osmometer. Readings were converted to pressure units by using the van't Hoff equation. Turgor potential (Ψ_p) was determined using the relationship: $\Psi_p = \Psi_w - \Psi_\pi$. Plants were harvested at 10, 15 and 20 days after transplanting and separated into roots, stem and leaves. Data were analyzed using the GLM procedure (SAS Institute, Cary, NC, 1985). Four replicates per salinity treatment per species per harvesting date were used for growth analyses and water relations. Two-way analysis of variance was used to determine significant differences among accessions for various traits. Treatment means were compared using protected LSD test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Salinity significantly affected leaf water, osmotic and water potentials (Fig. 1). Differences among cultivated and wild accessions were significant at any salt concentration. Overall, wild species *P. acutifolius* G40169 and *P. filiformis* PI535309 had less negative values of water potential at 0, 30, 60 and 90 mM NaCl than cultivated accessions. Salinity decreased leaf osmotic potential in all species (Table 1). Differences among species were highly significant at 90mM NaCl. Wild *P. acutifolius* G40169 was unique in that it reached the highest osmotic potential, -1.14 MPa, at 90Mm NaCl; whereas the osmotic potentials of the other species ranged between –0.50 to –0.98 MPa at the same salt concentration. Leaf turgor potential was unaffected by 30 and 60 mM NaCl, but was increased between 0.5 and 0.89 and 0.52 to 1.14 MPa at 60 and 90 mM NaCl, respectively. Leaf water potential (Ψ_w) gradually declined during the first 14 days after salinization (-0.43 to –0.79 MPa), thereafter, a steady state was attained,

and except at 90 mM NaCl, which decreased Ψ_w further. Salinity also decreased leaf osmotic potential. This difference was reflected in average turgor potentials, which increased at 90 mM NaCl, particularly for wild *Phaseolus* species. Our data indicate that the decrease in leaf osmotic potential always exceeded that of leaf water potential. This resulted from the fact that plants adjusted osmotically. Under salinity, it can achieved either from the accumulation of high levels of inorganic ions, predominantly Cl^-, Na^+ and K^+ in their leaves (Bayuelo-Jiménez et al., 2003) or from net solute accumulation (Lazcano and Lovatt, 1997). In many plants net accumulation of osmotically active solutes allows turgor-dependent processes to continue to some extent under salt stress conditions.

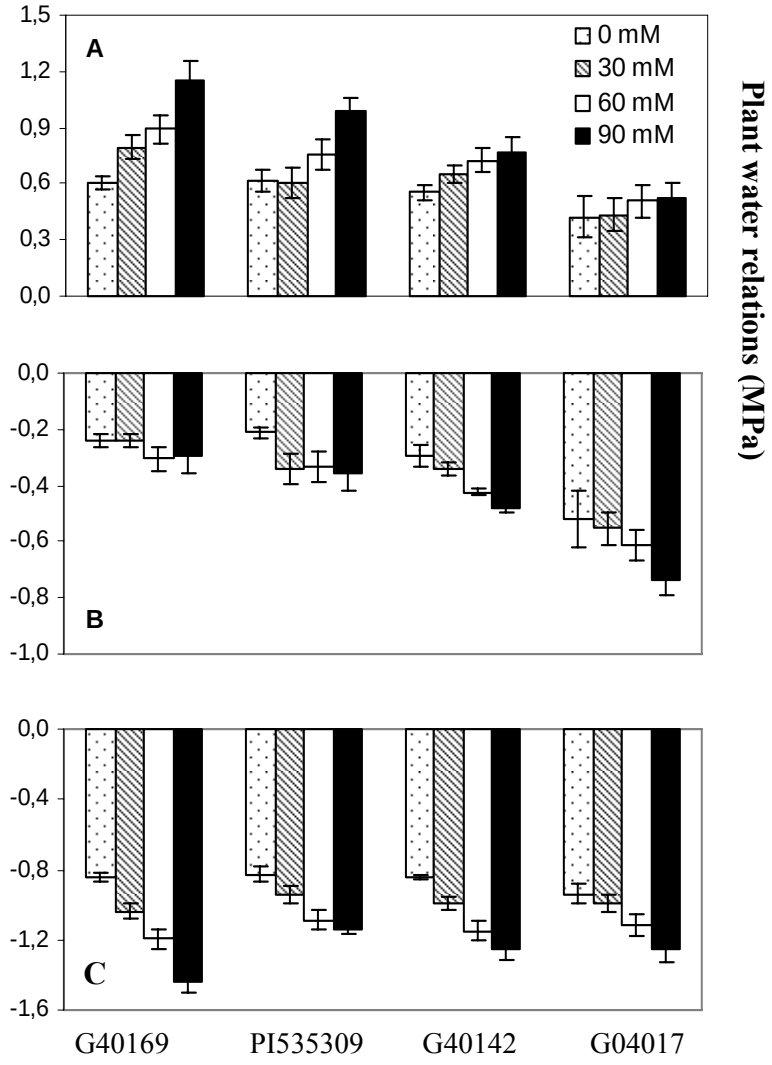

Fig. 1. Effects of increasing NaCl concentrations in the growth medium on leaf turgor potential (Ψ_p) (A); water potential (Ψ_w) (B), and osmotic potential (Ψ_π) (C) (in MPa) of *Phaseolus* species. Data correspond to the average of four measurements on different individual plants. Standard errors, when larger than symbols, are shown as vertical bars.

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SELECTION OF BEAN CULTIVARS FOR TOLERANCE TO WATER STRESS

G.B. Biudes[1], V. Moda-Cirino[1], Rogério T. Faria[2] and Ricardo T. Faria[3]

[1]Área de Melhoramento e Genética Vegetal do Instituto Agronômico do Paraná (IAPAR), Londrina – PR, Brasil, Caixa Postal 481, CEP 86001-970 (E-mail: vamoci@iapar.br); [2]Área de Engenharia Agrícola do IAPAR; [3]Departamento de Agronomia da Universidade Estadual de Londrina, Londrina – PR, Brasil, CEP 86051-990.

Introduction: In the regions of bean production, about 60% of the crops are under water deficit in any stage of development, which makes water shortage the second major constraint for crop production, after incidence of diseases (Singh, 1995). Common bean (*Phaseolus vulgaris* L.) is very sensitive species to water stress, particularly when it occurs at the reproductive stage, which leads to significant yield decrease (Molina et al., 2001). In spite that some agronomic practices, such as irrigation, can eliminate risk of water deficit, their high costs are prohibitive. Therefore, development of cultivars tolerant to water shortage is required for lower income farmers. In this context, this study had as an objective to evaluate the reactions of bean cultivars to water deficit, aiming to identify sources of tolerance to be used in breeding programs.

Material and Methods: Four bean cultivars of the commercial group Carioca were evaluated against the tolerant control IAPAR 81 (Moda-Cirino et al, 2001). All the cultivars were of type II- habit, indeterminate growth and intermediate cycle, about 88 days from emergence to physiological maturity. The experiment was conducted at the IAPAR Experimental Station, in Londrina-PR, Brazil (latitude 23° 30´S, longitude 51° 32´W), during the spring in 2004. The treatments consisted of different cultivars submitted to water stress or grown under adequate water regime. They were distributed in a split plot randomized block design, with three replications, with treatments allocated in the plots and cultivars in the subplots. For the treatment under water stress, water deficit started at flowering and lasted for 20 days. At that time, during rainfall or irrigation applications the plots were covered by moving shelters of 10m length, 5m width e 2,8m height, covered by a translucent roof. Weeds, pests and diseases were controlled following recommended practices. During the period of water stress soil moisture was measured once a week at depths 0-10cm, 10-25cm, 25-40cm and 40-60cm, using the gravimetric method. At physiological maturity, ten plants per subplot were collected to evaluate the following parameters: number of nodes at the main stem (NN), plant height (PH), number of pods per plant (PP), number of seeds per pod (SP), 100 seed mass (SM), grain yield per plant (PY) and total grain yield (TY). All the crop traits were submitted to analysis of variance using Genes program (Cruz, 2001). A reduction index (RI) was calculated for all cultivars to assess the effect of water stress on the crop traits according to the following expression: RI% = [100. (trait without stress - trait with stress) / trait without stress].

Table 1- Summary of the analysis of variance, genetic variation coefficient (CVg%), environmental variation coefficient (CVe%), genotypic determination coefficient (h^2%) for five bean cultivars of the group Carioca.

Source of variation	PH	NN	PP	SP	MS	TY	PY
Stress	**	**	**	*	**	**	**
Cultivars	**	**	**	**	**	**	**
Interaction	ns	**	**	ns	ns	ns	ns
CVg (%)	17,0	5,8	18,9	4,7	8,7	25,9	23,7
CVe (%)	7,7	3,7	7,1	5,8	3,7	10,0	9,9
h^2 (%)	96,7	93,5	97,7	79,5	97,2	97,6	97,2

ns: not significant; * signficant at 5% probability and ** signficant at 1% probability. PH: plant height (main stem height in cm), NN: number of nodes at the main stem; PP: number of pods per plant; SP: number of seeds per pod; MS: mean weight of 100 seeds (g); TY: grain yield (kg.ha^{-1}); PY: grain yield per plant (g).

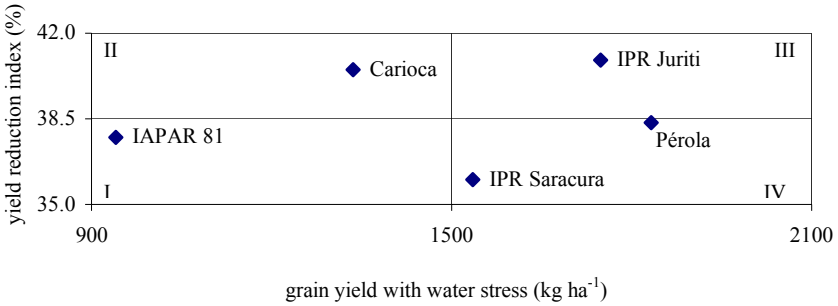

Figure 1**.** Relationship between yield reduction index (%) and grain yield with water stress (kg ha^{-1}), of common bean cultivars and lines, of the commercial carioca group.

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PERFORMANCE OF COMMON BEAN GENOTYPES FOR EARLY MATURITY IN SEMI-ARID AREAS OF EASTERN ETHIOPIA

Chemeda Fininsa and Bulti Tesso
Department of Plant Sciences, Haromaya University, P O Box 138, Dire Dawa, Ethiopia

Introduction: Drought and moisture stresses are primary limiting factors to crop production in Ethiopia. Common bean (*Phaseolus vulgaris* L.) production is constrained by recurrent droughts resulting in yield fluctuation from season to season. In bean growing semi-arid regions, rainfall is erratic in its distribution and the soil is often sandy with low moisture holding capacity [1]. The duration and distribution of rainfall during crop growing season are the key factors affecting crop productivity in dry-land agriculture [2].The erratic nature of rainfall distribution in the areas implies that water stress can occur at any time between emergence and maturity.

Developing early maturing and drought tolerant bean varieties in semi-arid areas of eastern Ethiopia is the most promising approach to expand common bean production, assure food self sufficiency, and satisfy the growing protein and cash source need. Earlier, variety development attention has been given only to high rainfall receiving potential areas, and the semi-arid areas were neglected. The objective of this study, therefore, was to evaluate the performance of common bean genotypes for early maturity under field conditions.

Materials and Methods: Fourteen bush type common bean genotypes, a standard (*Roba-1*), and local (*Red Wolaita*) checks were used. The genotypes were planted in plots of 1.2 x 4 m in four rows in RCBD in three replications at Babile (9° N 42° E, 1650 m.a.s.l., 600 mm annual rainfall, 22 °C mean annual temperature), eastern Ethiopia for five summer cropping seasons (1998-2002). Between row spacing was 0.4 m., and within row spacing was 0.1 m. No fertilizer was applied. The genotypes were sown each year between July 10 and 20, and harvested between 20 October and 10 November. Growth parameters (days to 50% flowering, maturity, height, and stand count), and seed yield and its components were considered for evaluation and selection of early maturing genotypes. Data were analyzed using analysis variance. Least significance difference (LSD) was used for mean separation.

Results and Discussion: The genotypes evaluated varied significantly ($P<0.01$) for their growth parameters (Table 1), and yield and its components (Table 2). Eleven genotypes flowered earlier than the standard and local checks, among which seven of them flowered in less than 50 days. Most of the genotypes matured in less than 85 days, and earlier than the checks. MAM-25 and MMS-25 genotypes gave better grain yield by producing 614 kg/ha, 551 kg/ha, respectively compared to others. These two genotypes mature in about 84 days, and have larger seed size. These early flowering and maturing genotypes have a good potential to escape terminal drought frequently occurring in the semi-arid areas of eastern Ethiopia. Moreover, the genotypes could also be grown in potential common bean growing areas of the country in years of drought years. Therefore, MAM-25 and MMS-25 can be promoted for earliness, and relatively better yield in the semi-arid and less fertile areas like Babile and Jijiga in eastern Ethiopia.

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Table 1. Growth characteristics of common bean genotypes evaluated for early maturity at Babile for five cropping seasons (1998-2002). Values are mean of five seasons.

Genotype	Stand count at emergence	Plant height (cm)	Stand count at harvest	Days to flowering	Days to maturity
RAB-473	59.0	26.7	31.7	58.9	89.9
TLP-11	62.0	28.9	33.9	59.7	94.3
MAM-25	66.1	22.8	46.3	48.5	84.3
MMS-25	65.5	22.3	46.9	47.0	82.5
ARA-3	65.5	21.4	38.8	51.5	82.6
EAP-7	64.6	26.1	28.8	58.9	91.7
NAG-242	67.1	29.8	36.9	59.1	88.1
G-11229	65.1	21.7	41.0	51.0	83.5
G-5481	60.9	23.7	45.9	46.0	79.9
G-14913	65.3	25.8	47.6	50.4	82.9
G-8043	64.9	21.8	45.4	46.0	79.9
G-18135	62.7	22.1	43.5	46,7	79.1
G-13220	63.4	21.2	44.4	47.1	79.8
G-14253	63.1	22.8	43.7	48.1	80.8
Red Wolaita	63.1	30.8	36.7	54.1	86.9
Roba-1	61.9	29.0	34.9	59.7	90.7
Mean	**63.3**	**24.8**	**40.4**	**52.0**	**84.8**
SE	1.4	0.9	1.9	0.8	1.0
LSD (0.01)	5.1	3.6	7.4	2.8	3.8
CV (%)	8.4	15.1	19.1	5.6	4.8

Table 2. Yield and its components of common bean genotypes evaluated for early maturity at Babile for five cropping seasons (1998-2002). Values are mean of five seasons.

Genotype	Number of pods/plant	Number of seeds/pod	100-seed weight (g)	Yield (kg/ha)
RAB-473	5.5	4.8	21.5	396.9
TLP-11	6.0	4.4	20.7	495.5
MAM-25	5.1	4.4	34.3	614.2
MMS-25	5.3	4.0	34.6	551.3
ARA-3	5.1	3.9	33.5	494.4
EAP-7	6.1	4.7	19.1	324.9
NAG-242	5.3	5.5	19.7	458.5
G-11229	7.4	4.9	13.7	382.6
G-5481	4.5	4.0	33.1	462.0
G-14913	4.8	4.1	32.3	447.4
G-8043	4.1	3.9	36.0	470.2
G-18135	4.2	3.5	36.0	444.6
G-13220	4.4	4.0	33.4	472.6
G-14253	4.2	3.5	33.3	420.6
Red Wolaita	5.1	5.2	22.7	450.5
Roba-1	5.7	5.2	18.2	432.2
Mean	**5.2**	**4.4**	**27.6**	**457.4**
SE	0.3	0.2	0.5	38.7
LSD (0.01)	1.3	0.9	2.0	142.8
CV (%)	25.6	21.0	7.7	32.8

DROUGHT RESISTANCE IN DIFFERENT MARKET CLASSES OF DRY BEAN

Margarita Lema*, Henry Terán, Marie Dennis, Craig Robinson, and Shree P. Singh
University of Idaho, 3793 North 3600 East, Kimberly, ID 83341-5076.
*Corresponding author (mlema@kimberly.uidaho.edu).

INTRODUCTION

Dry bean (*Phaseolus vulgaris* L.) production in the Western U.S. mostly occurs under intensively irrigated systems, although there is also some irrigation-assisted and dryland production. The ratio of agricultural over total water use is higher than 80% in most Western states, irrigation water supplies are highly variable, and farmers are often forced to apply less irrigation than desirable.

Net water requirement for optimum production of a 90-to 100-day dry bean crop ranges from 250 to 500 mm depending upon the length of growing season, climate, and cultivar. In general, dry bean is relatively more sensitive to water-stress or drought in pre-flowering and reproductive periods. Moderate to high drought stress reduces root growth, nodulation and biological nitrogen fixation, nutrient and water uptake and utilization, biomass or overall plant growth, number of seeds and pods, harvest index, seed yield, and seed quality including color, shape, weight, nutritive value, and recovery percentage. In the Western U.S., among various classes of dry bean, the large-seeded dark and light red and white kidney, cranberry, and other Andean bean are most susceptible to drought followed by small-seeded black and navy bean (Singh et al., 2001). The highest levels of drought resistance were found in medium-seeded cultivars of pink, red, pinto, and great northern market classes belonging to the Durango race that were introduced from the semi-arid central and northern Mexican highlands in the Western U.S. by the Native Americans. Native Americans and early settlers have grown dry bean under non-irrigated, unfertilized, and pesticide-free subsistence production systems for centuries. These dry bean cultivars are characterized by indeterminate, prostrate, semi-climbing growth habit Type III with pod concentration in the lower part of the canopy. In a recent study in Idaho, these landraces (e.g., Common Red Mexican) or old cultivars (e.g., 'Othello') exhibited the highest drought resistance (Muñoz-Perea et al., 2005). However, they are highly susceptible to diseases such as *Bean common mosaic virus*, common bacterial blight, rust, and/or white mold. Our objective was to identify drought resistant breeding lines and cultivars in the North American Cooperative Dry Bean Nursery (CDBN).

MATERIALS AND METHODS

Ten large-seeded, 20 medium-seeded, and eight small-seeded dry bean breeding lines or cultivars comprising the 2005 CDBN were grown in drought-stressed (DS) and non-stressed (NS) environments at the University of Idaho Parma Research and Extension Center (703 m elevation) in 2005. A randomized complete block design with two replicates was used for NS, and DS plots had a single replicate. Each plot consisted of four rows 25 ft long spaced 22 inches apart. The DS and NS plots were separated by eight rows of UI 239 planted in DS to avoid lateral movement of water from the NS to DS plots. Pre-plant herbicides were incorporated. After germination, hand weeding was practiced to keep plots free from weeds. Both plots received standard fertilizer and gravity irrigation was used for five irrigation each of 10 hr run (total 229 mm water) for the DS and nine irrigation each of 18 hr run (total 775 mm water) for the NS plots. Post-emergence cultivation and other management practices were similar to those commonly applied to dry bean in the Treasure Valley. For seed yield measurement, the two

central rows were cut at maturity, threshed 8 to 10 days latter, cleaned, and weighed at 12% moisture. Also, 100-seed weight, days to maturity, and growth habit were recorded. All data were analyzed using the SAS (v 9.1) PROC GLM statistical package.

RESULTS AND DISCUSSION

The mean reduction in seed yield in DS was 38% indicating a moderate drought stress. However, the mean reduction for large-seeded was 61%, for medium-seeded was 35%, and for small-seeded was 16%. Furthermore, the seed yield reduction values ranged from 90% (1863NS vs. 194DS kg ha^{-1}) for large-seeded 'Mogul' to –1% (2500NS vs. 2523DS kg ha^{-1}) for medium-seeded 'Merlot' (Table 1). Breeding line R02002 had 0% reduction. In general, large-seeded dry bean had the lowest mean seed yield in both the DS and NS environments. Pinto 'Canyon' followed by Othello was the highest yielding in NS and USRM 20 followed by 'Condor', Othello, and 115M had the highest yield in DS environment. From our previous studies (Singh et al., 2001) we had known that the medium-seeded dry bean had the highest and large-seeded the lowest levels of drought resistance in southern Idaho. Exceptionally high levels of drought resistance exhibited by small-seeded black bean Condor, 115M, CO27864, and CO11113 and white 'Seabiskit' and 'Sea Hawk', among others, are worthy of special mention because these are the first group of dry bean in their market class with such a high yield and drought resistance in the Western U.S. Nonetheless, further testing of these in replicated trials across contrasting DS and NS environments would be required to ascertain their drought resistance. Pinto Othello along with Common Red Mexican landrace and great northern 'Matterhorn' have shown high levels of drought resistance in Idaho (Muñoz-Perea et al., 2005). The feasibility of breeding for yet higher levels of drought resistance and yield potential from small-seeded x medium-seeded interracial crosses should be worth investigating. Similarly, the usefulness of these high yielding drought resistant small- and medium-seeded breeding lines and cultivars for improving yield potential and drought resistance of large-seeded dry bean needs to be determined.

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Table 1. Mean maturity, 100-seed weight, seed yield and percent reduction (PR) for selected dry bean breeding lines and cultivars from the CDBN evaluated in non-stressed (NS) and drought-stressed (DS) environments at Parma, Idaho in 2005.

Identification	Color	Maturity (days)	100-seed weight		Yield (kg ha^{-1})		PR (%)
			NS	DS	NS	DS	
Canyon	PT	102	34	31	4727	2923	38
Othello	PT	90	39	37	4610	3175	31
Merlot	RD	96	33	37	2500	2523	-1
USRM-20	RD	96	37	39	3841	3292	14
R02002	RD	98	35	32	2218	2207	0
115M	BK	99	21	19	4185	3034	28
Condor	BK	99	20	21	3290	3234	2
Seabiskit	SW	101	18	17	2917	2770	5
Sea Hawk	SW	98	24	22	2673	2488	7
UCD 9830	RK	103	42	42	1969	1602	19
Mogul	RK	99	47	40	1863	194	90
Mean		98	36	32	2882	1826	38
LSD (0.05)			4.2	18.3	1610	1788	

DYNAMICS OF DAY AND NIGHT WATER POTENTIAL AMONG LEAVES OF COMMON BEAN UNDER SOIL-WATER DEFICIT

Galván-Tovar, Marcos[1,7]; Kohashi-Shibata, Josué[2]; López-Castañeda, Cándido[3]; Acosta-Gallegos Jorge [4]; †Martínez-Garza, Ángel[5], García-Esteva, Antonio[6]
[1,2,3,5,6]Campus Montecillo, Colegio de Postgraduados. 56230 Montecillo, Edo. de Méx., México. Fax. (595) 95 2 02 47 y [4]Programa de Frijol. Campo Experimental Bajío, INIFAP. 38000. Apdo. Postal 112. Celaya, Gto. México. [7]Autor for correspondence: mgalvant@yahoo.com.

Introduction. The dynamics of the water potential among common bean (*Phaseolus vulgaris* L.) leaves at different hours during the night period (NP) and day period (DP) has not been well studied. Therefore, a study was undertaken to determine whether a water potential gradient or a potential equilibrium exist among the first four compound leaves (CL) of the main stem, as well as the dynamics of the water potential during the NP and DP.

Material and methods. The plants were grown in a greenhouse, one plant per pot in sandy loam soil. When the third CL was just completely expanded, normal watering (NW) and withheld water (WW) treatments were begun. At the beginning of permanent wilting of the first CL under WW, called here stress level 1 (SL-1), or the third compound leaf, called stress level 2 (SL-2), the water potential of the leaves (Ψ_{leaves}) was determined for both treatments every four hours of the NP (19:00, 22:00, 01:00, 05:00 h) and DP (10:00, 12:00, 14:00, 16:00 h). The evapotranspiration and vapor pressure deficit (VPD) were also determined.

Results and discussion. When SL-1 and SL-2 were reached, the Ψ_{leaves} in NW y and WW in the NP and in the DP showed no differences. In WW in the SL-2, Ψ_{leaves} differences occurred at 01:00 and 05:00 h of the NP. Such differences were due to the low values of CL-1 and CL-4 water potentials respectively. In general, Ψ_{leaves} did not show a gradient in the SL-1 and SL-2 in NW and WW at any time during the NP and DP (Figure 1). This response was the opposite of what was theoretically expected (Begg y Turner, 1976), but these results agree with those of McElrone *et al*. (2003), who determined that the different leaves of the main stem of *Parthenocissus quinquefolia* L. Planch. (Vitaceae) were at equilibrium at midday.
In WW as well as in NW, the average water potential per plant (Ψ_{plant}) increased during the NP to reach its highest value at 01:00 and 05:00 h. Since during NP, transpiration is minimal, it would be expected that Ψ_{plant} would be highest at dawn (Sellin, 1996) or several hours before. Under conditions of WW, the Ψ_{plant} increased slightly during the NP but diminished during the DP. In this period there were no changes registered among hours (Figure 2). It is probable that this was due to the closing of the stomata. During the DP in SL-2, the Ψ_{plant} in WW showed clear differences among hours, where the Ψ_{plant} decreased from a maximum at 10:00 h to a minimal value at 14:00 h, then again increased to a higher value at 16:00 h (Figure 2). The decrease of Ψ_{plant} during the DP in NW might be due to the increase the rate of transpiration, which was not compensated for water absorption by the roots. Consequently a transient water deficit was produced in the plant (Galston *et al*, 1980; Slatyer, 1967).

Conclusions. a) In WW and NW, the Ψ_{leaves} reached equilibrium in the four hours of the NP and DP, without being affected by evapotranspiration and the VPD. In NW this equilibrium was not affected by the level of stress. b) In WW and NW, the average water potential per plant increased

as VPD increased in NP, while as the water content of the soil decreased in NW, the plant water potential remained constant during the DP.

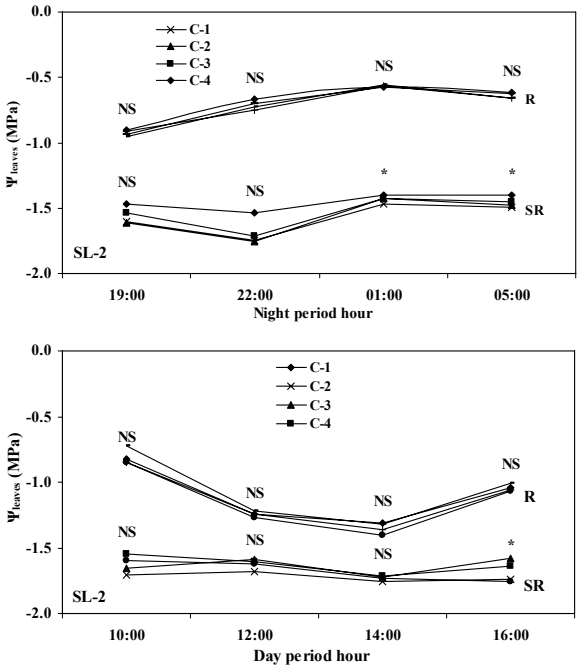

Figure 1. Water potential among leaves (Ψ_{leaves}) at various times during the night and day periods, under normal watering (NW) and withheld water (WW), at the beginning of permanent wilting of the compound leaf (CL-3). Each point represents the average of four observations. C-1, C-2, C-3, C-4 = compound leaf one, two, three and four. * = significant differences. NS = non-significant differences among leaves at the same time and with the same water treatment.

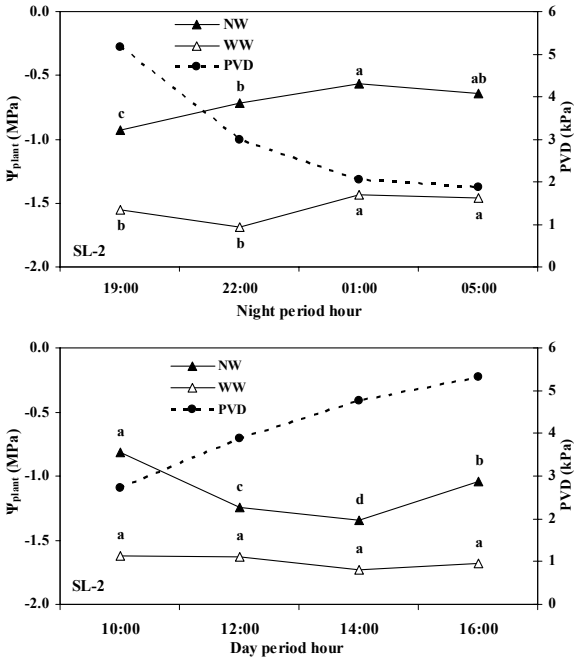

Figure 2. Average water potential per plant (Ψ_{plant}) at various times during the night and day periods, under normal watering (NW) and withheld water (WW), at the beginning of permanent wilting of the compound leaf (CL-3) and its corresponding vapor pressure deficit (VPD). Each point represents the average of four observations from four leaves of each plant and four replications for each leaf (16 observations). Means of water potential between times for each treatment followed with same letter are not significantly different at $P \leq 0.05$ using the Tukey test.

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CHLOROPHYLL CONTENT IN BEAN (*PHASEOLUS*) IN SALINE SOIL WITH FOLIAR-APPLIED OF IRON SULFATE

María Teresa Rodríguez-González*, J. Alberto Escalante-Estrada*, Mario Gutiérrez Rodríguez* y Ricardo Vega Muñoz*.

*Orientación en Botánica.Ecofisiología de Cultivos. Campus Montecillo.Colegio de Postgraduados, Montecillo, Edo. de México, CP 56230, México.

INTRODUCTION

Saline soils with high pH that exhibit Fe deficiency, present a problem for producers in low-rainfall climates throughout the world. When sorghum and corn are sowed in this soils, they show chlorosis (Godsey, 2003). One of the most used method for Fe deficiency correction, is foliar application of Fe solutions of ferrous sulphate (Fe $SO_4 \cdot H_2O$ or Fe $SO_4 \cdot 7H_2O$). This correction method usually alleviates chlorosis. The aim of this research was to evaluate several treatments of ferrous sulphate foliar application, to minimize the chlorosis, caused by Fe deficiency in bean, in saline soil with high pH.

MATERIAL AND METHODS

Research was carried out in Montecillo, Mexico (19º 98'W and 2250 m of altitude), with dry climate BS1, during the rainy season. The treatments were three cultivars of bush bean (*Phaseolus vulgaris* L.) Bayomex, and Canario-107 (determinate, Type I) and Criollo (indeterminate, Type II); one cultivar of (*Phaseolus coccineus* L.) Ayocote (indeterminate type II); two population densities: 6.25 (80X25 cm) and 12.50 (40X25cm) plants m^{-1} and three levels foliar sprays of Fe SO_4: 0, 2 and 4g L^{-1} water, applied each 15 days after emergence (on June 19 th ,2000), for five times. The soil characteristics were a clay soil; pH 8 to 8.7 and E.C. 7 to 14 dSm^{-1} and a percentage of exchangeable sodium of 9.73 to 37.When the soil is moist from rain the E.C. is reduced to 2 dSm^{-1}. All experiment was fertilized with 100-100-00 NPK. The experimental design was split plot with four replicates. The chlorophyll content in the four cultivars of bean was determined by two methods: destructive "slow" method (24 hours, Inskeep and Bloom, 1985) and fast method (five minutes), using the chlorophyll meter or SPAD meter (SPAD-502, Minolta Ramsey,NJ). In each plot, four readings were taken at random from the uppermost fully expanded leaf, first the SPAD lectures, then the samples for "destructive" method (two circles of one cm of diameter each one, for each sample). The analysis of chlorophylls were made to the 76 and 90 days after sowing (das), in reproductive stage.

RESULTS AND DISCUSSION

The readings of the SPAD can be reliable estimators of Chlorophyll content in bean plant, since both variables show a high relation (r^2=0.87 * to 0, 92 **, Table1).

Table 1. Relation Chlorophyll content and SPAD lectures in bean Cultivars. Equations based on two dates of Chlorophyll analysis.

Cultivar	**Equation: Chlorophyll content (mg L^{-1}) *vs.* SPAD lectures.**	**R^2**	**Prob F.**
Bayomex	Y= 1.0006X-22.809	0.87	*
Criollo	Y= 0.6438X-7.0894	0.90	**
Canario-107	Y= 0.6572X-7.9262	0.92	**
Ayocote	Y= 0.6296X-7.1863	0.90	**

*, ** F>0.05 and 0.01, respectively.

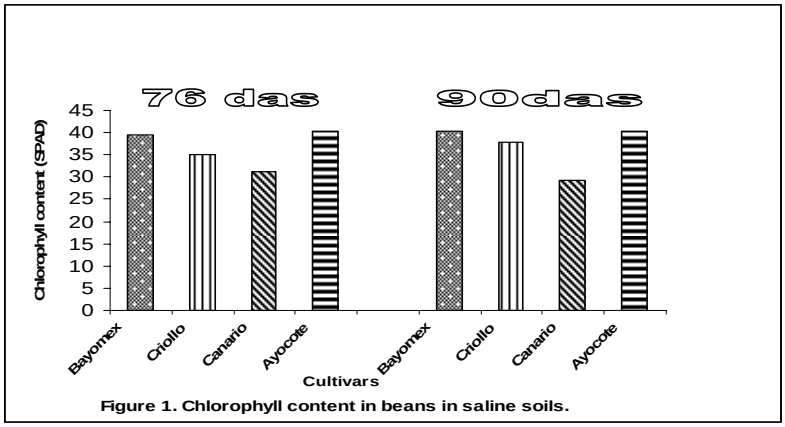

Figure 1. Chlorophyll content in beans in saline soils.

Thus, in figure 1, it was observed at 76 and 90 (das), that Ayocote showed the highest chlorophyll content, followed of Bayomex and Criollo; and the lowest corresponded to Canario. These differences were significant (P>0.05). With the application of ferrous sulphate, the plants showed greater content of Chlorophyll. Bayomex and Ayocote with 2 g of ferrous sulphate L^{-1} showed the highest values of Chlorophyll (19 mg L^{-1} in average) and Canario (without ferrous sulphate) the lowest (11.8 mg L^{-1}). Finally, these results suggest variations in chlorophyll content between cultivars. With the ferrous sulphate can prevent chlorophyll degradation in the leaves of bean and so get a longest duration of the photosynthetic activity. The response to ferrous sulphate will depend of the cultivar in study.

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DIVERSITY IN TOLERANCE BETWEEN *PHASEOLUS VULGARIS* AND *PHASEOLUS COCCINEUS* GENOTYPES TO SALINITY

Mario Gutiérrez Rodríguez, José Alberto Escalante Estrada

Programa de Botánica, Colegio de Postgraduados. Carretera México-Texcoco Km. 36.5, 56230, Montecillo, México. México

Introduction

Salinity affects one third of the world's irrigated land, especially in arid and semi-arid areas. It is one of the main causes that reduce crop yield because some crops are sensitive for growing under saline conditions (Maas, 1996). The specie *Phaseolus vulgaris* L. is considered as sensitive to salinity while *Phaseolus coccineus* L. is considered as tolerant (Subbarao and Johansen, 1994). The objective of the present work was to determine yield differences among genotypes of *P. vulgaris* L. and *P. coccineus* L. grown under saline field conditions.

Material and methods

The study was carried out in Montecillo, Mexico (19º19' N, 98º54' W, 2250 m of altitude) during rainy season (June-September, 2004) and with a temperate climate. Seeds of thirteen genotypes of *Phaseolus vulgaris* L. (Bayo-18, Bayomex, Canario-107, Flor de Durazno, Flor de Mayo, Negro 98, Morito, Ojo de Cabra, Peruano, Pinto, Pinto Cabaña, Promesa, and Zacatecas) and three genotypes of *Phaseolus coccineus* L. (Ayocote Blanco, Ayocote Morado, and Ayocote Negro) were sown in a soil with a pH of 6.8-7.5 and an electro-conductivity of 2-5 dS m^{-1}. The design was a random block with four replications, and a plant density of 12.5 plants m^{-2}. All the plots were fertilized with 100-100-00 NPK. Seed yield, biomass (mean of 4 plants) and other yield components were measured at physiological maturity. SPAD-502 was used to estimate chlorophyll content on the central leaflet during the anthesis stage.

Results and discussion

The *P. vulgaris* and *P. coccineus* genotypes showed diversity in yield and biomass production under saline field conditions (Table 1, 2). However, the *P. coccineus* genotypes had a higher yield and biomass production than did *P. vulgaris* genotypes. The *P. coccineus* genotypes showed a high seed weight, and height. The high number of seeds of *P. vulgaris* genotypes had low weight.

The final seed yield of *P. vulgaris* genotypes was related to low tolerance under saline growing conditions. In addition, the salinity reduced the days to reach anthesis and maturity compared with the *P. coccineus* genotypes (Table 1, 2). The *P. coccineus* plants reached anthesis at the same time like the most sensitive genotypes of *P. vulgaris* (Flor de Durazno, Canario-107, and Peruano) but they showed the longest time (days) between anthesis and maturity.

The most resistance *P. vulgaris* genotypes (i.e., Zacatecas, Ojo de Cabra, and Morito) showed the highest days to reach anthesis and maturity (Table 1).

The most sensitive genotypes to salinity showed low chlorophyll content while the most tolerant showed a higher content.

Conclusions

In conclusion, *P. coccineus* is more tolerant to saline field conditions than *P. vulgaris* showing high yield, and a longer growing season from emergency to the physiological maturity.

Table 1. Seed yield, biomass and other components measured at physiological maturity in genotypes of *Phaseolus vulgaris* and *Phaseolus coccineus* grown under saline field conditions. Montecillo, México.

Cultivar	Yield (g pl^{-1})	Biomass (g pl^{-1})	100 seeds (g)	Height (cm)	Pods Pl^{-1}	Seeds pod^{-1}	Anthesis (days)	Maturity (days)	Chl-Ant (SPAD)
Phaseolus coccineus									
Ayocote Blanco	52.2	137.7	200.4	129.0	10	2.6	46	122	**32.5**
Ayocote Morado	45.1	144.3	175.6	155.0	9.8	2.6	50	120	34.6
Ayocote Negro	41.8	110.4	170.9	128.0	10.0	2.4	52	119	35.8
Phaseolus vulgaris									
Zacatecas	25.0	51.2	45.8	83.0	19.2	2.9	82	105	32.9
Ojo de Cabra	23.4	36.4	49.1	65.6	158	3.9	78	98	32.3
Morito	290.1	620.4	53.7	77.0	12.6	4.3	75	100	30.2
Bayo-18	23.0	42.7	28.9	70.8	20.2	3.9	75	96	29.2
Bayomex	19.4	32.7	42.9	65.4	10.8	4.3	69	99	31.8
Negro 98	19.3	32.7	37.1	42.2	10.8	3.9	76	95	29.3
Promesa	17.4	31.7	45.5	48.8	10.6	3.7	80	96	21.1
Pinto Cabaña	15.7	33.0	28.5	59.6	16.2	3.8	74	94	29.1
Pinto	12.1	27.6	30.9	50.6	11.8	3.4	76	95	37.1
Flor de Mayo	11.5	18.7	25.5	32.4	12.4	3.9	72	105	27.0
Flor de Durazno	11.1	20.4	34.5	36.4	9.0	3.6	46	84	33.5
Canario-107	7.6	12.9	38.0	29.0	8.6	2.4	46	84	25.2
Peruano	5.4	14.6	41.6	25.4	5.6	2.3	45	88	25.5
LSD	9.7	17.8	26.1	21.9	3.1	0.62	5.2	8.9	15.2

Chl-Ant, chlorophyll estimation at anthesis.

Table 2. Average of yield among genotypes of *Phaseolus vulgaris* and *Phaseolus coccineus* grown under saline field conditions. Montecillo, México.

Specie	Yield (g pl^{-1})	Biomass (g pl^{-1})	100 seeds (g)	Height (cm)	Pods Pl^{-1}	Seeds pod^{-1}	Anthesis (days)	Maturity (days)	Chl-Ant (SPAD)
Phaseolus coccineus	48.3a	130.7a	182.3 a	137.3 a	9.9b	2.5 b	49.3 b	120.3 a	34.3 a
Phaseolus vulgaris	16.7b	31.8b	37.7 b	50.6 b	11.9a	3.4 a	64.2 a	89.1 b	28.5 b

†Different letters indicate statistical significant differences (Tukey, $p \leq 0.05$). In a previous report we established that the low-yielding genotypes of *P. vulgaris* and *P. coccineus* showed low gas exchange induced by the saline conditions (Gutierrez & Escalante, 2005).

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EVALUATION OF LIMAS UNDER IMPOSED ENVIRONMENTAL STRESS

John Rayapati and Brett Despain
Archer Daniels Midland Company, Edible Bean Specialties Research, 19332 Homedale Rd. , Caldwell , Idaho, 83607 U.S.A. rayapati@admworld.com

Introduction: Seed production of lima beans is conducted in the high desert areas of Treasure Valley Idaho. The furrow irrigated high desert environment is used to minimize seed borne diseases.

Methods: Four varieties were grown in the same field and harvested at the same time near Caldwell Idaho. The experiment was first conducted in 2004 and repeated in 2005. Standard treatment was planted May 20th and irrigated weekly until harvest. Drought treatment was planted at the same time as standard and irrigation was stopped at 50 days after planting. Early planting was irrigated weekly but planted 20 days earlier in 2004 and 15 days earlier in 2005. Four row plots with three replications were used. Seed yield in kg/hectare can be estimated by multiplying g/plot by 1.7.

Results:

Table 1. Seed yield (g/plot) of 4 varieties in 6 environments

	2005			2004		
Variety	**Early**	**Drought**	**Standard**	**Early**	**Drought**	**Standard**
M15	921	59	622	1090	1324	891
Kingston	1169	105	421	1019	926	573
Maestro	1070	114	633	1480	1519	1009
Meadow	1341	221	935	1489	1582	1075
Average	1125	125	652	1269	1338	887
Standard Deviation	175	33	142	550	281	225
%CV	16%	26%	22%	43%	21%	25%

Table 2. Comparisons of two methods of ranking cultivars

Variety	**Arithmatic AVG**	**Geometric AVG**	**(Z +3) Adaptability Calculation**	**(CVz) Stability Coefficient**
M15	818	593	2.37	31%
Kingston	702	551	2.12	32%
Maestro	971	748	3.13	13%
Meadow	1107	942	4.38	20%

Regression analysis between Geometric Average and Adaptability Calculation

$Y = 174.4X + 185.35$
$R^2 = 0.9961$
$p = 0.00196$

Table 3. Analysis of seed yield (g/plot) of limas in standard (STD), droughted (DRT) and early-planted (ELY) environments in 2004 and 2005.

Comparison	Mean	Mean	t Statistic	t Critical	p value	Significant Difference
DRT0405 vs STD0405	731	770	0.15	1.83	0.4415	no
DRT04 vs STD 04	**1338**	887	2.44	1.94	0.0253	yes
DRT05 vs STD05	125	**652**	4.74	2.13	0.0045	yes
ELY0405 vs STD0405	**1197**	770	3.78	1.76	0.0010	yes
ELY05 vs STD05	**1125**	652	3.43	1.94	0.0070	yes
ELY04 vs STD04	**1269**	887	2.28	1.94	0.0313	yes

Conclusions

1. Seed production of green baby limas in the Treasure Valley of Idaho can be improved by planting 15 to 20 days earlier than the usual May 20th planting date for dry beans.

2. Limiting irrigation can increase yield of Limas in some but not all environments.

3. The adaptability calculation of Airton et al. 2005 correlates well with geometric mean. The adaptability calculation uses deviations from an environment mean, and geometric mean does not. The stability coefficient of the Airton method (which is the coefficient of variation of the adaptability calculation), offers an additional tool for identification of lines with stable traits.

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EFFECT OF TWO TILLAGE SYSTEMS ON STRUCTURAL SOIL PROPERTIES AND GRAIN YIELD OF DRY BEANS UNDER RAINFED CONDITIONS IN NORTH-CENTRAL MEXICO[1]

E.S. Osuna-Ceja[2], J.S Padilla-Ramírez[2], E. Martínez-Meza[2], M.A. Martínez-Gamiño[2] and J.A. Acosta-Gallegos[2]

[1]Research partially financed through the project 175 (SAGARPA-CONACYT). PRECI 6055905A. [2]Bean Program INIFAP. Apdo. Postal 20 Pabellón de Aretaga Aguascalientes, Mexico. C.P. 20660.esosuna@yahoo.com

Introduction. Soil degradation and low precipitation patterns are among others limiting factors in rainfed bean production on semiarid lands of North-Central Mexico (1). Soil degradation involving conventional methods of soil tillage have shown negative influences in grain yield of bean by modifying structural soil properties. Structural soil properties affected by conventional tillage are soil water transmission, retention and storage characteristics (2). Besides soil physical properties also textural soil characteristics influence rainfall reception and infiltration capacity. The infiltration rate is a function of total porosity, the relative proportion and continuity of macropores and the stability of aggregates (3). Rainfall shortage in growing season of bean is a recurrent phenomenon in semiarid North-Central Mexico. The occurrence of plant water stress as a consequence of lower soil moisture availability during flowering and seed maturation stages may cause a reduction in grain yield. Grain yield of bean under rainfed conditions may be enhanced by utilizing Improved (drought resistant) bean varieties in conjunction with both conservation tillage systems and reception and conservation of soil water techniques. The objective of this study was to analyze the combined effect of tillage systems and a rainwater harvesting *in situ* technique ("pileteo") on some structural soil properties and grain yield of bean.

Materials and Methods. A field study was conducted at "Sandovales", Aguascalientes INIFAP-Experimental Substation in North- Central Mexico on a sandy clay loam soil with pH value of 7.9 and < 1% organic matter during summer 2005. This area is characterized as semiarid land. Mean annual precipitation is 450 mm and an altitude of 2000 m a. s .l. Plot size was 1 ha. The tillage methods examined were conventional tillage (CTP), conventional moldboard plowing followed by two diskings and reduced tillage (RTP), chisel plowing followed by two diskings, the soil is not turned at all; both tillage methods were combined with a rainwater harvesting *in situ* technique (P ="pileteo"). This last consisted of pulling soil on the bottom between rows for building micro-catchments along rows (50 cm apart) by using a kind of mechanical shovels ("pileteadora") attached to the rear rigid tines of cultivator. The "Flor de Mayo Sol" (FMS) improved bean variety was utilized. It was planted on 5 July 2005 in rows spaced 76 cm apart and resulted in average population of 100,000 plants ha^{-1}. Plot was cultivated twice during the growing season. Soil physical and yield parameters evaluated on the end of the growing season were: Saturation water content (θ_s), soil penetration resistance (R_p), bulk density (ρ_b), total soil porosity (f_t), soil water index (V_w), soil air index (V_a), soil solids index (V_s), empty space ratio (e) (4) and average grain yield of bean ($\hat{Y}_g$) respectively. Variability of soil and yield parameters were determined by using classification criteria proposed by (5). It is coefficient of variability (CV) < than 15% is classified low, 16-35% as moderate and > 35% as high variability respectively. Soil physical and yield parameters were evaluated by descriptive statistics and "t" test ($\alpha = 0.05$). The relationships between e and ρ_b and $\hat{Y}_g$ were evaluated by linear correlation.

Results and Discussion. The mean and median values of soil physical and yield parameters evaluated were similar for both tillage systems. These findings indicate that data follows a normal distribution (Table 1). Coefficient of Variability (CV) of soil and yield parameters and its classification according to Wilding (1985) were: R_p parameter from RTP resulted with the highest coefficient of variability (51%); therefore, it was classified as high. CV of R_p, V_a and $\hat{Y}_g$ values from CTP were 19.48, 27.18 and 27.18% respectively and CV of V_a and $\hat{Y}_g$ values from RTP were 17.65 and 29.63%, both cases were classified as

moderate variability. CV of ρ_b, V_s, f_t, e, V_w and θ_s values with a range of 2.58 -11.11% from CTP and 2.78 – 7.96% from RTP both were rated as low variability (Table 1). Negative correlation between e and ρ_b values was found (Figure 1a). In contrast, positive correlation between e and $\hat{Y}_g$ values was found (Figure 1b). R_p value from CTP significantly higher than R_p value from RTP. This, in part, explain reduction in V_w and θ_s mean values from CTP. No significant differences in $\hat{Y}_g$ were found among tillage systems ("t" test $\alpha = 0.05$). This no significance in grain yield was similar as that reported for soybean by (6). In general, descriptive statistics analysis results showed low variability among soil and grain yield parameters evaluated in both CTP and RTP systems. Since "Pileteo"(**P**) was included along with both tillage systems as standard technique; therefore, there was not any discussion about this.

Table 1. Descriptive statistics of soil physical properties and grain yield of dry beans resulting from conventional and reduced tillage systems under rainfed conditions, North-Central Mexico. 2005

Parameters	N	Mean	Median	Sd	CV %	Minimum	Maximum
CONVENTIONAL TILLAGE (CTP)							
R_p (Pa)	10	242.32 **a**	248.38	65.87	27.18	145.39	339.25
ρ_b (Mgm^{-3})	10	1.700 **a**	1.707	0.044	2.58	1.637	1.775
f_t (cm^3cm^{-3})	10	0.346 **a**	0.346	0.017	4.91	0.317	0.370
Θ_s (cm^3cm^{-3})	10	0.369 **b**	0.369	0.041	11.11	0.305	0.426
V_w (cm^3cm^{-3})	10	0.269 **b**	0.269	0.022	8.18	0.234	0.299
V_a (cm^3cm^{-3})	10	0.077 **a**	0.077	0.015	19.48	0.056	0.104
V_s (cm^3cm^{-3})	10	0.654 **a**	0.654	0.017	2.60	0.630	0.683
E (cm^3cm^{-3})	10	0.530 **a**	0.530	0.040	7.54	0.465	0.588
$\hat{Y}_g$ (Kg $parc^{-1}$)	10	0.093 **a**	0.086	0.028	30.10	0.054	0.142
REDUCED TILLAGE (RTP)							
R_p (Pa)	10	155.08 **b**	139.33	79.10	51.00	72.70	327.13
ρ_b (Mgm^{-3})	10	1.689 **a**	1.689	0.047	2.78	1.607	1.754
f_t (cm^3cm^{-3})	10	0.350 **a**	0.350	0.018	5.14	0.325	0.381
Θ_s (cm^3cm^{-3})	10	0.421 **a**	0.421	0.030	7.12	0.379	0.475
V_w (cm^3cm^{-3})	10	0.296 **a**	0.289	0.015	5.07	0.275	0.322
V_a (cm^3cm^{-3})	10	0.054 **a**	0.057	0.016	29.63	0.027	0.081
V_s (cm^3cm^{-3})	10	0.650 **a**	0.650	0.018	2.77	0.618	0.675
e (cm^3cm^{-3})	10	0.540 **a**	0.540	0.043	7.96	0.482	0.617
$\hat{Y}_g$ (Kg $parc^{-1}$)	10	0.085 **a**	0.084	0.015	17.65	0.065	0.114

Where: $\mathbf{R_p}$ = soil penetration resistance, $\boldsymbol{\rho_b}$ = bulk density; $\boldsymbol{f_t}$ = total soil porosity, $\boldsymbol{\theta_s}$ = Saturation water content; $\mathbf{V_w}$= soil water index, $\mathbf{V_a}$ = soil air index, $\mathbf{V_s}$ = soil solids index, **e** = empty space ratio and $\hat{\mathbf{Y}}_\mathbf{g}$ = average grain yield.

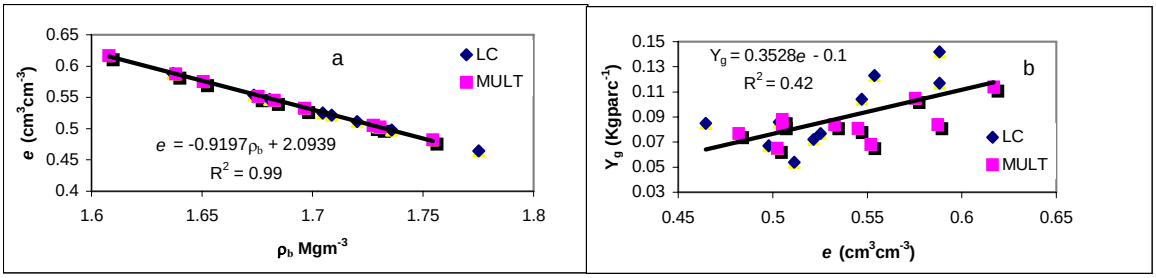

Figure. 1a and b, Relationships between (ρ_b) bulk density (Mgm^{-3}) and (e) empty space ratio (cm^3 cm^{-3}) and their effect on ($\hat{Y}_g$) grain yield of bean (Kg $parc^{-1}$) under rainfed conditions.

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BEANS (*PHASEOLUS VULGARIS* L.) YIELD IN RELATION TO GROWTH HABIT, PLANT DENSITY AND NITROGEN FERTILIZATION.

J. Alberto Escalante-Estrada [1], Maria Teresa Rodríguez- Gonzalez [1] and L. Enrique Escalante E.[2]

Orientación en Botánica. Ecofisiologia de Cultivos. Campus Montecillo. Colegio de Postgraduados Montecillo Mex. 56230 Mexico (1). Colegio Superior Agropecuario del Estado de Guerrero. Iguala Gro. México. (2)

INTRODUCTION

The optimum plant density than can be sustained also depends on environmental resources available per example, high yield of beans can be attained with high plant density and nitrogen supply (Escalante and Kohashi, 1993). The optimum sole-crop density for beans varies according to growth habit. The sole-crop optimum density for bush beans (types I and II) is approximately double that of climbing beans (24 plants per m2) and prostrate and semi-climbing (types III) are intermediate between these (Wooky and Davies, 1991). The objective of this study was to determinate the response of beans to plant density and nitrogen in relation to beans growth habit.

MATERIALS AND METHODS

The experiment was conducted in the field during the rainy season in a clay loam soil and 0.50 ppm of NO_3 initial (depth of 30 cm), temperate climate and 2,240 m of altitude. The beans cultivars: Cacahuate 72 (C72) a type I determinate bush bean (light seed with red line, 45 days to flowering and 94 days to maturity); Michoacan 12-A-3 (M12) a Type II indeterminate bush bean (black seed, 56 days to flowering and 114 days to maturity); and Bayo Madero (BM) a type III indeterminate bush bean (yellow seed, 44 days to flowering and 112 days to maturity) were sown on may 8, 2003. The plant densities (PD) were: a) 8.3 (15 x 80 cm); b) 12.5 (10 x 80 cm); c) 16.6 (15 x 40 cm); d) 25.0 (10 x 40 cm); and 50 plants per m^2 (5 x 40 cm). The nitrogen levels were 0 and 100 kg per ha (0 and 10 g per m^2). Treatments were allocated in a split-split-plot design with 4 replicates. A regression analysis for seed yield (dry weight, 10% humidity) in relation to PD and nitrogen was applied for cultivar.

RESULTS AND DISCUSSION

The cultivars showed different model of response for the relationship seed yield (SY) and plant density. In the C72 (type I) and BM (type III) the best fitted model was y=a + b ln (x);but en M12 (type II) was a quadratic model ($y=a+bx+cx^2$). The table 1 shows the equations for the cultivars in study. The N supply increased the SY for each density and cultivar. So, highest SY was 249 g m^{-2} for C72 and 50 plants m^{-2}; 254 g m^{-2} for M12 and 25 plants m^{-2}; and 193 g m^{-2} for BM and 50 plants m^{-2} although the difference with 25 plants m^{-2} was just 4%. The results suggest that the response in the beans seed yield to plant density can be relational with the growth habit and that apparently the nitrogen supply do not change this relation.

Table 1. Response of beans (*Phaseolus vulgaris* L.) to plant density and nitrogen supply. Chapingo-Montecillo. 2003.

Cultivar	Kg de N ha^{-1}	Equation: Seed yield (g m^{-2})vs. plant density (plants m^{-2})	R^2	Prob. F
Cacahuate 72	000	Y= -40.3 + 61.1 ln (x)	0.98	**
	100	Y= -72.8 + 81.4 ln (x)	0.94	**
Michoacán 12-A-3	000	$Y= -4.6 + 14.5x- 0.22x^2$	0.95	**
	100	$Y= 58.8 +12.8x-0.19x^2$	0.97	**
Bayo Madero	000	Y= 29.4 + 38.9 ln (x)	0.99	**
	100	Y= 65.4 + 36.9 ln(x)	0.99	**

**Prob F >0.01

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NATURAL ROCK OF IPIRÁ USED AS SOIL CONDITIONING CROPPED WITH COMMON BEAN

Itamar Pereira de Oliveira; Daniel Pettersen Custódio, Belmiro Pereira das Neves, Renato Sérgio Santos Mota

EMBRAPA Arroz e Feijão.
C.P. 179 75 375-000 Santo Antônio de Goiás. BRAZIL
Phone: 62 3533 2158; Fax: 62 3533 2100; E-mail: itamar@cnpaf.embrapa.br

Natural Rock of Ipirá presents a mineral composition containing aluminum and silicate and any mineral elements able to influence the soil chemical characteristics and to behave as soil conditioning. As low mineral concentration fertilizer, this bentonite clay can deliver available plant nutrients in proportion, facilitated by soluble flux the balance of soil solution nutrients that feeds the plant needs trough root system (Rossi,2004; Gopinath et al., 2003)). Then, high amounts of natural fertilizer source must be used to supply the crop needs (Anônimo, 2004). Also, natural conditioning can act as soil amendment similar to organic matter stabilizing the soil mineral composition avoiding toxic effects of aluminum, iron and manganese on bean plants. Therefore, conditioning material acts in soil improving its soil physical characteristics facilitating the vegetal development and the plant food. Nowadays any Brazilian states have stimulated technical researches using soil conditioning in mineral nutrition of plant, soil amendment and pottery. This goal research was to test the Natural Rock of Ipirá on the soil exchangeable complex cropped with common bean.

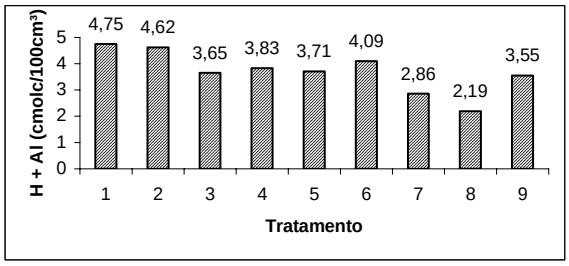

FIGURE 1. Concentration of soil acidifying (H+Al).

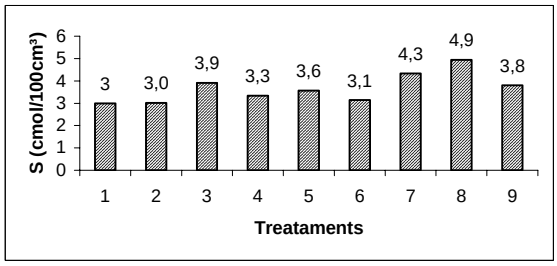

FIGURE 2. Sum (S = Ca+M+K) of soil cations in plots treated with any fertilizer doses.

The experiment was carried out in Saint Antonio de Goiás- Goiás State in one acid Oxisol, poor in organic matter, in phosphorus, calcium, magnesium, manganese and zinc. The following treatments were studied: 1 = check treatment, 2 = low fertilizer dose - 250 kg/ha of 4:30:16 (N, P_2O_5, K_2O), 3 = 300 kg/ha of Natural Rock of Ipirá used as fertilizer, 4 = 300 kg/ha of Natural Rock and 250 kg/ha of 4:30:16 (N, P_2O_5, K_2O) , 5 = limed soil and 300 kg/ha of Natural Rock of Ipirá plus 250 kg/ha of 4:30:16 (N, P_2O_5, K_2O). 6 = application of 1,000 kg/ha of Natural Rock of Ipirá.7 = application of 3,000 kg/ha of Natural Rock of Ipirá .8 = 9,000 kg/ha of Natural Rock of Ipirá and 9 = application of 27,000 kg/ha of Natural Rock Ipirá.

Higher H+Al concentrations (Figure 1) were observed in check plot. This result means that Natural Rock of Ipirá works as soil conditioning unconcerned with the amounts applied. In the contrary, higher S values (Sum of Ca + Mg + K) were observed where natural fertilizer was present (Figure 2).

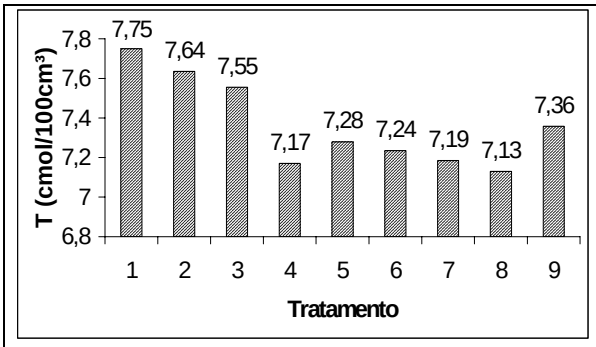

FIGURE 3.Variations in the cation exchange capacity (CEC) of soil.

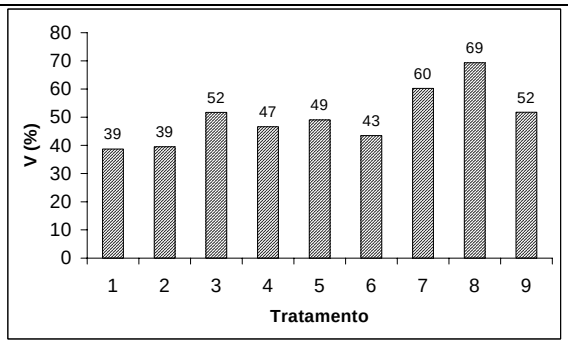

FIGURE 4. Base saturation (V) of soil.

Higher cation exchange capacity values were observed in check plot and in parcels were low doses of natural fertilizer were applied (Figure 3). In the other manner, these results can be attributed to the higher Al+H concentrations observed in the Figure 1. The CEC (Ca+Mg+K+Al+H) increased deeper in relation to A+H than in function of Ca+Mg+K concentrations. These results can be observed in Oxisol, from savanna soil, similar to that used in this research (Thung and Oliveira, 1998). Also, base saturation (V = S/T) presented higher values in plots where higher doses of Natural Rock of Ipirá were applied. Base saturation can be used as indicative of soil amelioration (Figure 4). In general way, the Natural Rock of Ipirá, can be used as soil conditioning in combination with low doses of fertilization in soil presenting low fertility to produce grain for the man and animal. It is believable that best results can be obtained in medium and high-soil fertility by using natural source as soil fertilizer.

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NATURAL ROCK OF IPIRÁ EFFECTS ON COMMON BEAN (*PHASEOLUS VULGARIS*) PRODUCTION AND SOIL ACIDITY

Itamar Pereira de Oliveira, Daniel Pettersen Custódio, Belmiro Pereira das Nesves,

Renato Sérgio R. Santos

EMBRAPA Arroz e Feijão.
C.P. 179 75 375-000 Santo Antônio de Goiás. BRAZIL
Phone: 62 3533 2158; Fax: 62 3533 2100; E-mail: itamar@cnpaf.embrapa.br

The Natural Rock of Ipirá is the commercial name of a product that presents as basic component the bentonite (Anônimo, 2006) that comes being tested with positive results on areas of crop exploration. The bentonite is a clay found in natural deposits presenting variable composition in accordance with local clime conditions from where the original material was formed. In water presence, the bentonite particles hydrate and expand forming a colloidal supension. In the maximum expansion, these particles move freely and due to the electric loads that they possess, form organized structures. Depending on the rock exchangeable cation, the bentonites can be calcic or sodic (Gopinath et al., 2003). They present characteristic stability and sometimes form porous surface quickly and has the capacity to liquefy and to form gel depending on the local humidity. Some of these clays are used as repellent pesticide in biological control of jungle plagues, medicinal plants and in traditional crops (Rossi, 2006). This research purposes is to test the efficiency of the Natural Land of Ipirá as fertilizer in the development and productivity of bean crop in comparison with commercial fertilizer applied in amounts based on Brazilian official recommendation.

The experiment was carried out in Santo Antonio de Goiás- Goiás State, Brazil in one acid Oxisol, poor in organic matter, in phosphorus, calcium, magnesium, manganese and zinc. The following treatments were studied: 1 = check treatment, 2 = low fertilizer dose - 250 kg/ha of 4:30:16 (N, P_2O_5, K_2O), 3 = 300 kg/ha of Natural Rock of Ipirá used as

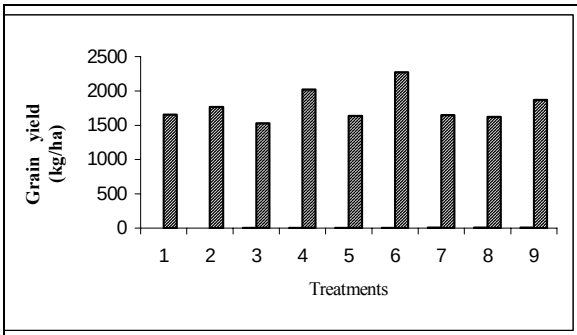

FIGURE 1. Effects of Natural Rock of Ipirá on grain yield of bean (kg/ha).

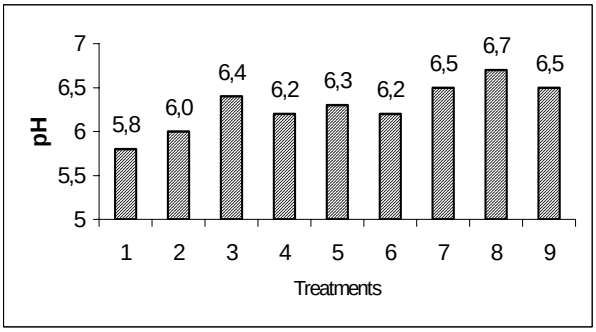

FIGURE 2. Effects of Natural Rock of Ipirá on soil pH (1:2,5 soil: water).

fertilizer, 4 = 300 kg/ha of Natural Rock and 250 kg/ha of 4:30:16 (N, P_2O_5, K_2O), 5 = limed soil and 300 kg/ha of Natural Rock of Ipirá plus 250 kg/ha of 4:30:16 (N, P_2O_5, K_2O), 6 = application of 1.000 kg/ha of Natural Rock of Ipirá, 7 = application of 3,000 kg/ha of Natural Rock of Ipirá,

8 = 9,000 kg/ha of Natural Rock of Ipirá and 9 = application of 27,000 kg/ha of Natural Rock Ipirá.

The common bean was cultivated as irrigated crop under central pivot. The parameters of production and soil characteristics observed were grain yield, pH, soil concentration of Ca and Mg. The bean production was influenced by the Natural Rock of Ipora application (Figure 1). High production was obtained by applying high amounts of Natural Rock of Ipirá but the production obtained by application of 300 kg/ha of Natural Rock plus 250 kg/ha of 4:30:16 (N, P_2O_5, K_2O) was similar to that obtained in parcels where 1,000 kg/ha of Natural Rock of Ipirá were applied. Higher soil pH, Ca and Mg values were observed in plots where Natural Rock of Ipirá was applied (Figure 2).

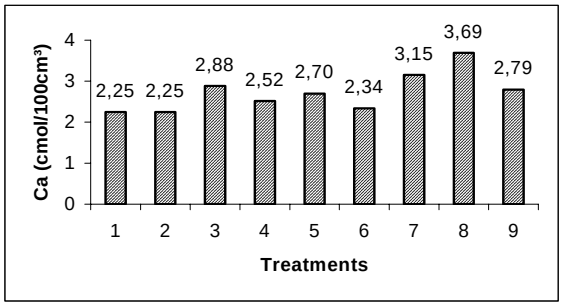

FIGURE 3. Effects of Natural Rock of Ipirá on soil calcium (Ca) concentrations.

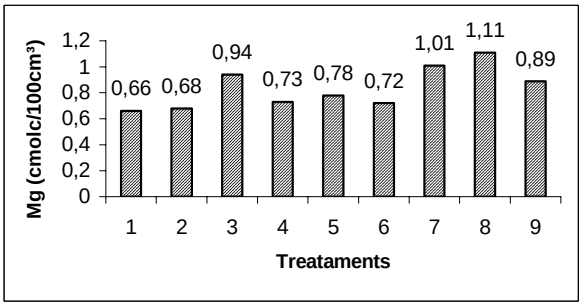

FIGURE 4. Effects of Natural Rock of Ipirá on soil magnesium (Mg) concentrations.

Bean crop is known by its needs to high soil fertility. As the results obtained, the best grain yields were observed in plots that present the highest pH (Figure1) values and Ca and Mg (Figures 3 and 4) concentrations. . Both calcium and magnesium are considered important nutrients for bean crop. On the other hand, in pH between 5.7 and 6.8, the majority of nutrients are disposable for plants (Thung & Oliveira, 1998). The best grain production in this research was observed in parcels presenting soil pH from 6.2 to 6.7. The Natural Rock of Ipirá functioned as soil conditioning, contributing for the high bean grain production.

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LAMBSQUARTER (*CHENOPODIUM ALBUM*) AND NUTSEDGE (*CYPERUS* SPP) INTERFERENCE IN DRY BEAN YIELD

Alberto Pedreros and Juan Tay
INIA Quilamapu
Casilla 426, Chillán, Chile
e-mail : alberto.pedreros@inia.cl

Introduction

Production of dry beans is affected by several flush of weeds during crop development, and yield can decreases more than 60% under central valley conditions of Chile. On the other side, needs of irrigation to obtain high yield, increases emergence of new weed flushes, after herbicides spraying. Use of herbicide is the most important weed control system, however species such us lambsquarters (*Chenopodium album*) is not controlled by treatments, while nutsedge (*Cyperus* spp) is able to regrowth from vegetative structures (Pedreros, 1993; Tay et al., 2005) This condition means that, in spite of excellent weed control by herbicides, both species are interfering with growing of bean, and at harvest time, high populations of them affects harvest. The objective of these experiments was to evaluate the competitive effect of uncontrolled lambsquarters and nutsedge plants on dry bean production.

Materials and Methods

Bean cvs. Curi-INIA and Blanco-INIA were planted during 2000-2001 and 2002-2003 seasons to evaluate competitive effect of different densities of nutsedge and lambsquarters respectively, on dry yield. An additive design with four replications in a randomized complete block was utilized in both experiments. Both seasons, beans were planted the first week of November, at densities of 30 plants per m2 with a distance of 0.5 m between rows, in plots 5 m long by 2 m wide. Nutsedge and lambsquarters plants were maintained at densities of 0, 1, 2, 4, 16, 64 y 128 plants per m^2 during all season, considering those plants emerged in a 12 cm wide band around each bean row. This simulated weeds not controlled mechanically. Grasses were controlled with clethodim at 0.24 kg/ha, while broadleaves were controlled with fomesafen at 0.25 kg/ha. New weed flushes were hand removed every three weeks until harvest. Two central rows were used to record production in which bean yield was analyzed using regression analysis.

The relation between weed density with crop yield has been described by the rectangular hyperbolic curve (Cousens, 1985). This model predict crop response yield as follows:

$$Y = Ywf\ (1-id/100(1+id/a))$$

where Y is the predicted yield as function of weed density, Ywf is the estimated weed-free crop yield, *i* is the initial slope or the percentage of crop loss per unit of weed population as *d* approaches to zero, *a* is the maximum corn lost yield loss as *d* approaches to infinity, and *d* is weed density. In these experiments yield was expressed as a percentage of lost by the model:

$$Yl = id/(1+ id/a)$$

where Yl is the percentage corn yield loss.

Results

The relation between bean yield and nutsedge density is presented in Figure 1. The hyperbolic model fitted the data with and equation of high significance ($P<0.001$). Estimating bean yield due to nutsedge population and fitted with equation, showed an $r^2 = 0.83$. This equation, in this experiment, predicts that 1 nutsedge/m^2 will reduce bean yield in 4.9% and 10 nutsedges/m^2 will reduce in 29%. The maximum expected loss, in this experiment with 128 nutsedges per m^2, was about 59% or 1800 kg ha.

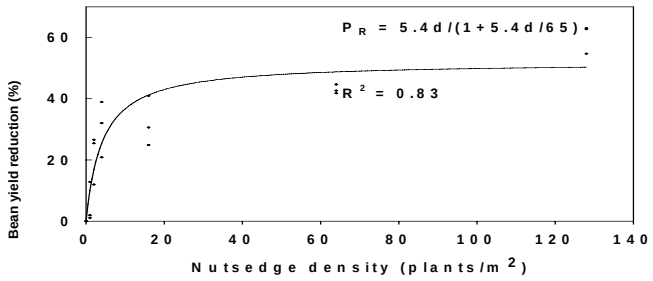

Figure 1. Effect of nutsedge density on dry bean yield reduction, Chillan, Chile 2000-2001.

The relation between lambsquarters density and dry bean reduction yield is presented in Figure 2. The hyperbolic model fitted the data with high significance ($P<0.001$). Estimating bean yield due to lambsquarters population and fitted with equation, showed and $r^2 = 0.82$. This equation, predicts that 1 lambsquarterse/m^2 will reduce bean yield in 1.1% and 10 lambsquarters/m^2 will reduce in 8.9%, this mean 32 and 270 kg/ha respectively. The maximum expected loss, in this experiment with 128 lambsquarters per m^2, was about 33% or 1000 kg/ha of dry bean.

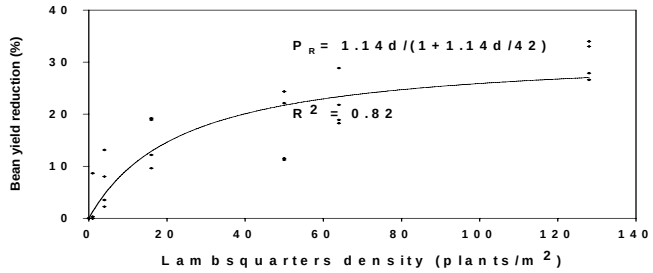

Figure 2. Effect of lambsquarters density on dry bean yield reduction, Los Angeles, Chile 2002-2003.

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RESPONSE OF DRY BEANS TO TILLAGE SYSTEMS AND *IN SITU* RAINWATER CONSERVATION TECHNIQUE IN THE SEMIARID HIGHLANDS OF MEXICO[1]

J. S. Padilla-Ramírez[2], E. Martínez-Meza[2], E. S. Osuna-Ceja[2], M. A. Martínez-Gamiño[2] and J. A. Acosta-Gallegos[2]

[1]Research partially financed through the project 175 (SAGARPA-CONACYT). PRECI 6055905 A.
[2]INIFAP Bean Research Program Group. Apdo. Postal 20 Pabellón de Arteaga Aguascalientes, México. C.P. 20660. jsaulpr@yahoo.com.

Introduction. The state of Aguascalientes, is located within the semiarid highlands of Mexico, where dry beans in one the most important crops. In Aguascalientes there are almost 100 thousands hectares cultivated under rainfed conditions and dry beans has occupied approximately 13% of that area last five years period (2000-2004). The average seed yield of dry beans in the state is about 327 kg ha^{-1}, and it is one of the lowest in region. This low yield of dry beans has been attributed to several factors such as: climatic restrictions especially moisture stress, degraded soil conditions because of inadequate soil management practices and use of non improved dry bean varieties. Conservation tillage systems that contribute to minimize soil erosion and reduce costs in semiarid conditions have been reported to increase productivity of rainfed crops (2). Similarly, soil water conservation practices that help to reduce runoff and increase water infiltration have shown positive effect on dry beans production under semiarid conditions. The utilization of early and drought resistant dry bean varieties has been suggested as an alternative to reduce production risks in this area (1). Thus, the objective of this study was to evaluate an integral dry beans production strategy to stabilize crop production by including improved bean varieties in combination with conservation tillage systems and soil water conservation techniques.

Materials and Methods. The study was conducted at the region known as "El Llano" located in the northeast of Aguascalientes state (21° 54' N; 102° 04' W and 2000 masl) during the summer of 2005. Experiments were conducted on four sites (El Tildio I, El Tildio II, El Copetillo and Sandovales). Plots were planted on July 5th at the beginning of the rainfall season. Size of Plots consisted of one hectare each. Three technological components were evaluated: 1) Dry bean cultivars: "Flor de Mayo Bajio" (FMB) and "Flor de Mayo Sol" (FMSol); 2) Tillage methods: Conventional Tillage (CT) including disc plowing plus harrowing and "Multiarado" (Mult) which has a wider horizontal knives to break ground without soil inversion and 3) *In situ* water conservation: Ridges (R) (mound of soil raised approximately 20 cm high from the bottom of the furrows during crop cultivation) and No Ridges (NR). Ridges were raised 3.0 m apart along rows by using a kind of mechanical shovels ("pileteadora") attached to the rear rigid tines of cultivator at the time of crop cultivation. Ridges were made during the first (20 to 25 days after planting) or second cultivation (35 to 40 days after planting) or in both, except at "El Copetillo" where not ridges were made. The treatments were established on strips of six to eight furrows 0.76 m wide and 100 to 150 m long. Grain yield was estimated from four to six samples of 6.08 m^2 (2 x 0.76 x 4.0) per treatment.

Results and Discussion. Clear differences were observed in the grain yield of dry beans among experimental sites, being the highest "El Tildio I" with an average of 996 kg ha^{-1}, while at "El

Tildio II" the grain yield averaged 390 kg ha^{-1} (Table 1). These differences could be attributed to the amount and distribution of rainfall at the specific site, since in the area an uneven space pattern of the rainfall in not uncommon. Another possible reason for the low grain yield at "El Tildio II" may be due to the presence of weeds, which were more a problem in this site than all others. The cultivars showed an interaction with the location, since FMB had the highest grain yield at "El Tildio I and II", whereas seed yield of FMSol was higher at "EL Copetillo" and "Sandovales". The grain yield average considering all locations was slightly higher for FMSol (721 kg ha^{-1}) as compared to FMB (690 kg ha^{-1}). Regarding to the Tillage methods, only at "EL Tildio II" grain yield was greater with "Multiarado", while in the rest of the experimental sites CT exceed the "Multiarado". However, the grain yield average of the four sites was similar (CT=704 kg ha^{-1} vs Mult=707 kg ha^{-1}). It is important to mention that "Multiarado" can reduce the time and costs of soil preparation since it is wider than plowing discs. The i*n situ* water conservation practice (Ridges) to improve water infiltration increased grain yield of dry beans from 11 to 40% depending of date when ridges were raised. The greatest increase was observed when ridges were raised along with the first cultivation. This suggest that ridges must be raised at early stages of bean crop to extend the availability of soil moisture. These results are similar to those reported from Durango and Chihuahua states were grain yield of dry beans was increased 30% and 6.5 to 122% respectively (3). In contrast, corn yield under rainfed conditions was not increased by ridges when rainfall was low (200 mm) and unevenly distributed during crop growing season (4). The results suggest that timing implementation of *in situ* water conservation practices such as ridges in conjunction with early and drought resistance dry bean cultivars and conservation tillage systems could be a beneficial integrated strategy to minimize environmental risks and ensure crop production for the semiarid conditions of Mexico highlands.

Table 1. Mean grain yield of dry beans (kg ha^{-1}) for the three technological components evaluated in each experimental site at "El Llano", Aguascalientes, Mexico. 2005

Technological Component	Experimental Site				Average
	El Tildio I	El Tildio II	El Copetillo	Sandovales	
Cultivar:					
FMB	1035[ǂ]	416	527	782	690
FMSol	957	365	581	980	721
Tillage Methods:					
CT	1028	333	560	893	704
Mult	964	447	547	868	707
Water Conservation:					
NR	931	428	554	789	676
R at 1st cultivation	---	---	---	956	956
R at 2^{ed} cultivation	1061	353	---	834	749
R at 1st and 2^{ed} cultivation	---	---	---	942	942

[ǂ] Each value represent the mean of 4 or 6 samples per treatment of two rows 4.0 m length (6.08 m^2).

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ORGANIC PRODUCTION EVALUATION OF SNAP BEAN (CV UEL-2).

BRITO[1], O. R.; MIGLIORANZA[1], E.; NAGASHIMA[2], G. T.; FEY[2], R.; CORTE[2], A. D.
[1]Agronomy Department of the State University of Londrina, PR, Brazil. [2]Graduate student.

The employment of organic residues is associated to the improvement of the biological activity, fertility and soils productivity. According IGUE AND PAVAN (1984), the animal manures contain all the plant essential nutrients, in variable amounts. The poultry manures are richer in nitrogen and phosphorus and are fast decomposition, while the ruminant manures are poor in these nutrients and your decomposition is slower (PENTEADO 2000). In the literature, there are few recommendations for organic residues employment in the snap bean culture (SANTOS et al. 2001). This work was carried out with the objective of evaluating the effects of organic (poultry and ruminant manures) and chemical fertilization on snap bean (cv UEL-2) culture.

MATERIAL AND METHODS

The experiment was carried out in a greenhouse at State University of Londrina, Paraná, Brazil (Latitude 23 29'41,4 " S and 50 12 ' 5,5 " W). Pots with capacity for 3,5 kg soil, that was collected of the superficial layer (0,0 - 0,2m) of a typical clay oxisoil (dusky-red Latosol) were used. Two plants of snap bean were cultivated (cv UEL-2) in each pot. The experimental design was randomized blocks with six treatments (Control (C), Broiler litter (BL) = 6.0 $Mgha^{-1}$, Layer manure (LM) = 3,0 $Mgha^{-1}$, Bovine manure (BM) = 8,0 $Mgha^{-1}$, Ovine manure (OM) = 6,0 $Mgha^{-1}$, and Chemical fertilizer (CF) = 0,4 $Mgha^{-1}$ of the 00-18-08) and four replications. The organic fertilizers were incorporate to the soil while the chemical fertilizer was applied to depth 0,05m. The results of different manures analyses are in the Table-1

Table 1. Results of manure chemical analysis

Manures	N	**P**	**K**	**Ca**	**Mg**
	----------		**g Kg^{-1}**	----------	
BM	**18.10**	**2.88**	**3.02**	**9.60**	**3.72**
BL	**25.70**	**8.96**	**9.76**	**13.23**	**3.76**
OM	**23.27**	**11.00**	**66.73**	**19.63**	**8.44**
LM	**26.46**	**2.11**	**28.73**	**89.89**	**14.92**

BM=bovine manure BL= Broiler litter OM=Ovine manure LM= Layer manure

During the experimental period, humidity of each pot was maintained in 70% of the maximum capacity of soil water retention. Nitrogen fertilization was applied with 30 kg ha^{-1} of N (BRITO et al. 2003) to the 42 days after the sowing (DAS) with the objective of avoiding yellowing and fall of leaves. To the 52 **DAS**, the plants of each pot were crop, being evaluated the height, leaf area and pods production. The obtained data were submitted to the variance analysis and the averages of the treatments were compared by the Tukey test to 5% of probability.

RESULTS

In greenhouse, were not verified significant effects of fertilizer on plants height (Table 3), however, the general average of plants height was 0,50m, being superior to the 0,40m presented by ATHANÁZIO et al (1998) that characterized the cultivar as medium load.

Table 2. Averages values for height, leaf area and pods dry matter production of the snap beans (cv UEL-2), to the 52 DAS**.**

Treatments	Height (cm)	Leaf area (cm^2 $plant^{-1}$)	PDMP (g $plant^{-1}$)
Control	49.3[1] A	693.23 B	3.07 B
CF	51.3 A	845.91 AB	3.53 AB
BM	48.8 A	690.30 B	2.96 B
BL	50.3 A	750.89 B	3.49 AB
OM	49.3 A	747.08 B	3.53 AB
LM	51.6 A	958.52 A	4.15 A
CV (%)	4.22	8.91	11.97

[1] Averages followed of the same letter in the columns do not differ to each other for Tukey test to 5%. **DAS** = Days after sowing. **PDMP**= pods dry matter production

The largest leaf area per plant (958.52 cm^2) was obtained in the treatment with layer manure that differed significantly of all the other treatments, except for chemical fertilization. This result is agreement with those observed by SANTOS et al. (2001) that attributed to the layer manure highest capacity to supply the nutrients to the plants.

The pods dry matter production per plant also went higher for treatment with layer manure application that differed significantly of the controls and bovine manure treatments, probably due to the highest supply of nutrients of this residue (SANTOS et al., 2001).

CONCLUSION

The organic fertilization with layer manure resulted in higher medium values for leaf area and pods dry matter production per plant.

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EFFECT OF RHIZOBIA INOCULATION ON SEED IRON AND ZINC

[1]P.M. Kimani, [2]S. Beebe and [2]M. Blair

[1] Dept of Crop Science, University of Nairobi, P.O Box 29053, Nairobi, Kenya
[2]CIAT, Apartado Aereo 6713, Cali, Colombia

Bean crops rely on symbiotic fixation to meet part of the nitrogen requirements. The balance is obtained from the soil. Nitrogen deficiency is the most important constraint to bean production in sub-Saharan Africa causing annual losses of more than 389,000 t per year. N is deficient in bean producing areas of Madagascar, South Africa, Zimbabwe, Malawi, Tanzania, Rwanda, Burundi, DR Congo, Uganda, Angola, Kenya and Ethiopia. Most of the farmers do not apply fertilizers to their bean crops. This implies that productivity of the micronutrient rich beans being developed for smallholder farmers in Africa and other regions of the world will depend on their ability to produce in soil low in N, and on symbiotic fixation. However, it is not known how inoculation with rhizobia may influence the seed iron and zinc concentration. Our objective was to study the effect of inoculating bean genotypes with rhizobia on seed iron and zinc concentration in some promising micronutrient dense bean lines.

Materials and Methods

Twenty-seven bean lines were evaluated at two locations in Kenya during the long rain season (April-July) and short rain season (November-February) of 2002-2003. The test lines which included genotypes low and high in seed iron and zinc concentration were of diverse seed sizes and seed colour. They included landraces and released commercial cultivars from eight countries in East and Central Africa (Ethiopia, Kenya, Uganda, Rwanda, DR Congo, Madagascar, Sudan and Tanzania). The factorial experiments were laid out in a split plot design with three replicates. Rhizobial treatments were the main plots. The treatments were: rhizobial inoculation only, rhizobial inoculation plus 50 kg P ha^{-1} and control plots without inoculation or P application. An effective rhizobial strain was obtained from MIRCEN program, Dept of Soil Science, University of Nairobi. Bean lines were the subplots. Each entry was sown in four, 5m rows. Normal agronomic practices were followed. Data was recorded from the inner two rows. Seed harvested from these plots was analyzed for iron and zinc concentration at CIAT, Cali, Colombia. Data was analyzed using Genstat statistical software (Release 6.1).

Results and Discussion

Results showed that there were highly significant differences due to location, rhizobial treatments and genotypes for duration to flowering, maturity, 100-seed mass and grain yield (Table 1). There were significant location x rhizobia inoculation, genotype x rhizobia inoculation, location x genotype and three-way interaction for duration to flowering and maturity, and grain yield. Significant rhizobia x location x genotype interaction was detected for 100-seed mass. This implied that genotypes responded differently to rhizobia treatments and responses also varied with locations. Gofta was the best yielding genotype over the two locations.

Seed iron increased with inoculation. The best ten lines for iron concentration are shown in Table 1. Ayenew had the lowest mean iron concentration among the test lines and was included in this table for comparison. Seven of the ten lines showed increase in iron concentration with inoculation with and without P. These were HRS545, K132, Selian 97, Gofta, MCM 2001, Lingot Blanc and Maasai Red. Three lines (Simama, Roba-1 and Mexican 142) showed no response to inoculation. HRS 545 had the highest iron concentration. In contrast, inoculation with rhizobia depressed seed zinc concentration in most of the genotypes. These results indicate that inoculation can maximize the genetic potential for iron seed concentration. Genotypes that are responsive to inoculation offer a new opportunity for enhancing the seed iron concentration.

Table 1. Days to flower, maturity, 100-seed mass, grain yield and effect of Rhizobia inoculation on seed iron and zinc concentration in bean lines grown at two locations over two seasons in Kenya.

Genotype	Days to 50% flowering	Days to maturity	100-seed mass (g)	Grain yield (kg ha-^{1})	#Fe (ppm)			#Zn (ppm)		
					0	Rh	Rh + P	0	Rh	Rh + P
HRS 545	41	83	18.2	55	59	69	111	38	33	36
K132	41	82	41.0	847	78	64	82	32	30	31
Simama	41	82	41.3	998	87	71	66	34	33	31
Selian 97	41	82	39.6	937	70	68	73	35	34	34
Gofta	41	82	33.2	1269	67	76	66	37	34	34
MCM 2001	43	84	24.4	1019	62	81	65	31	28	32
Roba-1	44	85	17.0	1128	76	71	61	33	31	32
Mex 142	43	85	17.2	795	69	69	68	34	33	34
Lingot Blanc	39	80	38.8	563	69	72	66	34	30	32
Maasai Red	43	84	21.4	995	56	75	63	34	34	34
Ayenew	39	81	29.9	241	60	50	53	31	28	32
Trial mean	42	83	28.1	900	62	66	64	33	32	33
Rep/locations										
Locations (L)	**	**	**	*						
Rh levels (Rh)	**	**	**	**						
Genotypes (G)	**	**	**	**						
L x Rh	*	*	NS	**						
L x G	**	**	**	**						
Rh x G	**	**	**	**						
L x Rh x G	**	**	**	**						
Error										

Data for Kabete long rain season only
*, ** : Significant at 5 and 1% probability levels, respectively; NS= not significant

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EFFECT OF INOCULATION OF BEAN AND SNAP BEAN

Vera Milić, Mirjana Vasić and Jelena Marinković
Institute of Field and Vegetable Crops, M. Gorkog 30,
21000 Novi Sad, Serbia and Montenegro milicv@ifvcns.ns.ac.yu

Microorganisms are the living part of soil and they actively participate in its forming. Microbiological processes in soil are conditioned by organic matter content, and each soil type fertility is connected to its microflora activity. Depending on types of microorganisms which are a part of microbiological fertilizers, activating favourable microbiological processes in soil and increasing their effectiveness can be influenced. There are symbiotic relations between plants and root nodule bacteria in soil under leguminoses. Specific root nodule bacteria for bean and snap bean are *Rhizobium leguminosarum bv. phaseoli,* and nitrogen quantity these bacteria can fix is between 20 and 115 kg N ha $^{-1}$ (Rennie and Kemp, 1983; Rushel *et al,* 1982). According to Plancquaret (1999), bean needs 300 kg N ha $^{-1}$ which is partly provided by symbiotic nitrogen fixation. If there are less than 10 cells per soil gram in soil of autochthonous population of *Rhizobium* adding bacteria, mostly by seed inoculation, increases root nodulation. Present autochthonous root nodule bacterial strains in soil can be less effective than strains added by seed inoculation. By adding NS-Nitragine For Bean a more effective symbiotic community is formed in relation to uninoculated seed, i.e. larger and more quality bean yield is produced (Milić *et al*).

Activity of *Rhizobium leguminosarum bv. phaseoli* depends on many environmental factors in soil, and especially on soil acidity. Because bean is grown on different soils, it is necessary to produce such a mixture of microorganisms that the commercial preparation can be used as inoculant on different soil types or inoculants which contain strains of these microorganisms for soils of extreme acidity values.

Materials and Methods

NS-Nitragin For Bean And Snap Bean is a commercial preparation made in Microbiological Section of the Institute of Field and Vegetable Crops in Novi Sad. Selected strains of *Rhizobium leguminosarum bv phaseoli* (strains 1, 2, 3 and 4) which are a part of the microbiological fertilizer (NS-Nitragin For Bean And Snap Bean) have been tested on nutritive substratum Demolon of different pH values (pH meter Cyber Scan 510) (substratum pH fixed with HCl 1% and NaOH 10%). Previously a starting culture of each strain has been sown (in 200 cm^3 of liquid medium) after 72 hours of culture growing at 28-30 C^o from each starting culture 1 cm^3 has been taken sterile and transported into new liquid medium Demolon of different pH values. After 72 hours of culture growing at 28-30 C^o in shaker, out of each liquid medium of different pH values number of cells of tested strains of *Rhizobium leguminosarum bv phaseoli* have been determined by method of thinning from the starting culture to 10 $^{-13}$.

Inoculation of bean seed of variety Aster and Zlatko, and snap bean of variety Bergold was performed directly prior to sowing by the microbiological fertilizer NS-Nitragin which contains mixture of strains *Rhizobium leguminosarum bv. phaseoli* (1,2,3,4) specific for bean and snap bean. At Experimental Fields in Rimski Šančevi, Vojvodina Province (soil type chernozem Tab.2) during 2005, a trial has been set by random block system in 4 repetitions with and without microbiological fertilizer NS-Nitragin. At the end of vegetation, number od pods and grains and grain mass per plant were determined.

Results and discussion

All strains of *Rhizobium leguminosarum bv phaseoli* (1, 2, 3, 4) are good both for acid and neutral soils. Strain 1 develops weakly on acid medium to pH 5; it reaches greatest development at wider acidity area from pH 6 upwards. Strain 2 has a broader development optimum, beginning at pH 5. Strains 3 and 4 have a narrower value of maximum development at different pH levels. Strain 3 is the most tolerant to environmental acidity change. Strain 4 does not develop at all on extremely acid medium. Strains 3 and 2 tolerate acid environment from pH 4, and strains 1 and 4 grow better on weakly alkaline and neutral mediums (Table 1).

Table 1. Number of microorganisms Rhizobium leguminosarum bv phaseoli on different pH value mediums

Strains	pH 4	pH 4,5	pH 5	pH 5.5	pH 6	pH 6,5	pH 7
1	$1x10^{1}$	$4,66x10^{5}$	$2x10^{4}$	$8,66x10^{7}$	$2,2x10^{12}$	$4,06x10^{12}$	$2,86x10^{12}$
2	$7,66x10^{2}$	$4,33x10^{7}$	$5,53x10^{13}$	$6,43x10^{13}$	$6,9x10^{13}$	$6x10^{12}$	$1,66x10^{9}$
3	$3,3x10^{10}$	$3x10^{9}$	$2,66x10^{11}$	$5,66x10^{12}$	$19,33x10^{13}$	$9,66x10^{12}$	$17x10^{12}$
4	Does not grow	$5,66x10^{5}$	$6,2x10^{13}$	$1,66x10^{12}$	$1,33x10^{11}$	$1,66x10^{9}$	$2,66x10^{10}$

Table 2. Basic soil chemical properties prior to sowing

pH		$CaCO_3$	Humus	Total	$AL\text{-}P_2O_5$	$AL\text{-}K_2O$
in KCl	in H_2O	%	%	N %	mg/100g	mg/100g
7,28	8,01	10,50	2,52	0,161	27,2	21,8

Gathered results have shown that inoculation with strains mixture (NS-Nitragin) had a positive influence on tested parameter of bean yield, while the effect of inoculation was diverse depending on plant genotype. On average, with all tested genotypes inoculation had effect on increased pod and grain number per plant, as well as grain mass per plant, i.e grain yield (Tab.3).

Table 3. Average number of pods, grains and grain dry matter mass per plant with inoculated and uninoculated varieties of bean and snap bean

Varieties	Pod Number		Grain Number		Grain Mass (g)	
	-N	+N	-N	+N	-N	+N
Aster	14,33	14,67	38,00	45,33	13,46	16,21
Bergold	15,22	17,50	37,52	48,33	16,17	18,39
Zlatko	4,80	7,75	13,60	18,50	6,11	9,42
AVERAGE	11,45	13,31	29,71	37,39	11,91	14,67

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FIELD EVALUATION OF BEAN ROOT ARCHITECTURE

J.P. Lynch[1], E. Peralta[2], G. Abawi[3] and E. Falconí[2]

[1] Department of Horticulture, Penn State, University Park, PA 16802 USA, [2] Instituto Nacional de Investigaciones Agropecuarias (INIAP), Estación Experimental "Santa Catalina," Casilla Postal 340, Quito, Ecuado, [3]Department of Plant Pathology, New York State Agricultural Experiment Station, 630 West North Street, Geneva, New York 14456, USA

Objective: To conduct a quick evaluation of root architectural traits of selected lines in a drought nursery at the Tumbaco research station of INIAP.

Methods: Plants were evaluated at stage R7 (mid pod-fill) on March 23 2006, soil was a well-fertilized Udand, silt loam texture, located at Tumbaco research station of INIAP near Quito, Ecuador, 0° 13' 0S by 78° 24' 0W, 2355 masl. For each plot 2-3 plants were excavated from each of three locations in the plot. A shovel was used to loosen soil within 15-20 cm of shoot, then roots were gently excavated from below by hand. All excavations were performed by one person (JPL) for consistency. A rating scale of 1-9 was used to rank the length and branching of taproots, basal roots, adventitious roots, and root rot (rated by GA) as follows:

Shallowness	Depth of the entire root crown, from 1 = horizontal to 9 = vertical
Taproot length	1= 20-30 cm (depth of excavation) to 9 = no taproot left
Taproot branching	1= multiple lateral branches with up to 4 orders of branching to 9 = no lateral branching
Basal root length	1= 20-30 cm (width of excavation) to 9 = 2-3 cm
Basal root number	Actual number of basal roots per plant
Basal root branching	1= multiple lateral branches with up to 4 orders of branching to 9 = no lateral branching
Root rot	1-3 = good roots/resistant germplasm: 3-6 = moderately diseased/tolerant; and 6-9 = poor/susceptible (van Schoonhoven and Pastor-Corrales, 1987).
Adv. root length	1=15-20 cm to 9 = 1 cm
Adv. root number	1= 10 adv. Roots per plant to 9 = none
Adv. root branching	1= multiple lateral branches with up to 4 orders of branching to 9 = no lateral branching
Nodulation	1= over 50 large pink nodules to 9 = no nodules

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Results

Field Evaluation of Bean roots

Genotype	# plants	shallowness	taproot length	taproot branching	Basal root length	Basal root number*	Basal root branching	Adventitious root length	Adventitious root number	Adventitious root branching	nodulation	rot
Yonguilla	7	3	4	8	5	4	8	2	8	6	7	3
B. Fanesquero	7	2	8	9	5	4	8	6	8	6	8	3
Canario del Chota	7	1	8	9	4	6	2	2	8	5	8	2
Canario Surco 26	7	7	4	7	3	5	8	6	8	6	8	4
Banco Belen	7	1	8	8	4	7	8	6	8	7	9	4
Surco 23	7	4	2	2	4	4	8	1	3	2	7	4
Paragachi	7	6	7	8	4	6	7	6	7	3	7	5
Je.Ma.	7	3	8	8	3	5	8	1	3	2	7	5
Negro San Luis	6	7	3	5	4	3	7	4	4	2	7	5
Concepción	7	4	6	5	4	6	8	7	8	7	8	5
L88-63	7	3	5	2	4	2	8	3	8	3	6	3
mean	7	4	6	6	4	5	7	4	7	4	7	4

* : basal root number is the actual number of roots per plant rather than a 1-9 rating

1. Genotypes varied substantially for root traits.
2. 'Surco 23' red mottled seed type stood out has having the strongest taproot system combined with one of the two strongest adventitious root systems. The combination of a strong taproot and strong adventitious roots should make this genotype capable of acquiring water under drought conditions as well as exploring the topsoil for nutrients, which is consistent with comments by growers that this genotypes is "less thirsty" than others.
3. 'Je. Ma.' red mottled variety was another outstanding genotype, having a strong adventitious root system combined with long basal roots.
4. 'L88-63' black bean had a moderately deep taproot system with abundant lateral branching from the taproot- a trait that may contribute to its drought tolerance.
5. 'Negro San Luis' and 'Canario Surco 26' were the two deepest-rooted genotypes. 'Negro San Luis' combined a fairly deep and well-branched taproot with well-branched adventitious roots. In contrast, the depth of 'Canario Surco 26' derived from long, deep basal roots.
6. Genotypes either favored basal roots or taproots/adventitious roots but not both, supporting the hypothesis that there is resource competition among root classes.
7. The only genotype with well-branched basal roots was 'Canario del Chota', which also had the best rating for root rots, suggesting that root rots destroy the fine lateral branches of basal roots in this soil. In this context the development of adventitious roots would be important to recover the root foraging capacity of the root system once the basal root system had been damaged.

Conclusions

1) Despite lack of true replication and crude excavation technique, we observed substantial genetic variation for root architecture that is consistent with reported drought tolerance of several genotypes. This indicates that root architectural evaluations are useful in understanding genotypic variation for drought tolerance and root rots.

2) In this environment a good root ideotype would be a strong taproot system for drought tolerance combined with a strong adventitious root system to escape root rots. This is the architectural pattern of 'Surco 23' and 'Negro San Luis'. Under intense droughts this ideotype may fail due to desiccation of adventitious roots.

MAPPING AND SEGREGATION DISTORTION ANALYSIS IN THE G2333 X G19839 RECOMBINANT INBRED LINE POPULATION OF COMMON BEAN

I.E. Ochoa[1], O.E. Checa[2], S.E. Beebe[3], J.P. Lynch[1], M.W. Blair[3]

[1] Department of Horticulture, Pennsylvania State University, University Park, PA 16802 USA,
[2] Universidad de Nariño, Pasto, Colombia, [3]Centro Internacional de Agricultura Tropical (CIAT), AA 6713, Cali, Colombia.

Introduction

Mapping populations are a critical genetic tool for gene tagging and quantitative trait loci studies but also serve to analyze the frequency of segregation distortion, where this genetic phenomenon is the deviation from expected values of observed allelic frequencies. Segregation distortion is a common aspect of mapping population analysis, especially for recombinant inbred line or double haploid populations and has important implications for linkage mapping: namely, in map regions suffering from segregation distortion, the estimate of recombination fraction might be biased leading to the inaccurate calculation of marker linkage and map distances in some map segments with a high proportion of distorted markers; however, the order of the markers seems not to be particularly affected by the inaccuracy in map distance introduced by segregation distortion when these are corrected for by two-point analysis (Lashermes et al., 2001). The number of genetic factors causing segregation distortion in a given distorted region might also affect the estimate of map distance; nevertheless, Lu et al. (2002) speculated that as a general rule only one gametophytic or sporophytic incompatibility factor is present per region of segregation distortion. The objective of this study was to develop a new linkage map of *Phaseolus vulgaris* L. with PCR-based markers, and to identify sets of markers showing segregation distortion.

Materials and Methods

A $F_{5:8}$ recombinant inbred line (RIL) population of 84 genotypes was derived from the cross between G2333 and G19839 (from Mesoamerica and Andean gene pool respectively) and used for this study. Four different types of DNA based markers were analyzed: (1) 106 genomic and gene-coding microsatellites (Blair et al., 2003); (2) 34 RAPD fingerprints (primers) that had been previously tested in CIAT mapping populations (M. W. Blair, unpublished data); (3) 15 SCAR markers (http://www.ars.usda.gov/SP2UserFiles/person/3848/pdf/Scartable3.pdf/); and (4) 3 STS markers linked to seed coat color. In addition the biochemical marker phaseolin (Phs) and the morphological marker flower color (V) were scored. The PCR amplifications of microsatellites markers were performed as described by Blair et al. (2003). Linkage analysis was conducted using the software MAPMAKER/EXP 3.0 (Lander et al., 1987) with the Kosambi mapping function. Groups of markers were assigned to particular linkage groups if at least two microsatellites were reported previously from that linkage group (Blair et al., 2003). These were then used for the placement of additional markers based on the most-likely interval at a minimum LOD of 2.0. The best marker order of the linkage groups was determined using a minimum LOD of 4.0. For each of the 160 polymorphic markers evaluated in the RIL population, a chi-square goodness-of-fit test against an expected 1:1 (A:B) ratio of parental alleles was carried out in order to identify markers showing segregation distortion. Heterozygous individuals were not considered in the analysis.

Results and Discussion

One hundred forty nine out of 160 polymorphic markers evaluated were assigned into 11 linkage groups for the G2333 x G19839 RIL population. The total cumulative map length was 1175 cM (Figure 1). We found 46.3% (69) of the total markers being mapped showing segregation distortion. Considering all loci together, there was preferential transmission of maternal alleles from G2333 (53.5% vs. 46.5) over paternal alleles from G19839 (χ^2 = 473.7; Table 1). This significant preferential transmission of maternal alleles occurred on every linkage group (Table 1) except for linkage groups B4 and B11 where the expected 1:1 ratio was observed. Linkage group B9 showed the highest proportion of maternal alleles (67.6%). In our map, distorted markers were unevenly distributed among the 11 bean linkage groups (Figure 1). The highest proportion of skewed markers occurred on linkage groups B2 with 12 out of 16 markers distorted and B5 with 10 out of 11 markers distorted, while the fewest distorted markers were found on linkage groups B1, B4, and B11 with 1 or 2 loci each (Figure 1). Following the criteria proposed by Xu et al. (1997), we identified four well defined segregation distortion regions (SDRs) located in the bottom half of linkage group B2, the entire middle section of linkage group B5, the top half of linkage group B9, and the bottom half of linkage group B10 (Figure 1). We speculate that the high proportion of distorted markers and the preferential transmission of maternal alleles from G2333 would have been due to selection against G19839 gametes and alleles in the environmental conditions (average temperature and altitude) in which the RIL population was developed at CIAT where conditions might have been more favorable to the Mesoamerican alleles. This might be particularly true because the parents represented different gene pools, and adaptation to different agroecologies.

Table 1. Transmission of alleles in recombinant inbred population of G2333 x G19839.

	Linkage group											
	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	Total
G2333 allele (A)	332	753	645	677	552	551	563	780	580	603	401	6437
G19839 allele (B)	238	373	472	741	253	466	367	453	188	269	373	4193
χ^2 (1:1)	15.5	128.2	26.8	2.9	111.1	7.1	41.3	86.7	200.1	127.9	1.0	473.7
$P_{\alpha>0.05}$; 1*df*	***	***	***	ns	***	**	***	***	***	***	ns	***
Allele in excess	A	A	A	-	A	A	A	A	A	A	-	A

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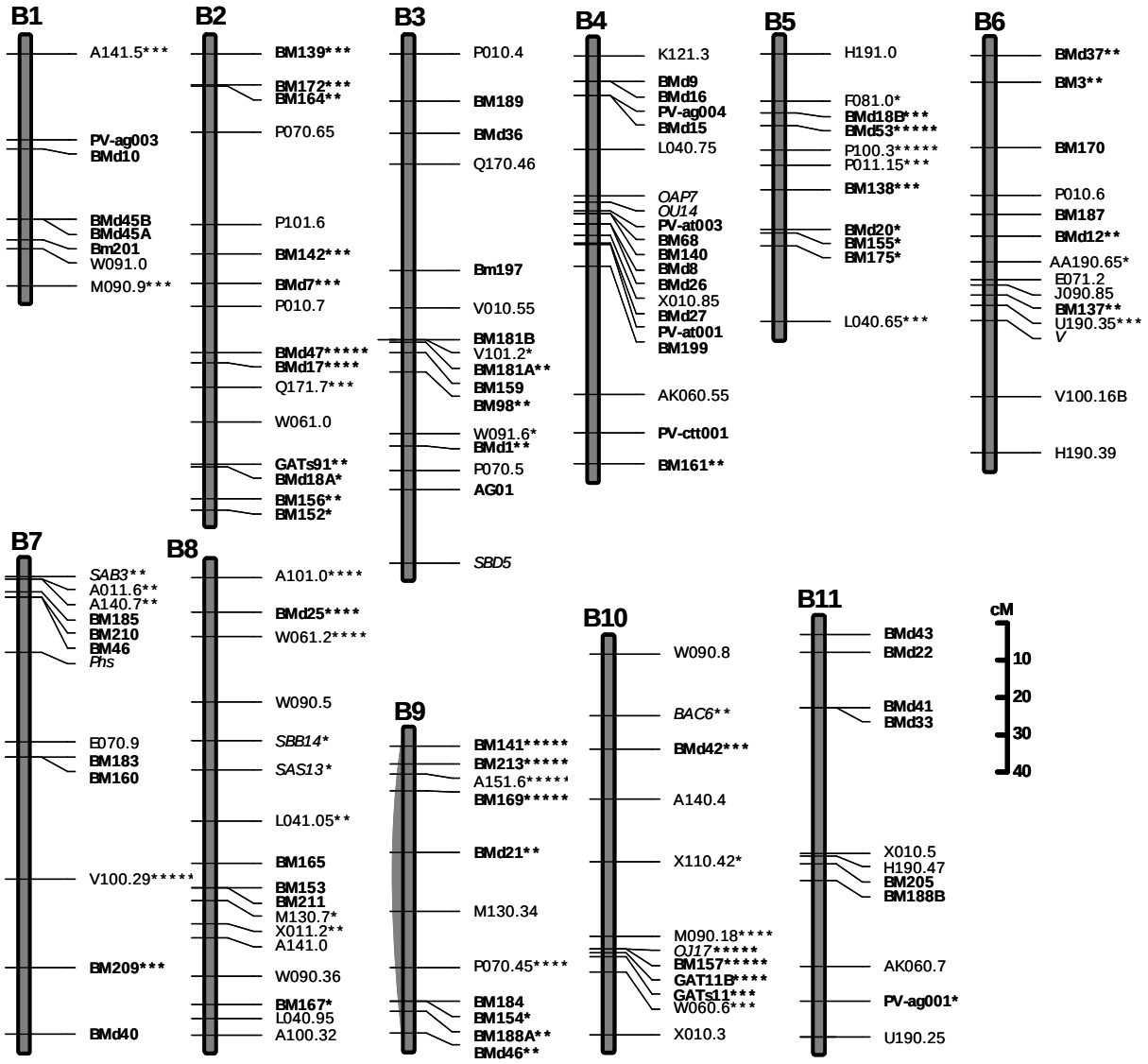

Figure 1: Genetic map of the G2333 x G19839 RIL population showing distribution of molecular markers displaying segregation distortion. Markers displaying significant distortion at 5%, 1%, 0.1%, 0.01%, and 0.001% are represented by *, **, ***, ****, and ***** respectively.

FARMERS' PARTICIPATION IN COMMON BEANS BREEDING IN SOUTH ETHIOPIA: THE WAY FORWARD

Asrat Asfaw, Daniel Dauro, Gashahune Wolde and Fistum Alemayehu
Awassa agricultural research center, P.O.Box 6, Awassa, Ethiopia

INTRODUCTION

Common beans breeding program in the country has developed many productive varieties that could increase yield per unit area following conventional breeding approach. However, there has been a very limited uptake of improved bean varieties by smallholder farmers', especially women in the south region. This could be due nonparticipation of the farmers and other actors in the variety development process that attributed in lack of acceptable characteristics of the varieties such as seed color, size, cooking time, taste, poor adaptation or inadequate diversity to meet local preferences of bean farmers and consumers. Participating farmers in the breeding process helps to fit the crop to specific needs and uses of farmers' communities (Ceccarelli *et al.*, 2000) and improve cultivar adoption (Horne and Stur, 1997). Farmer participation is a powerful tool to achieve a meaningful orientation of a breeding program (Weltzien/Smith *et al*. 1999). Participatory plant breeding involves scientists, farmers, and others, such as consumers, extensionists, vendors, industry, and rural cooperatives in plant breeding research and it is termed participatory because many actors, and especially the users, can have research role in all major stages of the breeding and selection process (Sperling *et al.*, 2001). In order to reach many resource poor farmers including women in marginal heterogeneous environments and to incorporate farmers' diverse traits to meet their specific preferences participatory bean breeding with women and smallholder farmers was initiated.

MATERIALS AND METHODS

Participatory plant breeding approach was attempted on common bean at two villages/communities, Remeda and Korangoge near Awassa in Sidama zone of the South Regional State of Ethiopia. Remeda represented the tepid to cool moist and humid mid-highland whereas Korangoge represented the hot to warm sub-moist lowland areas of bean production in the region. Forty-four farmer selectors (22 each from two villages) represented by the community evaluated and then selected bean lines on-station from initial diverse germplasm pool of 147 lines at first selection cycle in 1999. In the following three years (2000 - 2002), the farmers evaluated their selected lines on their farms and retained promising lines at the end of each selection cycle according to their own selection criteria. The best selections were verified for their yielding potential in larger plot both on-station and on-farm during 2003. The materials used for initial selection cycle (C_1) in 1999 belg (short rainy season) were 147 entries comprised of 8 climbers, 36 large seeded and 103 small-medium seeded beans from the regional program of the Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia. This germplasm pool included bean lines of a wide range of seed colours, seed sizes and growth habits.

RESULTS

The number of lines selected by a farmer ranged from 5 - 51 and on average a farmer selected 15 lines at first selection cycle in 1999. At final section cycle i.e., in 2002, the number of lines selected by a farmer ranged from 1 - 4 and on average a farmer selected 2 lines (Figure 3). This

indicates that the range and average number of lines selected by a farmer decreased from first to last selection. At the final selection i.e., cycle-5, the 27 farmer-selectors from two villages retained in general 18 large and 17 small-medium seeded beans i.e., 35 lines out of the original 147 lines. The number of lines farmers retained for planting increased from 1 to 4. Number of small and medium sized bean lines retained by farmers increased from 1 to 3 and that of large seed size beans increased from 0 to 3. This revealed that participating farmers in breeding process created access for the communities to improved bean germplasm and increased intra-varietal diversity at on-farm level at the trial sites where there has low level of bean agro-biodiversity.

The most important criteria mentioned by farmers in their selection of lines from the initial germplasm pool in cycle-1 in 1999 and in subsequent selection cycles were growth habit, plant height, pod load, pod length, pod clearance from the base, early maturity, seed color, seed size and seed yield. Subsequent interviews with farmers revealed that seed color and seed yield were their decision-making criteria to retain or reject a line, the remaining criteria being descriptor to select a good line.

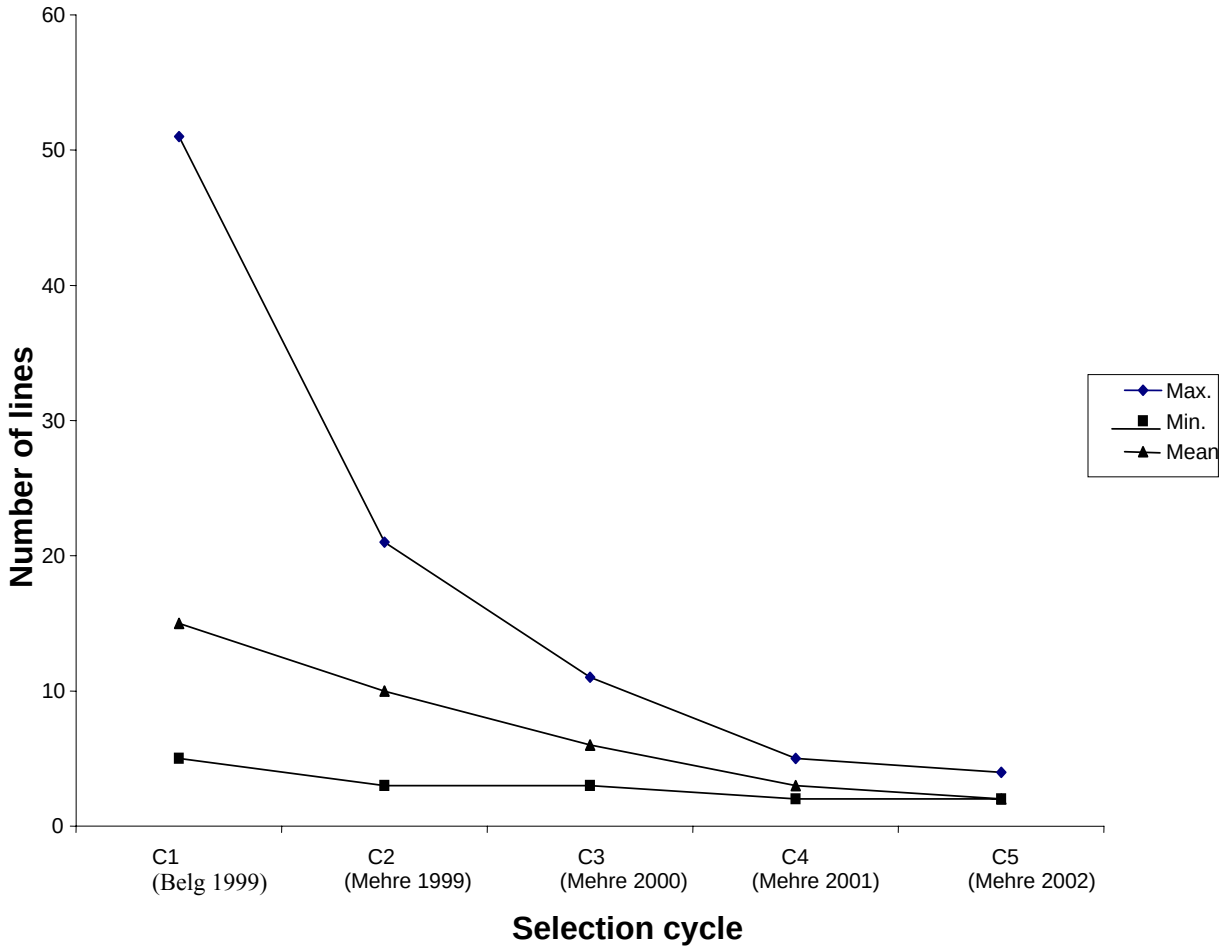

Figure 1. Range and average number of lines selected by a farmer per selection cycle (C)

In the year 2003, XAN-319 and RAB-585 from small seed class and AFR-702 and OBA-4 from large seed class which were selected by majority of farmers were planted on verification plot. The mean grain yield and culinary traits of the farmers' varieties on verification plots is given on Tables 1. The farmers selected varieties RAB-585 (small red) and AFR-702 (large red kidney) out yielded their respective checks at on-station and on-farm mean performance.

In general involving farmers in bean breeding can improve variety development as the farmers are capable of identifying superior lines that meet their specific requirements within relatively short period and increase the chance of adoption of new varieties by other farmers in a community.

Table 1. Mean grain yield (kg/ha) and culinary quality of PPB varieties grown on verification plot in the year 2003

Varieties	On-station yield (kg/ha)				On-farm yield (kg/ha)				100 seed wt (gm)	% of non-soakers	Cooking time (minute)
	Awassa	Inseno	Remeda	Mean	Remeda	Wondo Tika	Korangoge	Mean			
RAB-585	3066.6	1829.4	5000.0	3298.7	3490.0	2170.0	2040.0	2566.7	26.1	1.0	14
XAN-314	3087.2	2215.1	3900.0	3067.4	2200.0	1390.0	2030.0	1873.3	24.1	5.25	17
Omo-95 *	2988.3	2096.9	4400.0	3161.7	3000.0	1305.0	2600.0	2301.7	24.4	0	13
Red wolayta *	3001.5	-	2050.0	2525.8	1300.0	440.0	-	870.0	25.9	0.25	15.7
AFR-702	2672.8	2116.2	4600.0	3129.7	3180.0	2080.0	1800.0	2353.3	46.7	0	23.3
OBA-4	2600.2	1835.2	3900.0	2778.5	2025.0	1710.0	1330.0	1688.3	49.7	0	20
Ibado*	2833.9	1666.3	4700.0	3066.7	2070.0	2000.0	2000.0	2023.3	49.2	0	17.3
Brownspeckled *	1891.5	1433.9	4400.0	2575.1	1800.0	1630.0	1700.0	1710.0	39.2	0	14.3

* Checks

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THE IMPACT OF COMMON BEAN RESEARCH IN MICHIGAN

R.H. Bernsten, C. Ragasa, and M. Maredia, Dept. of Agricultural Economics, Michigan State University, East Lansing, MI 48824

This study documents trends in MI's bean subsector, assesses the impact of MSU's bean breeding program, and proposes policy recommendations for strengthening MI's bean industry.

Methodology

To gain insights regarding bean industry trends, interviews were conducted with MSU's bean breeder and staff of the MI Crop Improvement Association, MI Bean Commission, MI Bean Shippers Association, and elevators responsible for bean seed sales and purchases. USDA and MI Agricultural Statistics data were analyzed to document area, yield, and production trends. Seed sales data were collected from 4 elevators and analyzed to document varietal adoption. MI growers were surveyed by mail to solicit their assessment of varietal adoption and constraints. Historical bean yield data (multi- location trials), obtained from the Production Research Advisory Board, were analyzed to quantify the impact of varietal improvement on yield.

Major Findings

Trends in MI's Bean Industry: While MI is the 2nd most important bean-producing state (2002), its share of the U.S. bean area (18% in1987-89;15% in 2000-02) and production (18% in 1987-89; 12% in2000-02) declined in recent years. Since the late-1980s, MI's harvested area declined by 2%/year–from 277,000 (1987-89) to 223,000 ac (2000-02). Since the late-1980s (1987-89 vs. 2000-02), the area planted to major market classes shifted substantially. While navy beans declined in importance (-8%/yr, 79% vs. 35% of harvested area), black beans increased in importance (+15%/year, 4% vs. 32%), as did pinto (2% vs. 5%), cranberry (4% vs. 8%), red kidney (7% vs. 11%), and small red (3% vs. 4%) beans. MI's production is concentrated in the Saginaw Valley–7counties account for 85% of production. The number of growers declined from 3,100 in 1993 to 2,100 in 1997. While MI yields increased by 2%/year (1987-89 to 2000-02), yields varied greatly from year-to-year and there was no significant difference in the rate of yield gain among market classes. There is growing seed industry consolidation–4 elevators sold about 90% of bean seed purchased in 1998-2001 and Archer Daniels Midlands accounted for 50-60% of sales. There is growing consolidation in marketing–3 elevators accounted for about 80% of grain purchases and ADM accounted for about 50%. The canning industry is growing in importance– about 90% of MI's bean crop is sold to canners, but the share varied by market class. Prices trended downward since the 1980s (1-4%/yr), but varied by year and market class.

MSU's Bean Breeding Program and Seed Multiplication (since 1980): Each year, promising material generated by MSU's breeding program--along with private lines/varieties–are entered in multi-location (6 sites) PRAB field trials, which are independently managed by the MBC.

Varietal Releases Since 1980: MSU's bean breeding program released 29 varieties in 9 market classes: navy (9), black (6), pinto (3), LRK (3), DRK (2), cranberry (2), great northern (2), white kidney (1), small red (1). MSU foundation seed is multiplied by the MCIA and sold to private seed companies, who produce certified seed under contract with growers in the western U.S. and sell the seed at elevators–both in MI and other states.

Producer Mail Survey: To collect producer-level data, a survey was mailed (2003) to 1,250 growers included on the MBC's mailing list. Growers returned 616 surveys, but only 215 provided useable responses--many growers no longer planted beans. On average, growers farmed 1,015 ac, planted 250 ac of beans, and had produced beans for 32 years. Growers reported that to adopt a new variety, it must yield 100-300 lbs/ac more than their current variety. Most growers (86%) purchased new seed annually. Key factors that growers considered in deciding which market class to plant included the availability of a contract (48%) and the expected price (44%). Their most important sources of information about new varieties were an elevator (54%) and the MBA's newsletter (24%). While 22% of growers planted a MSU variety during the past 5years, the adoption rate varied by market class. In choosing a new variety, growers primarily considered yield, canning quality, disease resistance, and erect architecture. Regarding constraints, growers cited drought (50%; once/5 years, resulting in a 67% yield loss) and excess rain (33%; twice/5 years, resulting in a 39% yield loss). Growers cited low/erratic prices and elevator consolidation as key threats to the bean industry. About one-half (47%) of the growers planned to reduce their bean area in 2003 and plant more soybeans.

Contributions of MSU's Bean Breeding Program: Analysis of elevators seed sales data (1997-2001) indicated that MI growers planted an average of 48,280 ac/year to MSU varieties (17% of the bean area). MSU varieties' share varied by market class: DRK (85%), great northern (78%), white kidney (49%), cranberry (33%), LRK (26%), black (18%), navy (6%), pinto (<1%) beans. In addition, growers outside of MI (MN, ND, Ontario, NY) planted an average of 116,102 ac/year of MSU varieties–1.6 times the area planted by MI growers. Analysis of pedigree data indicated that MI growers planted an average of 76,100 ac/year of private varieties that included at least 25% MSU germplasm. Data were not available to estimate ac planted outside of MI to private varieties with MSU germplasm, but it is likely significant.

Average Yield Advantage of All Commercial Varieties: Analysis of PRAB trial data (1985-2001) found that new releases produced statistically significant higher yields (compared to vintage varieties) in all market classes, except black and great northern beans. Log regression analysis indicated that the annual rate of yield gain (genetic improvement) was greatest for navy (0.7%), LRK (0.6%), DRK (0.5%), pinto (0.5%), and cranberry (0.4%) beans.

Economic Benefits (1985-2001): Benefit-cost analysis indicated that the present value of all genetic improvements in MI ranged from $400,000 to $600,000/year, depending on the yield assumption. The share of this total contributed by MSU's breeding program (MSU releases, plus MSU germplasm in private varieties) ranged from $240,000 to $360,000/year. The internal rate of return (discounted costs & benefits) to investments in bean research in MI ranged from 4.5% to 26.8%, depending on the yield assumption. Since this estimate does not include seed sales in other states–which were 1.6 times greater than MI sales–this figure substantially underestimates the economic rate of return to investments in varietal development in MI.

Policy Implications: Clearly, investing in bean varietal development has had a high payoff. The public sector (universities) has a comparative advantage in breeding, while the private sector has a comparative advantage in seed marketing. In a mature bean industry, such as in MI, there is a continuing need for public sector research to address the varietal requirements of niche/small markets (great northern, DRK) that the private sector is not interested in targeting and to produce improved germplasm with traits that private sector firms can incorporate into their crossing programs. As the canning industry is the primary market for beans, there is a need to strongly promote public varieties to both canners and elevators. Canners prefer specific varieties, so elevators promote the varieties that canners prefer, plus their own private varieties.

BRSMG PIONEIRO: NEW CARIOCA COMMON BEAN CULTIVAR RESISTANT TO ANTHRACNOSE AND RUST, FOR THE SOUTHERN OF BRAZIL.

Maurílio Alves Moreira, Everaldo Gonçalves de Barros, José Eustáquio de Souza Carneiro, Fábio Gelape Faleiro, Luís Cláudio de Faria, Geraldo Estevam de Souza Carneiro, Maria José Del Peloso, Trazilbo José de Paula Júnior, Ângela de Fátima Barbosa Abreu, Magno Antônio Patto Ramalho, Leonardo Cunha Melo, João Bosco dos Santos, Carlos Agustín Rava, Joaquim Geraldo Cáprio da Costa, Aloísio Sartorato, Josias Correa de Faria.

Universidade Federal de Viçosa (UFV), 36570-000 Viçosa, MG, Brazil
Embrapa Arroz e Feijão, Caixa Postal 179, 75375-000 Santo Antônio de Goiás, GO, Brazil.

The Southern region of Brazil, that comprises the States of Paraná, Santa Catarina and Rio Grande do Sul, produces about 24,7% of the total national bean production.The prevalent climatic conditions of this region, with moderate temperatures and high humidity, favor disease development such as anthracnose. In this way, one of the main goals of the bean breeding programs in Brazil, is to give emphasis to the development of bean cultivars with resistance to this disease associated to grain type for each region consumers preferences and high average yield. *Colletotrichum lindemuthianum* presents a great pathogen variability making necessary, in the breeding program, the combination of different resistance genes.

BRSMG Pioneiro was obtained in the bean breeding program of the Instituto de Biotecnologia Aplicada à Agropecuária, University of Viçosa, Viçosa, MG, using the backcross method assisted by molecular markers. The original cross involved the genitors Rudá and Ouro Negro. Subsequently, three backcross cycles were performed to the recurrent genitor Rudá. In each backcross cycle the F_1 plants were inoculated with a mixture of pathotypes of the fungus *Uromyces appendiculatus*, the causal agent of bean rust, and with the pathotype 89 of the fungus *C. lindemuthianum*. By using molecular markers, from the resistant plants, it was identified those with the nearest genotype to the recurrent genitor for the next backcross cycle. After the third backcross cycle, 15 selected plants were self pollinated and 50 $F_{2:3}RC_3$ families were evaluated to their resistance to *U. appendiculatus* and to the pathotype 89 of *C. lindemuthianum*. Only 13 families were resistant and presented no segregation to both pathogens. Vi 4899 was selected as the nearest genetically lines to the recurrent genitor. During 2000 and 2001, it was also evaluated field trials, in 16 different environments in Brazil's Southern region, together with other nine breed lines and cultivars Pérola and Carioca used as control in a complete randomized block design with three replications in the State of Paraná (9), Santa Catarina (2) and Rio Grande do Sul (5).

In the 16 field trials line Vi 4899 showed to be 13% superior in an average yield when compared to the average yield of the controls Carioca and Pérola (Table 1). This data, together with the resistance (Table 2) of this line, allowed its release for planting during the wet and the dry seasons in the States of Rio Grande do Sul, Santa Catarina and Paraná, with the fantasy name of BRSMG Pioneiro.

Cultivar BRSMG Pioneiro was evaluated together with its genitors, cultivars Ruda and Ouro Negro, under artificial inoculation, for disease resistance to ten pathotypes of *U. appendiculatus* and 18 pathotypes of *C. lindemuthianum*. It showed to be resistant to all *U. appendiculatus* races and susceptible only to the pathotype 65 of *C. lindemuthianum* (Table 2). In field trials, it was susceptible to angular leaf spot and to common bacterial blight.

BRSMG Pioneiro presents a type II indeterminate habit growth, with a short-to-medium guide and erect stem and branches in the majority tested environments. During all growing cycle (average of 79 days from emergency to physiological maturity), it also showed to be resistant to lodging. The cultivar BRSMG Pioneiro presents a carioca grain type (cream-beige) with an average of 100 grains weight of 20 g (Table 3). It also presents an excellent cooking quality that fulfill all requirements of the market demand. Genetic seed stocks are maintained by Embrapa Rice and Beans and basic seed is available at Embrapa Technology Transfer.

Table 1. Average grain yield of the cultivar BRSMG Pioneiro compared to the average yield of control cultivars (Carioca and Pérola) in the Valor de Cultivo e Uso - VCU field trials in Southern Brazil, during the years of 2000 and 2001.

State	BRSMG Pioneiro ($kg.ha^{-1}$)	Control average yield ($kg.ha^{-1}$)	Relative yield (%)	Number of environments
Rio Grande do Sul	1626	1384	117	5
Santa Catarina	3522	3010	117	2
Paraná	2141	1951	110	9
Total average yield	2153	1906	113	-

Table 2. Disease reactions of cultivars BRSMG Pioneiro, Rudá and Ouro Negro inoculated with different pathotypes of *C. lindemuthianum* and *U. appendiculatus*.

Colletotrichum lindemuthianum				***Uromyces appendiculatus***			
Pathotype	Rudá	Ouro Negro	BRSMG Pioneiro	Pathotype	Rudá	Ouro Negro	BRSMG Pioneiro
7	rr	R/r	RR	54	RR	R/r	RR
23	RR	RR	RR	49	RR	R/r	RR
55	rr	RR	RR	58	RR	R/r	RR
64	RR	R/r	RR	52	RR	R/r	RR
65	rr	rr	rr	59	RR	R/r	RR
67	rr	RR	RR	45	RR	R/r	RR
73	rr	RR	RR	46	RR	R/r	RR
79	rr	RR	RR	50	RR	R/r	RR
81	rr	RR	RR	47	RR	R/r	RR
83	rr	R/r	RR	32	RR	R/r	RR
87	rr	RR	RR				
89	rr	RR	RR				
95	rr	R/r	RR				
102	R/r	RR	RR				
117	rr	R/r	RR				
119	RR	RR	RR				
343	R/r	R/r	RR				
453	rr	R/r	RR				

rr –susceptible ; RR – Resistant; R/r – segregant.

Table 3. Technological grain quality of BRSMG Pioneiro cultivar.

Cultivar	Cooking time (minute)	Soluble solids (%)	Protein content (%)
BRSMG Pioneiro	32.0	10.9	25.2
Pérola	29.0	9.6	21.3
Carioca	24.5	9.2	19.4

Institutions involved in the cultivar evaluation: Embrapa Arroz e Feijão; Embrapa Trigo; Instituto Agronômico do Paraná (Iapar); Centro Federal de Educação Tecnológica (Cefet) – Pato Branco-PR; Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (Epagri); Fepagro.

‘OURO VERMELHO’: NEW RED BEAN CULTIVAR FOR MINAS GERAIS, BRAZIL

José Eustáquio S. Carneiro[1], Lêlisângela Carvalho da Silva[2], Trazilbo José de Paula Júnior[3], Geraldo Antônio A. Araújo[4], Pedro Crescêncio S. Carneiro[5], Marcos Paiva Del Giúdice[6], José Ângelo N. Menezes Júnior[7], Magno Antonio Patto Ramalho[8], Maria José Del Peloso[9], Ângela de Fátima B. Abreu[10], Maurílio A. Moreira[11] and Everaldo G. de Barros[12]

[1, 2,4,6] Departamento de Fitotecnia, Universidade Federal de Viçosa (UFV), 36570-000, Viçosa, MG, Brasil; [3] Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG), Vila Gianetti, 47, 36570, Viçosa, MG, Brasil; [5] Departamento de Biologia Geral (UFV); [7,8] Departamento de Biologia, Universidade Federal de Lavras (UFLA), Lavras, MG, Brasil; [9,10] Embrapa Arroz e Feijão, Caixa Postal 179, 75375-000 Santo Antonio de Goiás, GO, Brasil; [11,12] Instituto de Biotecnologia Aplicada à Agropecuária (BIOAGRO), UFV

The common bean is cultivated throughout Brazil with the state of Minas Gerais ranking nationally as the second largest bean producer. The most important grains in the country belong to the Carioca and Black groups, which have been given special attention by bean breeding programs. However, there is a regional prevalence for particular common bean types, such as red bean, which is widely cultivated in the Zona da Mata of Minas Gerais state. This bean type occupies around 50% of the bean cultivated area in this region, and tends to expand to other regions in the state and even in the country (Soares et al., 2002). The cultivar Vermelhinho belongs to this class and is traditionally grown by small farmers. It is widely accepted, and for this reason its market price is higher than those obtained for other bean types. However, it is highly susceptible to the major bean pathogens. Thus, there is an enormous demand for improved red bean cultivars. Aiming to meet this demand, the bean breeding program of the Universidade Federal de Viçosa started to work with this type of bean, developing lines that can be potentially recommended. During 2002-2004, some of these lines participated in the national assays, and one of them, identified as line VR 6, was recommended under the name of Ouro Vermelho.

This cultivar is derived from the cross between AN9022180 and Vermelhinho with backcross for Vermelhinho. For conducting the population, the bulk method was used among families derived from F_3 plants, with generation advance being carried out until the $F_{3:7}$ generation and massal selection for the traits grain color and aspect. One of the families selected, identified in the national assays as line VR 6, gave origin to the cultivar Ouro Vermelho. From 2002 to 2004, the line VR 6 was jointly assessed with two checks (Vermelhinho and Vermelho 2157) and other 13 lines in 19 environments, comprising the municipalities of Viçosa, Coimbra, Ponte Nova, Leopoldina and Florestal, in the rain, dry and winter seasons. Considering the 19 environments assessed, the cultivar Ouro Vermelho showed an yield increase of 31% compared to the check Vermelhinho, whereas in the harvest evaluation, Ouro Vermelho out yielded the cultivar Vermelhinho by 106% in the rain season and by 18 and 35% in the dry and winter seasons, respectively (Table 1). The better performance of cultivar Ouro Vermelho in relation to cultivar Vermelhinho was much more expressive in the rain season, indicating that this cultivar, even under unfavorable conditions, showed higher production potential. As for grain quality (Table 2), Ouro Vermelho presents bright red grains and excellent cooking quality. Thus, the cultivar Ouro Vermelho is a new option for the bean producing areas in Minas Gerais state.

Indeterminate growth habit, type II plant, semi upright stand, and average flowering of 38 days and cycle (from emergence to harvest maturity) varying from 80 to 90 days, depending on the

planting season are traits presented by cultivar Ouro Vermelho, whose architecture is similar to that of Vermelhinho, but with smaller size and more upright stand. It also presents white-colored-flowers and red dish pink pods at physiological maturity and purplish-brown at harvest maturity, with elliptical and semi–full seeds.

Table 1 – Grain productivity averages (kg/ha) of red bean cultivars Vermelho 2157, Vermelhinho and Ouro Vermelho, evaluated in Minas Gerais, 2002-2004

Environments		Grain yield (kg/ha)			Relative production(%)[1]
Harvests	**Number of assays**	**Vermelho 2157**	**Vermelhinho**	**Ouro Vermelho**	
Rainy season (R)	4	1,590	817	1,687	106
Dry season (D)	10	2,157	2,190	2,581	18
Winter(W)	5	3,472	2,720	3,680	35
R + D+ W	19	2,383	2,040	2,682	31

[1] Grain yield increase percentage of cultivar Ouro Vermelho, compared to check Vermelhinho.

Table 2– Technological and nutritional quality of cultivar Ouro Vermelho grains, compared to cultivars Vermelho 2157 and Vermelhinho

Cultivar	Cooking time (min)	Soluble solids (%)	Whole grains (%)	Seed coats (%)	Protein (%)	100 Seed weight
Ouro Vermelho	30	12.6	98	9.6	25.7	27.6
Vermelho 2157	48	12.3	81	9.7	23.1	27.0
Vermelhinho	50	13.1	94	8.6	23.1	26.0

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UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
WASHINGTON, D.C. 20250
and
IDAHO AGRICULTURAL EXPERIMENT STATION
MOSCOW, IDAHO 83844

RELEASE OF COMMON BACTERIAL BLIGHT RESISTANT PINTO BEAN GERMPLASM LINES USPT-CBB-5 AND USPT-CBB-6

The Agricultural Research Service, U.S. Department of Agriculture, and the Idaho Agricultural Experiment Station announce the release of USPT-CBB-5 and USPT-CBB-6 pinto bean (*Phaseolus vulgaris* L.) germplasm lines with high levels of resistance to common bacterial blight caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*). Scientists participating in the development of this germplasm were Phil Miklas (USDA-ARS, Prosser, WA), James Smith (USDA-ARS, Stoneville, MS), and Shree Singh (University of Idaho). Common bacterial blight is a major seed-borne disease of common bean worldwide. The disease is endemic to pinto bean production regions in Colorado, Michigan, Minnesota, Montana, Nebraska, North Dakota, and Wyoming. Genetic resistance in the host provides the most effective control of this disease, and planting certified disease-free seed is critical. USPT-CBB-5 and USPT-CBB-6 possess two major QTL and perhaps other minor genes that confer a high level of resistance to *Xap*. Marker-assisted selection for the SAP6 and SU91 markers tightly linked with QTL derived from great northern landrace cultivar Montana No.5 and breeding line XAN 159, respectively, was used in development of USPT-CBB-5 and USPT-CBB-6 for combating this problematic disease of pinto bean in the U.S.

USPT-CBB-5 and -6 are $F_{1:5}$ lines derived from the cross G 17341/'Othello'//VAX 4/ 'Maverick'/3/89:980. G 17341 (CIAT accession no.) is a small seeded pinto breeding line developed at CIAT from segregating population provided by Dr. R.E. Wilkinson, Cornell University, New York. G 17341 has indeterminate growth habit Type III, with moderate resistance to common bacterial blight and *I* gene for resistance to *Bean common mosaic virus* (BCMV). Pinto Othello has early maturity, drought resistance and wide adaptation. VAX 4, with beige seed, is a germplasm line from CIAT with a high level of resistance to common bacterial blight. VAX 4 possesses both SAP6 and SU91 markers linked with major QTL for common bacterial blight resistance that ultimately derive from Montana No.5 and tepary bean (*P. acutifolius*), respectively. VAX 4 possesses *I* gene for resistance to BCMV. Maverick is a pinto bean well adapted in North Dakota. Maverick possesses *Ur-3* gene for resistance to bean rust, but is susceptible to BCMV. Pinto 98:980 is a breeding line from the University of Idaho with good yield potential and reduced lodging.

Marker-assisted selection was used to identify F_1 plants from the initial four-way cross for the presence of SAP6, SU91, and SW13 markers. The SW13 marker is linked with *I* gene. An F_1 plant from the four-way cross with all three markers was crossed to 89:980. An F_1 plant from this final cross (PS99-113E) with all three markers was advanced to F_2 which was planted in the field at Prosser, Washington, and screened for plant and seed type. Two $F_{2:3}$ progenies (PS99-113E-6 and PS99-113E-4) were tested for reaction to common bacterial blight in leaf inoculation tests conducted at the USDA-ARS Tropical Agriculture Research Station at Mayaguez, Puerto Rico. An individual F_3 plant (PS99-113E-4-5) with high level of resistance to

common bacterial blight was advanced to F_4. The other F_3 progeny was bulk-harvested (PS99-113E-6-B) because of uniform expression of resistance to common bacterial blight. The F_4 progenies were grown in the field at Prosser and harvested in bulk (PS99-113E-6-B-B and PS99-113E-4-5-B). The F_5 bulks were screened for leaf reaction to common bacterial blight in the University of Idaho greenhouse at Kimberly, Idaho. Individual F_5 plants (PS99-113E-6-B-B-1 and PS99-113E-4-5-B-6) with high levels of resistance and later confirmed to possess SAP6 and SU91 markers were selected to produce USPT-CBB-5 and USPT-CBB-6 that were subsequently increased for two generations and evaluated in multiple greenhouse tests for reaction to common bacterial blight and examined in the field for yield, plant type, and maturity.

USPT-CBB-5 and USPT-CBB-6, in a greenhouse leaf inoculation test conducted at Kimberly, ID, in December 2004, had mean disease scores of 4.9 based on a 1 to 9 scale where 1 is no common bacterial blight and 9 is completely susceptible. In comparison, 'Chase' pinto known to possess moderate resistance to common bacterial blight had a mean disease score of 7.2. In a repeated test in December 2005, USPT-CBB-5 scored 3.3 and USPT-CBB-6 scored 4.7 compared to 8.6 for Chase (note: other inoculation tests also indicated that USPT-CBB-5 is slightly more resistant than USPT-CBB-6). Thus, USPT-CBB-5 and USPT-CBB-6 exhibit a much higher level of resistance to common bacterial blight than any commercially available pinto cultivars. Both breeding lines also possess both the SAP6 and SU91 markers linked with major QTL for resistance derived from Montana No.5 (via VAX 4) and tepary bean (via VAX 4), respectively.

USPT-CBB-5 exhibits a Type III indeterminate vine growth habit. Its seed yield was 107% of Othello at Othello, Washington, in 2005. Average weight of 100 seeds was 35 g, the same as Othello. USPT-CBB-5 matured in 87 d, one day later than Othello. Few lines with high levels of resistance to common bacterial blight possess such early maturity, which makes USPT-CBB-5 a unique germplasm. Seed appearance was rated as commercially unacceptable because of blocky shape. USPT-CBB-5 also exhibits a hypersensitive resistance response to the NL-3 strain of *Bean common mosaic necrosis virus* (BCMNV) in Prosser greenhouse tests, which infers presence of the *I* gene for resistance to BCMV. This line is resistant to *Beet curly top virus* (BCTV).

USPT-CBB-6 exhibits a Type III indeterminate growth habit. Its seed yield was 146% of Othello at Othello, Washington, in 2005. Average weight of 100 seeds was 33 g, which is slightly smaller than Othello. USPT-CBB-6 matured in 99 d, compared to 86 d for Othello. This breeding line also performed well in a multiple stress plot at Prosser, Washington, in 2005, yielding 139% of Othello, which indicated resistance to drought. Seed appearance was rated as commercially acceptable. USPT-CBB-6 is susceptible to BCMV and BCMNV, but possesses resistance to BCTV.

USPT-CBB-5 and USPT-CBB-6 will be most useful for incorporating resistance to common bacterial blight in cultivars of pinto market class, but also other medium-seeded market classes of Middle American race Durango. Seed will be maintained by USDA-ARS at Prosser, WA, and provided in small quantities upon written request. We ask that appropriate recognition of source be given when this germplasm contributes to the development of a new cultivar or germplasm line.

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Director, Idaho Agricultural Experiment Station Date
University of Idaho

_____ _____

Deputy Administrator, Crop Production and Protection Date
Agricultural Research Service, U.S. Department of Agriculture

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
WASHINGTON, D.C. 20250
and
IDAHO AGRICULTURAL EXPERIMENT STATION
MOSCOW, IDAHO 83844

RELEASE OF COMMON BACTERIAL BLIGHT RESISTANT WHITE KIDNEY BEAN GERMPLASM LINE USWK-CBB-17

The Agricultural Research Service, U.S. Department of Agriculture, and the Idaho Agricultural Experiment Station announce the release of USWK-CBB-17 white kidney (*Phaseolus vulgaris* L.) germplasm line with a high level of resistance to common bacterial blight caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*). Scientists participating in the development of this germplasm were Phil Miklas (USDA-ARS, Prosser, WA), James Smith (USDA-ARS, Stoneville, MS), and Shree Singh (University of Idaho). Common bacterial blight is a major seed-borne disease of dry and snap beans worldwide. The disease is endemic to the U.S. bean production regions east of the continental divide and problematic in Colorado, Michigan, Minnesota, Nebraska, New York, North Dakota, and Wisconsin. Genetic resistance in the host provides the most effective control of this disease, and planting certified disease-free seed is critical. USDK-CBB-17 possesses two major QTL and perhaps other minor genes that confer a high level of resistance to *Xap*. Marker-assisted selection using the SAP6 and SU91 markers tightly linked with QTL derived from great northern landrace cultivar Montana No.5 and breeding line XAN 159, respectively, enabled us to expedite development of USWK-CBB-17 for combating this entrenched disease problem in the U.S.

USWK-CBB-17 (previously tested as PS99-499-3-2-B-2) is an $F_{1:5}$ line derived from the cross 98MSU-837// I9566-21-4-2/USLK-2. 98MSU-837 is an advanced white kidney breeding line from Michigan State University with high yield potential. USLK-2 is a light red kidney breeding line released by USDA-ARS (Prosser, WA) in 1999 that possesses *I* and *bc-3* genes for resistance to *Bean common mosaic virus* (BCMV) and *Bean common mosaic necrosis virus* (BCMNV). I9566-21-4-2 is an F_3 derived line from the cross 'Montcalm'/XAN 159 selected for the presence of SAP6 and SU91 markers and resistance to common bacterial blight in greenhouse leaf inoculation assays. XAN 159 with the pedigree UI-114/PI319441//PI319443/3/'Masterpiece' is an advanced breeding line from CIAT with resistance to common bacterial blight derived via interspecific hybridization with tepary bean (*P. acutifolius*). XAN 159 is the source of a major resistance QTL linked with the SU91 SCAR marker also developed at CIAT. Montcalm with the pedigree GN No.1/'Dark Red Kidney' is a dark red kidney cultivar from Michigan State University with moderate resistance to common bacterial blight conferred by a major QTL linked with the SAP6 SCAR marker (developed by USDA-ARS, Prosser, WA) that was derived from Montana No.5 via Great Northern No.1.

Marker-assisted selection was used to identify F_1 plants from the last cross for the presence of SAP6 and SU91 markers. An F_1 plant (PS99-499B) with both markers was advanced to F_2 which was planted in the field at Prosser, Washington, and screened for plant and seed type. An F_3 progeny from an F_2 single plant selection (PS99-499B-3) was tested for reaction to common bacterial blight in leaf inoculation tests conducted at the USDA-ARS Tropical Agriculture Research Station at Mayaguez, Puerto Rico. An individual F_3 plant (PS99-499B-3-2)

with high level of resistance and confirmed to possess SAP6 and SU91 markers was advanced to F_4. The F_4 progeny-row was grown in the field at Prosser and harvested in bulk (PS99-499B-3-2-B). The $F_{4:5}$ bulk was screened for leaf reaction to common bacterial blight in the University of Idaho greenhouse at Kimberly, ID. An individual F_5 plant (PS99-499B-3-2-B-2) with high level of resistance and later confirmed to possess SAP6 and SU91 markers was selected to produce USWK-CBB-17 that was subsequently increased for three generations and evaluated in multiple greenhouse tests for reaction to common bacterial blight and examined in the field for yield and maturity.

USWK-CBB-17, in a greenhouse leaf inoculation test conducted at Kimberly, ID, in December 2004, had a mean disease score of 4.8 based on a 1 to 9 scale where 1 is no visible common bacterial blight and 9 is completely susceptible. In comparison, the dark red kidney bean Montcalm had a mean disease score of 8.7. In a repeated test in December 2005, USWK-CBB-17 scored 3.9 compared to 8.0 for Montcalm and 7.7 for 'Beluga' large white kidney. USWK-CBB-17 possesses both the SAP6 and SU91 markers linked with major QTL for resistance derived from Montana No.5 (via Montcalm) and tepary bean (via XAN 159), respectively. Thus, USWK-CBB-17 exhibits a much higher level of resistance to common bacterial blight than commercially available kidney bean cultivars.

USWK-CBB-17 exhibits a Type I determinate bush growth habit typical of kidney bean. Yield was 98% of Beluga at Othello,Washington, in 2005. Average weight of 100 seeds was 41 g which is slightly less than the 44 g for Beluga. USWK-CBB-17 matured in 91 d, the same as Beluga. Seed appearance was rated commercially acceptable for the white kidney market class. USWK-CBB-17 also exhibits a hypersensitive resistance response to the NL-3 strain of *Bean common mosaic necrosis virus* (BCMNV) in Prosser greenhouse tests, which infers presence of the *I* gene for resistance to *Bean common mosaic virus* (BCMV). This line is also resistant to *Beet curly top virus*.

USWK-CBB-17 will be most useful for incorporating resistance to common bacterial blight in the white kidney market class, but also other large-seeded market classes of Andean origin as well. Seed will be maintained by USDA-ARS at Prosser, WA, and provided in small quantities upon written request. We ask that appropriate recognition of source be given when this germplasm contributes to the development of a new cultivar or germplasm line.

_____ _____

Director, Idaho Agricultural Experiment Station Date
University of Idaho

_____ _____

Deputy Administrator, Crop Production and Protection Date
Agricultural Research Service, U.S. Department of Agriculture

THE WASHINGTON AGRICULTURAL RESEARCH CENTER
WASHINGTON STATE UNIVERSITY
PULLMAN, WASHINGTON 99164

and

THE UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURE RESEARCH SERVICE
WASHINGTON, D.C. 20250

RELEASE OF 'SILVER CLOUD' WHITE KIDNEY DRY BEAN

A. N. Hang, P. N. Miklas, M. J. Silbernagel and G. H. Hosfield
Department of Crops and Soils, WSU-Prosser and USDA-ARS Prosser

The Agriculture Research Center of Washington State University and the Agricultural Research Service, U.S. Department of Agriculture jointly announce the release of 'Silver Cloud' a new large-seeded white kidney dry bean (*Phaseolus vulgaris* L.). This new release was developed to provide a high yielding, upright bush, mid season maturity, disease resistant white kidney for bean producing areas of the Northwestern states. Scientists participating in the development of this variety were A.N. Hang (Washington State University), P.N. Miklas (USDA-ARS Prosser) and M.J. Silbernagel (retired, USDA-ARS Prosser).

Silver Cloud is F_7 derived F_9 line from the cross 'Lisa'/'Linden' Lisa is a small-seeded white kidney mutant out of 'Royal Red', a dark red kidney bean cultivar with bean common mosaic virus (BCMV) and curly top virus (CTV) resistance developed by D.W. Burke. Linden developed at the University of California-Davis, has a large bright white seed, dominant *I* gene resistance to BCMV but susceptible to CTV. Silver Cloud has an upright growth habit and is resistant to lodging. Silver Cloud has the combined *I* and *bc-1* genes, which together condition resistance to BCMV and complete resistance to CTV. Silver Cloud is more tolerant to bean rust (*Uromyces appendiculatus* (Pers.:Pers.) Unger in test performed in Beltsville, MD. It was tested as USWA-70 in our advanced variety trial in Othello, Washington and was outyielded Lassen in many years. Under stress conditions of low residual soil nitrogen (~ 22 kg ha^{-1}) with no fertilizer applied, low soil moisture (irrigation water applies at ~50% of water used requirements based on evapo-transpiration schedules), and heavy root rot pressure, due mainly to *Fusarium solani*, Silver Cloud produced 19 and 59% higher yield than Lassen and Beluga, respectively. Silver Cloud is a medium to late maturity averaging 96 d, 8 d. later than Lassen. Silver Cloud produced an average of 2490 kg ha^{-1} and about 5 to 10 % higher yield than Lassen. Silver Cloud has an unusual attractive, large and shinny white seed that are bigger than Lassen, at 53.7 g 100 $seeds^{-1}$ compared to 47.0 g 100 $seeds^{-1}$. Silver Cloud is an acceptable canner in trial conducted by USDA-ARS and the Michigan Agricultural Experiment Station and in the NY Agricultural Experiment Station.

Silver Cloud has been released as a non exclusive public variety without Plant Variety protection. Breeder and foundation seed will be maintained by Washington Crop Improvement Association, Inc. Department of Crop and Soil Sciences, WSU Seed House, Pullman, WA 99164-6420.

SUBJECT MATTER INDEX - VOLUME 49

George Abawi
Dept. of Plant Pathology
NYSAES Cornell University
630 W. North St.
Geneva, NY 14456
Phone 315-787-2374
Fax: 315-787-2389
Email: gsa1@cornell.edu

Jorge A. Acosta Gallegos
Campo Exp. Bajio
Apartado Postal 112
CP 38110 Celaya, GTO MEXICO
Phone 595-42905
Fax: 595-46528
Email: jamk@prodigy.net.mx

M.W. Adams
5607 Colby Rd.
Crystal, MI 48818
Phone 989-235-6561
Fax: 989-235-6561

African Center for Crop Improvement
University of Kwazulu-Natal
P Bag X01, Scottsville 3209
Kwa Zulu Natal SOUTH AFRICA
Phone 27-33-2605274
Fax: 27-33-2605580

AGRO–HARIBEC Inc.
919 Route 239
St. Aime, Quebec
J0G 1K0 CANADA
Phone 450-788-2196
Fax: 450-788-2709
Email: Pierre@haribec.com

Homero Aidar
EMBRPA Arroz e Feijao
C. Postal 179
75375-000 Santo Antonio deGoias, GO
BRAZIL

Mary Catherine Aime
USDA, ARS, SBML
Rm 319, B011A
10300 Baltimore Ave.
Beltsville, MD 20705
Phone 301-504-5758
Email: Cathie@nt.ars-grin.gov

Richard F. Allison
Dept. of Plant Biology
Michigan State University
East Lansing, MI 48824-1312
Phone 517-432-1548
Fax: 517-353-1926
Email: allison@msu.edu

Antonio Alvarez Tortosa
Seminis Vegetable Seeds Iberica
PO Box 175
04700 El Ejido Almeria SPAIN
Phone 34950580012
Fax: 34950581162

Maurilio Alves Moreira
Instituto de Biotecnologia Aplicada a
Agropecuaria da UFV
Vicosa, M. G.
36571-000 BRAZIL
Phone 55-31-3855-2977
Fax: 55-31-3855-2864
Email: moreira@ufv.br

Ana Lilia Alzate Marin
Rue Guatambu 626
Bairro Jardim Recreio
CEP 14040-160 Ribeirao Preto
Sao Paulo BRAZIL
Phone 55-16630-8556
Email: anaalzate@rge.fmrp.usp.br

Manuel Amane
Coordinacao, Programa Feijao
I.N.I.A.
C.P. 3658 Mavalane,
Maputo MOZAMBIQUE

Warren Anderson
Campus Box 5, Dept. of AgriBus & AgriSci
Middle Tennessee State University
Murfreesboro, TN 37132
Phone 615-898-2408
Fax: 615-898-5169
Email: wanderso@mtsu.edu

Steve Antonius
600 N. 15th St.
Rochelle, IL 61068
Phone 815-562-1345
Fax: 815-562-1319
Email: Steve.antonius@delmonte.com

Carlos Manuel Araya F.
Univ. Nacional/Escuela de Ciencias Agrarias
Lab. De Fitopatologia
Apartado 86-3000, Heredia
COSTA RICA
Phone 506-277-3301
Fax: 506-261-0035
Email: caraya@una.ac.cr

Pedro Antionio Arraes Pereira
5601 Sunnyside Ave.
Room 4-1193
Beltsville, MD 20705-5741
Phone 301-504-4556
Fax: 301-504-4528
Email: paa@ars.usda.gov

J. W. Aylesworth
Box 98
Woodslee, Ontario
N0R 1V0 CANADA
Phone 519-975-2557
Fax: 519-975-1045

Parthiba M. Balasubramanian
Agriculture & Agri- Food Canada
Morden Res. Stat.
Unit 100 – 101 Route 100
Morden, MB R6M 1Y5 CANADA
Phone 204-822-7229
Fax: 204-822-7207
Email: parthibab@agr.gc.ca

Pedro Alberto Balatti
Inst. de Fisiologia Vegetal
Universidad Nacional de La Plata
CC327 LaPlata CP 1900 ARGENTINA
Phone 54-221-4236618
Fax: 54-221-4233698
Email: pbalatti@ceres.agro.unlp.edu.ar

Jim Ballerstein
NYSAES Dept. of Hort. Sci.
PO Box 462
Hedrick Hall
Geneva, NY 14456-0462
Phone 315-787-2223
Fax: 315-787-2216
Email: jwb2@cornell.edu

Mark J. Bassett
2900 NW 32nd St.
Gainesville, FL 32605
Phone 352-374-8264
Email: mark@righteousindignation.com

Messias Jose Bastos de Andrade
Departmento de Agricultra
Univ.. Federal de Lavras
CP 37, CEP 37200-000
Lavras-MG BRAZIL
Phone 35-3829-1327`
Fax: 35-3829-1301
Email: mandrade@ufla.br

Jean-Pierre Baudoin
Faculté Univ. des Sciences Agronomiques
Unité de Phytotechnie tropicale et d´Hort
Passage des Déportés
2 à B.5030 Gembloux BELGIUM
Phone 32081-622112
Fax: 32081-614544
Email: baudoin.jp@fsagx.ac.be

Jeannette Sofia Bayuelo-Jimenez
Inst. de Investigaciones Agro. Y For.
Universidad Michoacana de San Nicolas de Hidalgo
Km. 9.5 Carretera Morelia-Zinapecuaro
Tarimbaro, Michoacan CP 58880 MEXICO
Phone 54-443-2958323
Fax: 52-443-2958324
Email: Jsbayuelo2002@aol.com

Priscila Bazin
EMBRPA Arroz e Feijao
C. Postal 179
75375-000 Santo Antonio deGoias, GO
BRAZIL

Bean Coordinator
EARO Melkassa Research Center
P. O. Box 436
Nazreth, ETHIOPIA
Phone 251-2-112186
Fax: 251-2-113777

Bean Research Group
Awassa Research Center
P. O. Box 6
Awassa, ETHIOPIA

Bean Research Group
KARI-Katumani
Dryland Farming Res Sta
P. O. Box 340
Machakos, KENYA

Bean Research Group
KARI Reg. Research Centre
P. O. Box 27
Embu KENYA

Bean Research Group
KARI Reg. Research Centre
P. O. Box 169
Kakamega, KENYA

Bean Research Group
Min. of Agric. Research and Training Inst.
MARTI Uyole
P. O. Box 400
Mbeya TANZANIA

Bean Research Group, Director of Research
Alemaya Univ. of Agriculture
PO Box 138
Dire Dawa ETHIOPIA

James S. Beaver
Dept. of Agronomy & Soils
Univ. of Puerto Rico, Mayaquez
PO Box 9030
Mayaguez, PR 00681-9030
Phone 787-832-4040/2566
Fax: 787-265-0220
Email: j_beaver@hotmail.com

Steve Beebe
CIAT
1380 NW 78th Avenue
Miami, FL 33126-1606
Phone 1-650-833-6625
Fax: 1-650-833-6626
Email: s.beebe@cgiar.org

Beijing Book Co., Inc.
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Fax: 908-862-4201

Maurice R. Bennink
FSHN
Michigan State University
106F Trout Food Sci. Bldg
East Lansing, MI 48824-1224
Phone 517-355-8474 ext 103
Fax: 571-353-8963
Email: mbennink@msu.edu

Rick Bernsten
411 E Agriculture Hall
MSU
East Lansing, MI 48824
Phone 517-355-3449
Fax: 517-432-1800
Email: bernsten@msu.edu

Matgorzata. Berova
Agricultural University
Menoleleev St. 12
4000 Plovoliv BULGARIA
Phone 359326126
Fax: 35932832004

Kirstin Bett
Dept. of Plant Sciences
University of Saskatchewan
51 Campus Dr.
Saskatoon, SK S7N 5A8 CANADA
Phone 306-966-4947
Fax: 306-966-5015
Email: k.bett@usask.ca

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1463 Sofia BULGARIA

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Blackwood, NJ 08012
Phone 856-228-8900
Fax: 856-228-0977

Matthew W Blair
CIAT/Intl Center for Tropical Agriculture
1380 NW 78th Ave
Miami, FL 33126
Phone 650-833-6625 ext 3078
Fax: 650-833-6626
Email: m.blair@cgiar.org

Fred A. Bliss
Seminis Vegetable Seeds
37437 Highway 16
Woodland, CA 95695
Phone 530-669-6154
Fax: 530-666-6791
Email: fred.bliss@seminis.com

Roberto Bollini
Istituto Biologia e Biotechnologie, CNR
Via Bassini 15
20133 Milano, ITALY
Phone 390223699435
Fax: 390223699411

Lech Boros
Dept. of Seed Science & Techology
Plant Breeding Institute
05-870 Blonie POLAND
Email: l.boros@ihar.edu.pl

Lluis Bosch
CEIB-ESAB
Urgell, 187
Barcelona 08036 SPAIN
Phone 34-934137553
Fax: 34-934137501
Email: lluis.bosch-roura@upc.es

Andrea Brady
ADM
861 Sandcherry St.
London, Ontario N6H 5V1 CANADA
Phone 519-657-9064
Fax: 519-657-3837
Email: Andrea_brady@admworld.com

Mark A. Brick
Dept. of Soil & Crop Sciences
Colorado State University
Fort Collins, CO 80524
Phone 970-491-6551
Fax: 970-491-0567
Email: mbrick@lamar.colostate.edu

Osmar Rodirgues Brito
Universidade Estaudual de Londina
Dept de Agronomia
Londrina, Parana 86051-970 BRAZIL
Phone 552143371-4555
Fax: 552143371-4697
Email: osmar@uel.br

Jan Bert Brouwer
PO Box 910
Horsham,
Victoria 3402 Australia
Phone 61353846293
Email: janbertb@wimmera.com.au

Steve Brown
Jack's Bean Company LLC
402 N. Interocean, Box 327
Holyoke, CO 80734
Phone 970-854-3702
Fax: 970-854-3707
Email: steve@jacksbean.com

Bruno Campion
Inst. Sperimentale Orticoltura
via Paullese 28, 26836 Montanaso
Lombardo Lodi ITALY
Phone 39-0371-68171
Fax: 39-0371-68172
Email: Bruno.campion@libero.it

Francesc Casanas Artigas
Escola Superior Agricultura Barcelons
Campus de Baix Llobregat
Avinguda del Canal Olimpic s.n.
08860 Castelldefels SPAIN
Phone 34-935521215
Fax: 34-935521001
Email: francesc.Casanas@upc.edu

Oscar Checa
CIAT/Int. Center for Tropical Ag.
1380 NW 78th Ave.
Miami, FL 33126
Phone 650-833-6625
Fax: 650-833-6626
Email: ocheca@udenar.edu.co;
ochk@latinmail.com

Chef., Programme Legumineuses
FOFIFA
B.P. 1444 Ambatobe
Antananarivo 101
MADAGASCAR

Chief
Programme Haricot
ISABU-Mosso, BP 795
Bujumbura, BURUNDI

Dr Rowland Chirwa
Coordinator, SABRN
Chitedze Res. Stat.
P. O. Box 158
Lilongwe, MALAWI
Phone 781-182-76722
Fax: 781-182-782835

CIAT PAN-AFRICA Coordinator
Kwanda Agric. Research. Inst.
P. O. Box 6247
Kampala, UGANDA
Phone 256-41-567670
Fax: 256-41-567635

CIAT Regional Bean Programme
PO Box 2704
Arusha TANZANIA

Karen Cichy
Michigan State University
Plant & Soil Science Bldg
E. Lansing, MI 48824
Phone 517-355-6884
Fax: 517-337-6782
Email: cichykar@msu.edu

Robert B. Colville
1127 Westview Drive
Rochelle, IL 61068-1205
Phone 815-562-2980
Email: rbcysc@rochelle.net

Robert L. Conner
Morden Research Station
Unit 100 - 101 Route 100
Morden, Manitoba R6M 1Y5 CANADA
Phone 204-822-7221
Fax: 204-822-7207

Coordinator
Agric. Techn. Dev. Transfer
PO Box 255
Butare RWANDA

Coordinator, Bean Programme
KARI Hort Research Station
P. O. Box 220
Thika, KENYA

Jeremy Davidson
32 Blind Creek Rd.
R.D. 3
Blenheim NEW ZEALAND
Phone 03-570-5382
Email: Davidson.j@xtra.co.nz

Carlos Alberto De Bastos Andrade
Universidade Estadual de Maringa
Bairro Jardim Universitario
Av. Colombo 5790
CEP 87020-900
Maringa, PR BRAZIL
Phone 55442614407
Email: cabandrade@uem.br

Itamar Pereira de Oliveira
Embrapa Arroz e Feijao
Caixa postal 179
75375-000 San Antônio de Goiás/GO
BRAZIL
Phone 55-62-833-2181
Fax: 55-62-833-2100
Email: itamar@cnpaf.embrapa.br

Trazilbo Jose De Paula, Jr.
EPAMIG
Vila Gianetti 47
Vicosa, MG 36570-000 BRAZIL
Phone 55-313891-2646
Fax: 55-313899-5224

Antonio M. De Ron
Dept. of Plant Genetic Resources
PO Box 28
36080 Pontevedra SPAIN
Phone 34-986-854800
Fax: 34-986-841362
Email: amderon@mbg.cesga.es

Jose Eustaquio Souza Carneiro
Universidade Federal de Vicosa
Dept. de Fitotecnia
Vicosa, MG 36571-000 BRAZIL
Email: jesc@ufv.br

Pedro Crescencio Souza Carneiro
Departmento de Biologia Geral
UFV
Vicosa, MG 36570-000 BRAZIL
Phone 55133-8993368
Fax: 55313-8992549
Email: carneiro@ufv.br

Leslie L. Dean
Idaho Seed Bean Co., Inc.
P. O. Box 1072
Twin Falls, ID 83303-1072
Phone 208-734-5221
Fax: 208-733-1984
Email: LLBDean@mindspring.com

Daniel G. Debouck
GRU c/o CIAT Miami Branch
1380 NW 78th Avenue
Miami, FL 33126-1606
Phone 57-2-4450000
Fax: 57-2-4450073
Email: d.debouck@cgiar.org

Maria Jose del Peloso
EMBRAPA Arroz E Jeijao
C. P. 179
75 375-000 Santo Antonio De Goias
BRAZIL
Phone 62-533-2158
Fax: 62-533-2100
Email: mjpeloso@cnpaf.embrapa.br

Luis del Rio
Plant Pathology
N.D. State University
306 Walster Hall
Fargo, ND 58105
Phone 701-231-7073
Fax: 701-231-7851
Email: Luis.delRio-Mendoza@ndsu.nodak.edu

Alfonso Delgado-Salinas
Inst de Biologia, UNAM
Herbario Nac de Mexico, Apdo Pos 70-367
04510 Mexico, DF MEXICO
Phone 525-5424-3867
Email: adelgado@servidor.unam.mx

Patricia DeMark
ADM-JRRRC
1001 N Bruch College Rd
Decatur, IL 62521
Phone 217-451-2060
Fax: 217-451-2457
Email: Patricia_DeMark@admworld.com

Brett Despain
ADM –Edible Bean Specialties, Inc.
Box 117
Huston, ID 83630
Phone 208-455-7728
Fax: 208-459-3370
Email: brett_despain@admworld.com

Michael H. Dickson
Dept. Horticultural Sciences
NYS Agr Exp Station
Geneva, NY 14456
Phone 315-789-1996
Email: mandjdickson@aol.com

Dobroudja Agricultural Institute
Biblioteka
9520 GENERAL TOCHEVO BULGARIA
Phone 359-58-879234
Fax: 359-5731-4448

Kirk Dolan
Food Science & Human Nutrition
Michigan State University
208 Trout Bldg.
East Lansing, MI 48824
Phone 517-355-8474 X119
Fax: 517-353-8963
Email: dolank@msu.edu

Shigehiko Ebe
Lab of Bean Breeding
Tokachi Ag Expt Sta
Memuro-cho, Kasai-gun
Hokkaido 082-0071 JAPAN
Phone 81-155-62-2431
Fax: 81-155-62-0680
Email: ebesg@agri.pref.hokkaido.jp

J. Alberto Escalante Estrada
Especialidad de Botanica
IRENAT
Montecillo, Mex 56230 MEXICO
Phone 595-2-0247
Fax: 595-20247
Email: jasee@colpos.mx

Estacion Experimental Agropecuaria Salta INTA
Maria Elisa Maggio
Casilla de Correos 228
Salta 4400 ARGENTINA
Phone 54-387 4902214
Fax: interno 212
Email: memaggio@correo.inta.gov.ar

Kathryne Everts
27664 Nanticoke Rd.
Salisbury, MD 21801
Phone 410-742-8789
Fax: 410-742-1922
Email: everts@udel.edu

Luis Claudio Faria
EMBRPA Arroz e Feijao
C. Postal 179
75375-000 Santo Antonio deGoias, GO
BRAZIL
Email: lcfaria@cnpaf.embrapa.br

Juan Jose Ferreira Fernandez
SERIDA Apdo 13
Villaviciosa, Asturias SPAIN
Phone 34-85890066
Fax: 34-85891854
Email: jjferreira@serida.org

Iraja Ferreira Antunes
EMBRAPA / CPACT
Caixa Postal 403
96001-970 Pelotas, RS, BRAZIL
Phone 53-275-8434
Fax: 53-275-8412
Email: iraja@cpact.embrapa.br

Deidre Fourie
ARC-Grain Crops Institute
Private Bag X1251
Potchefstrom 2520 REPUBLIC OF SOUTH AFRICA
Phone 27-18-299-6312
Fax: 27-18-297-6572
Email: FourieD@arc.agric.za

Gary D. Franc
College of Agriculture-Plant Sciences
1000 E. University Ave, Dept. 3354
University of Wyoming
Laramie, WY 82071
Phone 307-766-2397
Fax: 307-766-5549
Email: francg@uwyo.edu

George F. Freytag
3808 Crescent Drive
Fort Collins, CO 80526
Phone 970-223-2189

Incoronata Galasso
CNR Instituto di Biologia e Biotecnologia Agraria,
Via Bassini15
20133 Milan ITALY
Phone 39-0223699435
Fax: 39-0223699411
Email: galasso@ibba.cnr.it

Nadejda Ganeva
IZK "Maritza" - Biblioteka
UL, "Brezovsko Shosse" 32, KL 3
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Gautier Semences SAS
Rte d' Avignon
B. P. No. 1
13630 Eyragues FRANCE
Phone 04-90-240-270
Fax: 04-90-240-271

Robert J. Gehin
Harris Moran Seed Co.
1677 Muller
Sun Prairie, WI 53590
Phone 608-837-6574
Fax: 608-837-3758

Jean Robert Gelin
Plant Sci. Dept.
NDSU
360B Loftsgard Hall
Fargo, ND 58105-5051
Phone 701-231-8148
Fax: 701-231-8474
Email: Jean.gelin@ndsu.nodak.edu

Dititar Genchev
Dobroudja Agricultural Institute
9520
General Tochevo BULGARIA
Email: genchev@dai-gt.dobrich.net

Paul Gepts
Agronomy and Range Science
One Shields Avenue
University of California
Davis, CA 95616-8515
Phone 530-752-7743
Fax: 530-752-4361
Email: plgepts@ucdavis.edu

Robert L. Gilbertson
Dept. of Plant Pathology
University of California
1 Shields Ave
Davis, CA 95616
Phone 916-752-3163
Fax: 916-752-5674
Email: rlgilbertson@ucdavis.edu

Chris Gillard
Ridgetown College
University of Guelph
120 Main St., E.
Ridgetown, ON N0P 2C0 CANADA
Phone 519-694-1632
Fax: 519-674-1600
Email: cgillard@ridgetownc.uoguelph.ca

Ramon Giraldez
Departamento de Biologia Funcional
Universidad de Oveido
33006 Oviedo SPAIN
Phone 34-985103594
Fax: 34-985103534
Email: giraldez@uniovi.es

Graciela Godoy-Lutz
406 Plant Science
Department of Plant Pathology
University of Nebraska
Lincoln, NE 68583-0722
Phone 402-472-5759
Fax: 402-472-2853
Email: ggodoy@unlnotes.unl.edu

Everaldo Goncalves de Barros
BIOAGRO . UFV
36571-000
Vicosa M. G., BRAZIL
Phone 55-31-899-2151
Fax: 55-31-899-2864
Email: ebarros@ufv.br

Maria Celeste Goncalves Vidigal
Av. Colombo 5790-cep:87020-900
Univ. Estadual de Maringa
Maringa - Parana BRAZIL
Phone 442635036
Fax: 442615599
Email: mcgvidigal@uem.br

Kenneth F. Grafton
Department of Plant Sciences
North Dakota State University
Fargo, ND 58105
Phone 701-231-8145
Fax: 701-231-8520
Email: k.grafton@ndsu.nodak.edu

Phillip Griffiths
NYSAES Dept of Hort. Sci.
PO Box 462
Hedrick Hall
Geneva, NY 14456-0462
Phone 315-787-2222
Fax: 315-787-2216
Email: pdg8@cornell.edu

Robert Hall
Dept. of Environmental Biology
University of Guelph
Guelph, Ontario N1G 2W1 CANADA
Phone 519-824-4120
Fax: 519-837-0442
Email: rhall@uoguelph.ca

Donald Halseth
15D Plant Sci Bldg
Cornell University
Ithaca, NY 14853-0327
Phone 607-255-5460
Fax: 607-255-9998
Email: deh3@cornell.edu

An N. Hang
Washington State University
24106 N Bunn Rd
Prosser, WA 99350
Phone 509-786-9201
Fax: 509-786-9370

Richard M. Hannan
Plant Introduction, USDA
Rm. 59, Johnson Hall, WSU
Pullman, WA 99164
Phone 509-335-3683
Fax: 509-335-6654
Email: hannan@wsunix.wsu.edu

Head, Library
Areka Agric Research Center
P. O. Box 79
Areka, North 0M0 ETHIOPIA

Bob Henson
Carrington Research Extension Center
PO Box 219
Carrington, ND 58421
Phone 701-652-2951
Fax: 701-652-2055
Email: bhenson@ndsuext.nodak.edu

Sanjuana Hernandez Delgado
Centro de Biotechnologia Genomica-IPN
Blvd. Del Maestro esq. Elias Pina s/n Col.
Narcisco Mendoza
88710 Reynosa
Tamaulipa MEXICO

David Holland
Washington State University
Dept. of Agric. & Resource Economics
Box 646210
Pullman, WA 99164-6210

Gerrit Hoogenboom
Dept. of Biological & Agricultural. Eng.
University of Georgia
Griffin GA 30223
Phone 770-229-3438
Fax: 770-228-7218
Email: gerrit@griffin.uga.edu

George L. Hosfield
208 Artists Alley
Blowing Rock, NC 28605
Phone 828-295-6727
Email: georgelouis@earthlink.net

Francisco J. Ibarra-Perez
INIFAP-Durango
Apartado Postal 186
Durango, Dgo. 34000 MEXICO
Phone 91-181-2-1044
Fax: 91-181-2-1133

Inst. for Wheat & Sunflower
"Dobroudja" - Biblioteka
Near General Toshevo BULGARIA

Tanja Vanjak-Kvaic Institute of Field & Vegetable Crops
Maksima Gorkog 30
21000 Novi Sad SERBIA AND MONTENEGRO
Phone 381-21-4898142
Fax: 381-21-624212
Email: tanja@ifvcns.ns.ac.yu

Instituto di Virologia Vegetale
Strada delle Cacce 73
10135 Torino ITALY
Phone 011-348-99-51

Carmen Jacinto-Hernandez
Apartado Postal 10
56230 Chapingo, Mex, MEXICO
Phone 595-4-2877
Fax: 595-4-6528

Antony Jarvie
PANNAR Limited
Box 19,
Greytown 3250 REPUBLIC OF SOUTH AFRICA
Phone 033 4131131
Fax: 033 4171208
Email: antony.jarvie@pannar.co.za

Frans J. Jongeleen
SVS Holland BV
JPO Box 22
1600 AA Enkhuizen NETHERLANDS
Phone 0220-357000
Fax: 0220-357035

K2 Seed Growers, Inc.
Box 206
19265 Darrow Lane
Huston, ID 83630
Phone 208-459-4420
Fax: 208-459-4457
Email: K2seeds@speedyquick.net

Yu Kangfu
Agriculture and Agri-Food Canada
Greenhouse and Processing Crops Research Centre
Harrow, Ont. N0R 1G0 CANADA
Phone 519-738-2251 ext. 479
Fax: 519-738-2929
Email: yuk@agr.gc.ca

Lawrence Kaplan
26 Parker St
Newton Centre, MA 02459
Phone 617-527-3449
Email: Lawrence.Kaplan@umb.edu

Ed Kee
16684 County Seat Hwy.
Georgetown, DE 19947
Phone 302-856-7303
Fax: 302-856-1845
Email: kee@udel.edu

John Keenan
5202 Fawn
Farmington, NM 87402
Phone 505-327-4219
Fax: 505-327-4219
Email: jgkeenan@sisna.com

James D. Kelly
Dept. of Crop & Soil Sciences
Michigan State University
East Lansing, MI 48824
Phone 517-355-0271 x1181
Fax: 517-353-3955
Email: kellyj@msu.edu

Paul M. Kimani
Dept of Crop Science-Kabete
Univ. of Nairobi
P. O. Box 30197
Nairobi, KENYA
Email: kimanipm@nbnet.co.ke

Joao Kluthcouski
EMBRPA Arroz e Feijao
C. Postal 179
75375-000 Santo Antonio deGoias, GO
BRAZIL

Ken Kmiecik
7202 Portage Rd.
DeForest, WI 53532
Phone 608-842-1411
Fax: 608-846-7892
Email: ken.kmiecik@seminis.com

Josué Kohashi Shibata
Centro de Botanica, Col. de Postgr.
Montecillo, Edo. de Mexico
C.P. 56230 MEXICO
Phone 1-595-9520200 ext 1318
Fax: 1-595-9520247
Email: jkohashi@colpos.mx

Chet Kurowski
Harris Moran Seed Company
9241 Mace Blvd
Davis, CA 95616
Phone 530-747-3216
Fax: 530-756-1016
Email: c.kurowski@harrismoran.com

Richard Larsen
USDA ARS
24106 N. Bunn Rd
Prosser, WA 99350
Phone 509-786-9259
Fax: 509-786-9277
Email: rlarsen@tricity.wsu.edu

Colin Leakey
The Close, 15 Cambridge Road
Girton
Cambridge CB3 OPN ENGLAND
Phone 1223-276866
Fax: 1223-565215

Margarita Lema-Marquez
Kimberly Research & Extention Center
University of Idaho
3793 North 3600 East

Kimberly, ID 83341-5076
Phone 208-423-6624
Fax: 208-423-6637
Email: mlema@kimberly.uidaho.edu

Librarian
CSIRO Livestock Industries
Queensland Biosci. Precinct
306 Carmody Rd.
St. Lucia QLD 4067 AUSTRALIA
Phone 6173214-2555
Fax: 6173214-2911
Email: qstcp@csiro.au

Library/Bibliotheque
Agriculture & Agri-Food Canada
Edifice Sir John Carling Building
Ottawa, ON K1A 0C5 CANADA

A.J. Liebenberg
ARC-Grain Crops Institute
Private Bag X1251
Potchefstroom 2520 REPUBLIC OF SOUTH AFRICA
Phone 27-18-299-6326
Fax: 27-18-297-6572
Email: LiebenbergA@arc.agric.za

Merion M. Liebenberg
ARC-Grain Crops Institute
P. Bag X1251
Potchefstroom 2520 REPUBLIC OF SOUTH AFRICA
Phone 27-18-299-6311
Fax: 27-18-297-6572
Email: LiebenbergM@arc.agric.za

Dale T. Lindgren
461 W University Dr.
University of Nebraska - West Central Center
North Platte, NE 69101
Phone 308-696-6706
Fax: 308-532-3823
Email: dlindgren1@unl.edu

Lucia Lioi
Plant Genetics Institute, CNR
Via Amendola 165/A
70126 Bari, ITALY
Phone 080-5583400
Fax: 080-5587566
Email: Lucia.lioi@igv.cnr.it

Ted Lund
Brotherton Seed Company
Box 1136
Moses Lake, WA 98837
Phone 509-765-1816
Fax: 509-765-1817
Email: ted@brothertonseed.com

Devanand Luthria
Food Composition Lab
Bldg. 161, BARC (East)
Beltsville, MD 20705
Phone 301-504-7247
Fax: 301-504-8314
Email: luthriad@ba.ars.usda.gov

Jonathan Lynch
Dept. of Horticulture
Penn State University
221 Tyson Building
University Park, PA 16802-4201
Phone 814-863-2256
Fax: 814-863-6139
Email: Jpl4@psu.edu

Robert Mabagala
Tanzania Bean CRSP, Dept. of Crop Science
Sokoine Univ. of Agr, P.O. Box 3005
Morogoro, TANZANIA
Phone 255-56-3661
Fax: 255-56-4645
Email: mabagal@suanet.ac.tz

F. Magayane
Sokine Univ. of Agriculture
Dept. of Crop Sci. & Prod.
Box 3005, Subpost
Morogoro TANZANIA

Steve Magnuson
1509 Stadium Ct
Lehigh Acres, FL 33971
Phone 239-693-5153
239-810-2944
Email: d.s.magnuson@att.net

Magrath Library
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George Mahuku
CIAT/Intl. Center for Tropcial Agriculture
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Phone 572-445-000
Fax: 572-455-0073
Email: g.mahuku@cgiar.org

Roxanne Mainz
Syngenta Seeds, Inc.
317-330th St.
Stanton, MN 55018-4308
Phone 507-663-7612
Fax: 507-645-7519
Email: roxanne.mainz@syngenta.com

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Blvd. Del Maestro esq. Elias Pina s/n Col.
Narcisco Mendoza, 88710 Reynosa
Tamaulipa MEXICO
Phone 899-9243627
Fax: 899-924-3627
Email: nmayekp@hotmail.com

M. Mbikayi Nkonko
Chief D' Antenne PNL/INERA MULUNGU
BP 327
Cyangugu RWANDA

Phil McClean
Department of Plant Sciences
North Dakota State University
Fargo, ND 58105-5051
Phone 701-231-8443
Fax: 701-231-8474
Email:
mcclean@beangenes.cws.ndsu.nodak.edu

Debra McLaren
Agric & Agri_Food Canada
Branon Research Center
PO Box 1000A, RR3
Brandon, MB R7A 5Y3 CANADA
Phone 204-578-3561
Fax: 204-728-3858
Email: dmclaren@agr.gc.ca

Rex Metzger
Kelley Bean Company
1520 Ave "B"
PO Box 83
Scottsbluff, NE 69361
Phone 308-635-2338
Fax: 308-635-2339
Email: rexmetzger@kelleybean.com

Thomas Michaels
Dept. of Horticultural Sci.
University of Minnesota
1970 Folwell Ave.
St. Paul, MN 55108
Phone 612-624-7711
Fax: 612-624-4941
Email: michaels@umn.edu

Charlotte M.S. Mienie
ARC-Grain Crops Institute
Private Bag X1251
Potchefstrom 2520 REPUBLIC OF SOUTH AFRICA
Phone 27-18-299-6315
Fax: 27-18-297-6572
Email: MienieC@arc.agric.za

Edison Miglioranza
Universidad Estsadual de Londrina
Depto de Agronomia
Londrina Parana 86051-970 BRAZIL
Phone 43-371-4697
Email: emiglior@uel.br

Phil Miklas
USDA, ARS, IAREC
24106 No. Bunn Road
Prosser, WA 99350-9687
Phone 509-786-9258
Fax: 509-786-9277
Email: pmiklas@pars.ars.usda.gov

Carol A Miles
WSU Vancouver REU
1919 NE 78th St
Vancouver, WA 98665-9752
Phone 3605766030
Fax: 3605766032
Email: milesc@wsu.edu

El Sadig S. Mohamde
Coordinator, Grain Legumes
Shambat Res. Sta.
P. O. Box 30
Khartoum N. SUDAN

Kennedy Muimui
Misamfu Regional Research Cntr.
PO Box 410055
Kasama ZAMBIA

Augustine Musoni
Chief, Programme Legumineuses

ISAR Rubona, B.P. 138
Butare RWANDA

James R. Myers
Dept. of Horticulture, ALS 4017
Oregon State University
Corvallis, OR 97331
Phone 541-737-3083
Fax: 541-737-3479
Email: myersja@bcc.orst.edu

Rosa Navarrete-Maya
Sur 121 MZ 17 L 14
Col. Juventino Rosas
D. F. 087000 MEXICO
Phone 6505975

Susan Nchimbi-Msolla
Sokine Univ. of Agriculture
Dept. of Crop Sci. & Prod.
Box 3005, Subpost
Morogoro TANZANIA

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Phone 31-475-599314
Fax: 31-475-591361
Email: S.vanroy@nunhems.com

Dawn S. Neuman
Dept. of Biol Sci, 4505 Maryland Pkwy.
University of Nevada-Las Vegas
Las Vegas, NV 89154-4004
Phone 702-895-3390
Email: neuman@ccmail.nevada.edu

James Nienhuis
Dept. of Hort, 1575 Linden Drive
University of Wisconsin
Madison, WI 53706
Phone 608-262-6975
Fax: 608-262-4743
Email: nienhuis@wisc.edu

F. Nuez
ETSIA-Dept. Biotechnologia
Camino de Vera 14
46022-Valencia, SPAIN
Phone 34-6-387-74-21
Fax: 34-6-387-74-29

NYS Agr. Exp. Station
Library
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Geneva, NY 14456
Phone 315-787-2214
Fax: 315-787-2276
Email: lib@nysaes.cornell.edu

Ivan E Ochoa
Dept. of Horticulture, PSU
102 Tyson Building
State College, PA 16803
Phone 814-863-2536
Fax: 814-863-6139
Email: Ieo101@psu.edu

Barry Ogg
Dept. of Soil & Crop Sciences
Colorado State University
Fort Collins, CO 80523-1170
Phone 970-491-6354
Fax: 970-491-0564
Email: beans@lamar.colostate.edu

Arie Oppelaar
SVS Holland Research Station Wageningen
Wageningse Afweg 31
6702 PD Wageningen HOLLAND

Pedro F. Ortega M.
Martires de Cananea 475
Col. Ley 57
Hermosillo, Sonora, 83110 MEXICO
Phone 52-662-261-0074
Fax: 52-662-261-0073
Email: ortega.pedro@inifap.gob.mx

E.S. Osuna-Ceja
Km. 32.5 Carretera Aguascalientes-Zacatecas
Pabellon de Arteaga, AGS
MEXICO
Phone 014659580167
Fax: 014659580167
Email: esosuna@yahoo.com

J. Saul Padilla-Ramirez
Revolucion 309 Col. Popular
Pabellon de Arteaga CP 20660
Ags MEXICO
Phone 465-958-0167
Fax: 465-958-0186
Email: jsaulpr@yahoo.com

James Palmer
Michigan Crop Improvement Assoc.
PO Box 21008
Lansing, MI 48909
Phone 517-332-3546
Fax: 517-332-9301
Email: palmerj@michcrop.com

Gloria Palomares
Dept. de Biotech, Univ. Politecnica
Camino de Vera 14
46022 Valencia, SPAIN
Phone 96-387-74-21
Fax: 96-3877429
Email: gpaloma@btc.upv.es

Soon O. Park

Texas Agricultural Res. Center
Texas A&M University
2415 East Highway 83
Weslaco, TX 78596-8399
Phone 956-969-5610
Fax: 956-969-5620
Email: so-park@tamu.edu

Soon Jai Park
Agriculture & Agri-Food Canada
Greenhouse & Processing Crops Res. Centre
2585 County Rd 20
Harrow, Ontario NOR 1GO CANADA
Phone 519-738-2251
Fax: 519-738-2929
Email: parks@agr.gc.ca

Parks Library
Acquisitions Department
Iowa State University
Ames, IA 50011-2140
Phone 515-294-2749
Fax: 515-294-7411

Marcial A Pastor-Corrales
USDA-ARS-Vegetable Lab
10300 Baltimore Ave.
Bldg. 010A Rm. 240 BARC-West
Beltsville, MD 20705
Phone 301-504-6600
Fax: 301-504-5555
Email: pastorm@ba.ars.usda.gov

Calvin H. Pearson
Fruita Research Center
1910 L Road
Fruita, CO 81521
Phone 970-858-3629
Fax: 970-858-0461
Email: calvin.pearson@colostate.edu

Eduardo Peralta Idrovo
Panamerican Sur km 14
Estacion Experimental Santa Catalina
INIAP
Quito ECUADOR
Phone 59322693360
Fax: 59322693360
Email: Legumini@pi.pro.ec

Bill Pfeil
Pure Line Seeds, Inc.
P. O. Box 8866
Moscow, ID 83843
Phone 208-882-4422
Fax: 208-882-4326
Email: pure@moscow.com

Florence Picard
Ets Vilmorin
49250 La Menitre FRANCE
Phone 02-61-79-41-79
Fax: 02-41-79-41-21
Email: florence.picard@vilmorin.com

Vicki J. Pierce
Del Monte Corporation
6580 Furlong Avenue
Gilroy, CA 95020
Phone 408-842-4180
Fax: 408-847-2768

Angela Rosa Piergiovanni
Germplasm Inst Cntr
Via Amendola 165/A
70126 Bari ITALY

K. N. Pillay
Pannar Research Services Pty LTD
P. O. Box 19,
Greytown, 3250 SOUTH AFRICA
Phone 27-33-4139645
Fax: 27-33-4171208
Email: kiru.pillay@pannar.co.za

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Univ. of Wisconsin, 584 Russell Lab
1630 Linden Drive
Madison, WI 53706
Phone 608-262-8698
Fax: 608-263-2626
Email: scloyd@library.wisc.edu

Pop Vriend Seeds B.V.
P. O. Box 5
1619 ZG Andijk NETHERLANDS
Phone 31-22859-1462
Fax: 31-22859-3354
Email: rcdkroon@popvriendseeds.nl

Tim Porch
USDA ARS SAA TARS
2200 P.A. Campos Ave., Suite 201
Mayaguez, PR 00680
Phone 787-831-3435
Fax: 787-831-3386
Email: maytp@ars-grin.gov

Richard Pratt
Horticulture & Crop Science
OSU/OARDC
1680 Madison Ave.
Wooster, OH 44691
Phone 330-263-3972
Fax: 330-263-3887
Email: Pratt.3@osu.edu

Emmanuel Prophete
Ministry of Agriculture
PO Box 2363
Port-au-Prince, HAITI
Phone 509-404-2193
Email: eprophete@hotmail.com

ProVita
PO Box 628
Kuna, ID 83634
Phone 208-463-7624
Fax: 208-442-6433
Email: Ron@Provita-Inc.com

Rosario Provvidenti
115 Ent-Plant Path Bldg.
NYS Agr. Exp. Station
Geneva, NY 14456
Phone 315-787-2316
Fax: 315-787-2389
Email: rp13@cornell.edu

Magno Antonio Patto Ramalho
Dept. de Biologia - UFLA
Cx. Pos. 3037
37200-000 Lavras, M.G BRAZIL
Phone 035-829-1352
Fax: 035-829-1341
Email: magnoapr@ufla.br

Robert E. Rand
1630 Linden Drive
University of Wisconsin
Madison, WI 53706
Phone 608-262-3269
Fax: 608-263-2626
Email: rer@plantpath.wisc.edu

Thomas Randgaard
Faribault Foods Inc.
128 NW 15th St.
Faribault, MN 55021
Phone 507-331-1400
Fax: 507-331-1457
Email: trandgaard@faribaultfoods.com

Jack Rasmussen
Plant Pathology
N.D. State University
306 Walster Hall
Fargo, ND 58105
Phone 701-231-1027
Fax: 701-231-7851
Email: Jack.Rasmussen@ndsu.nodak.edu

John Rayapati
JRRRC
1001 Brush College Rd.
Decatur, IL 62521-1656
Phone 217-451-4225
Fax: 217-451-4230
Email: rayapati@admworld.com

Ron Riley
Basin Seed Co.
1922 S Middleton Rd
Nampa, ID 83686
Phone 208-461-4656
Fax: 208-461-4439
Email: ron.riley@basinseed.com

A Paula Rodino
Dept of Plant Breeding
Carballeira 8-Salcedo
36 Pontevedra SPAIN
Phone 34-986-854800
Fax: 34-986-841362
Email: aprodino@mbg.cesga.es

Maria Teresa Rodriguez Gonzalez
Especialidad de Botanica
IRENAT
Montecillo, Mex 56230 MEXICO

Gonzalo Rojas-Cifuentes
Plant Sci. Dept
NDSU
360 B Loftsgard Hall
Fargo, ND 58105-5051
Phone 701-231-8148
Fax: 701-231-8474
Email: Gonzalo.rojas@ndsu.nodak.edu

Belinda Roman
Catedratico Auxiliar Depto. De Biologia
UPR-Cayey
Avenida Antonio R. Barcelo
Cayey, PR 00736
Phone 787-738-2161 ext. 2205
Email: romanbelinda@hotmail.com

Rigoberto Rosales Serna
Allende 519, Depto 6
Barrio La Conchita,, Texcoco
Estado de Mexico CP 56170 MEXICO
Phone 52-595-954-2964
Email: rigoberto_serna@yahoo.com

Juan Carlos Rosas
EAP/ZAMORANO
P. O. Box 93
Tegucigalpa, HONDURAS
Phone 504-776-6140 ext 2314
Fax: 504-776-6242
Email: jcrosas@zamorano.edu

Gerrit Ruiter
Holland-Select B.V.
PO Box 27
1619 ZG
Andijk HOLLAND
Phone 228-291578
Fax: 228-591755
Email: info@holland-select.nl

Marcelo O. Salgado
Adolfo Guemes 427-2 B
4400 - Salta ARGENTINA
Phone 5487-212506

Carmen Asensio Sanchez-Manzanera
SIDTA-Junta de Castilla y Leon
Ctra de Burgos km 118, Apdo. 172
47080 Valladolid SPAIN
Phone 34-983-414461
Fax: 34-983-414780
Email: Carmen.Asensan@cag.jcyl.es

Marta Santalla
Mision Biologica de Galicia
PO Box 28
36080 Pontevedra SPAIN
Phone 34 -986-854800
Fax: 34-986-841362
Email: msantalla@mbg.cesga.es

Aloisio Sartorato
EMBRPA Arroz e Feijao
C. Postal 179
75375-000 Santo Antonio deGoias, GO
BRAZIL
Phone 055-062-8332171
Fax: 055-062-8332100
Email: sartorat@cnpaf.embrapa.br

Hitoshi Sato
Tokachi Agri. Expt. Station
S9-2 Shinsei Memuro-Town
Hokkaido 082-0071 JAPAN
Phone 81-155-62-2431
Fax: 81-155-62-0680
Email: satohhs@agri.pref.hokkaido.jp

Jim Schild
University of Nebraska
Planhandle Res. & Ext. Center
4502 Ave. I
Scottsbluff, NE 69361-4907
Phone 308-632-1481
Fax: 308-632-1481
Email: Jschild1@unl.edu

Roger A. Schmitt
Del Monte Corp. Agr Res Ctr
205 No. Wiget Lane
Walnut Creek, CA 94598
Phone 925-944-7312
Fax: 925-942-0940
Email: roger.schmitt@delmonte.com

Eduardo C. Schroder
Dept. of Agronomy & Soils
University of Puerto Rico
Mayaguez, PR 00681-9030
Phone 787-832-4040 x3471
Fax: 787-265-0860
Email: e_schroder@hotmail.com

Howard F. Schwartz
Dept. of Bioagr. Sci. & Pest Mgmt.
C205 Plant Sciences
Colorado State University
Fort Collins, CO 80523-1177
Phone 970-491-6987
Fax: 970-491-3862
Email: howard.schwartz@colostate.edu

Seminis Vegetable Seeds
Ruelle Simon
80500 Guerbigny FRANCE
Phone 011-33-3-22-37-5000
Fax: 011-3-33-22-37-4810

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University Park, PA 16802-1808

Shree P. Singh
Kimberly Research and Extension
University of Idaho
3793 N. 3600 East
Kimberly, ID 83341
Phone 208-423-6609
Fax: 208-423-6559
Email: singh@kimberly.uidaho.edu

Rusty Smith
USDA-ARS-CG&PR
PO Box 345
Stoneville, MS 38776
Phone 662-686-5499
Fax: 662-686-5218
Email: rsmith@ars.usda.gov

Tom Smith
Plant Agriculture Dept.
Crop Science Bldg.
University of Guelph
Guelph, ON, N1G 2W1 CANADA
Phone 519-824-4120 ext58339
Fax: 519-763-8933
Email: thsmith@uoguelph.ca

Raymond C. Smith
Sunland Seed Pty Ltd.
P. O. Box 7,
Coopernook 2426 NSW AUSTRALIA
Phone 61-265-563234
Fax: 61-265563234

Societe Generale
3, rue Martin Luther King
Boite Postale 242
24010 Avignon Cedex 1 France
Phone 049-080-5700

Francesca Sparvoli
Instituto di Biologia e Biotecn. Agraria, CNR
Via Bassini15
20133 Milan ITALY
Phone 39-0223699435
Fax: 39-0223699411

Eben Spencer
ADM Edible Bean Specialties, Inc
Box 208
Oslo, MN 56744
Phone 218-695-5566
Fax: 218-695-5566
Email: spencer@invisimax.com

Doug Sprehe
Hylands Seeds Research
41703 US Hwy 2 SW
East Grand Forks, MN 56721
Phone 218-773-8834
Fax: 218-773-9809
Email: dsprehe@hylandseeds.com

Mark Stack
Southwestern Colo. Res. Center
Box 233
Yellow Jacket, CO 81335
Phone 970-562-4255
Fax: 970-562-4254
Email: mark.stack@colostate.edu

J. Rennie Stavely
2206 Apple Tree Lane
Silver Spring, MD 20905-4415
Phone 301-384-6853
Email: JRNGStavely@comcast.net

James R. Steadman
Dept. of Plant Pathology
University of Nebraska
406 PSH
Lincoln, NE 68583-0722
Phone 402-472-3163
Fax: 402-472-2853
Email: jsteadman1@unl.edu

Kathy Stewart-Williams
University of Idaho
3806 N. 3600 E.
Kimberly, ID 83341
Phone 208-423-6655
Fax: 208-423-6656
Email: williams@kimberly.uidaho.edu

Peter Stoffella
2199 South Rock Road
University of Florida
Fort Pierce, FL 34945-3138
Phone 772-468-3922
Fax: 772-468-5668

Diana Lilova Svetleva
25 'Gustav Vajgand' Str.
Plovdiv 4000, BULGARIA
Phone 359-32-227829
Fax: 359-32-633157
Email: svetleva@yahoo.com

Swets Information Services
160 Ninth Ave
Suite A
Runnemede, NJ 08078
Phone 856-312-2690
Fax: 856-312-2000
Email: info@us.swets.com

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1600 AA Enkhuizen
THE NETHERLANDS
Email: Annet.bos@syngenta .com

Alan Taylor
Horticultural Sciences
630 North St
Geneva, NY 14456-0462
Phone 315-787-2243
Fax: 315-787-2320
Email: agt1@cornell.edu

Steven R. Temple
Plant Science Department, UC Davis
Mail Stop One
One Shields Ave.
Davis, CA 95616
Phone 530-752-8216
Fax: 530-752-4361
Email: srtemple@ucdavis.edu

John Theuws
Kempen Laan 7
B-3600 Genk
BELGIUM
Phone 32-89-85-2931
Email: johntheuws@versateladsl.be

Alyson Thornton
Harris Moran
1677 Muller Rd.
Sun Prairie, WI 53590
Phone 608-837-6574
Fax: 608-837-3758
Email: a.thornton@harrismoran.com

Michael D. T. Thung
Embrapa Arroz e Feijao
Caixa Postal 17975375-000 Santo Antonio
De Goias/GO BRAZIL
Phone 55625332183
Fax: 55625332100

Adriana Tofino Rivera
Unidad Residencial Sultana Norte
Calle 31N #2AN-25 Apto 504B
Cali COLOMBIA
Phone 6675985
Email: aptr611@hotmail.com

Joseph Michel Tohme
C I A T
7343 NW 79th Terrace
Medley, FL 33166-2211
Phone 415-833-6625
Fax: 415-833-8826
Email: j.tohme@cgiar.org

Heloisa Torres da Silva
Rod. Goiania
St. Antonio de Goias, Go
CP 179
74001-970 Goiania, GO BRAZIL
Phone 062-8332150
Fax: 062-8332100
Email: heloisa@cnpaf.embrapa.br

S. J. Tsao
Dept. of Horticulture
#1 Sec 4 Roosevelt Rd.
Taipei, R.O.C. 106 TAIWAN
Phone 886-2-23630231 ext. 2526
Fax: 886-2-23625542
Email: jocelyn@ntu.edu.tw

Ray Tucker
Bush Bros. & Co
1016 East Wesgarber Rd
Knoxville, TN 37909-2683
Phone 865-909-8647
Email: rtucker@bushbros.com

Mark A. Uebersax
FSHN
204 G. Malcolm Trout Bldg.
Michigan State University
East Lansing, MI 48824-1224
Phone 517-355-8474
Fax: 517-353-8963
Email: uebersax@msu.edu

Michael Ugen
NARO
NAARI
P. O. Box 7084
Kampala UGANDA
Phone 256-41-567635

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2101 VLSB #6500
Berkeley, CA 94720-0001

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University Libraries
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PO Box 880410; 13 & R Sts.
Lincoln, NE 68588-0410

Juan-Tay Urbina
Estacion Exp. Quilamapu
Casilla # 426
Chillan CHILE
Phone 56-42-209700
Fax: 56-42-209599
Email: Jtay@quilamapu.inia.cl

Carlos Urrea
Panhandle Research & Extension Center
University of Nebraska
4502 Avenue I
Scottsbluff, NE
Phone 308-632-0556
Fax: 308-632-1365
Email: Currea2@unl.edu

USDA National Agric. Library
Current Serial Records, Room 002
10301 Baltimore Ave.
Beltsville, MD 20705
Fax: 301-504-5243
Email: wgelenter@nal.usda.gov

John Van Herk Jr.
Hyland Seeds
P. O. Box 130, 2 Hyland Dr.
N0P 1A0 CANADA
Phone 519-676-8146
Fax: 519-676-5674
Email: JVanHerk@Thompsonslimited.com

Bert Vandenberg
Dept. of Plant Sciences
51 Campus Drive, Univ of Saskatchewan
Saskatoon, SK S7N 5A8 CANADA
Phone 306-966-8786
Fax: 306-966-5015
Email: bert.vandenberg@usask.ca

Greg Varner
Saginaw Valley Research Farm
3066 S Thomas Road
Saginaw, MI 48609
Phone 989-781-0260
Fax: 989-781-5282
Email: varnerbean@hotmail.com

Carmen Asensio Vegas
Instituto Technologico Agrario
Ctra de Burgos km 118
Apdo. 172
47080 Valladolid SPAIN
Phone 34-983-414461
Fax: 34-983-414780
Email: asevegma@jcyl.es

Pedro Soares Vidigal Filho
Av. Colombo 5790-cep:87020-900
Universidade Estadual de Maringa
Maringa, PR 87020-900 BRAZIL
Phone 21442634407
Email: psvfilho@uem.br

Rogerio Faria Vieira
Grain Legume Researcher
EPAMIG - Vila Gianetti 47
Vicosa, MG 36571-000 BRAZIL
Phone 55-31-3891-2646
Fax: 55-31-3894-5224
Email: rfvieira@epamig.br

Clibas Vieira
Dept. de Fitotecnia
Univ. Federal de Vicosa
Vicosa, MG 36571-000 BRAZIL
Phone 55-31-3899-1169
Email: cbvieira@ufv.br

Oswaldo Voysest Voysest
1225 Bushnell St
Beloit, WI 53511
Phone 608-313-8606
Email: ovoysestv@aol.com

Wageningen UR Bibliotheek
66775
Postbus 9100
6700 HA Wageningen
NETHERLANDS

Dan Wahlquist
Syngenta Seeds, Inc.
6338 HWY 20 - 26
Nampa, ID 83687
Phone 208-465-8510
Fax: 208-467-4559
Email: dan.wahlquist@syngenta.com

J. G. Waines
Botany and Plant Sciences
University of California
Riverside, CA 92521-0124
Phone 909-787-3706
Fax: 909-787-4437
Email: giles.waines@ucr.edu

Ralph Waniska
Crop & Soil Sciences
TAMU
2474 Heep Center
College Station, TX 77843-2474
Phone 979-845-2985
Fax: 979-845-0456
Email: waniska@tamu.edu

David Webster
Seminis Vegetable Seeds
21120 Highway 30
Filer, ID 83328-5508
Phone 208-326-6136
Fax: 208-326-4326
Email: David.Webster@seminis.com

Dr. Molly Welsh
Curator, Phaseolus Collection
WRPIS
59 Johnson Hall
Pullman, WA 99164-6402
Phone 509-335-3692
Fax: 509-335-6654
Email: mmwelsh@wsu.edu

Jeffrey White
ALARC, USDA-ARS
21881 North Cardon Lane
Maricopa, AZ 85239
Phone 520-316-6368
Fax: 520-316-6330
Email: jwhite@uswcl.ars.ag.gov

Irvin E. Widders
Bean/Cowpea CRSP
Michigan State University
321 Agriculture Hall
E. Lansing, MI 48824
Phone 517-355-4693
Fax: 517-432-1073
Email: widders@msu.edu

Dale Williams
ND Foundation Seedstocks
Dept. of Plant Sciences, NDSU
166F Loftsgard
Fargo, ND 58105-5051
Phone 701-231-8140
Fax: 701-231-8474
Email: Dale.Williams@ndsu.nodak.edu

Jim Wyatt
University of Tennessee
605 Airways Blvd.
Jackson, TN 38301-3200
Phone 901-424-1643
Fax: 901-425-4760
Email: jwyatt6@utk.edu

Dongfang Yang
Dept. of Life Science
Hebei Normal University of Sci. & Tech.
Qinhuangdao, Changli 066600 PR CHINA
Phone 86-335-203-9046
Fax: 86-335-202-4487
Email: Yang_dongfang@hotmail.com

Yellowstone Bean Company
Rt. 1 - Box 1198
East Bridger, MT 59014
Phone 406-662-3622
Fax: 406-662-3679
Email: ybco@brmt.net

Mildred Zapata
Dept. of Crop Protection
Univ. of Puerto Rico
PO Box 9030
Mayaguez, PR 00680
Phone 787-265-8484
Fax: 787-265-3857
Email: Plant_Zapata@hotmail.com

BEAN IMPROVEMENT COOPERATIVE

2005 FINANCIAL STATEMENT

Balance on hand		$8,101.00
INCOME		
2005 Dues	3,876.00	
2005 Dues CD	495.00	
Back Issues	123.00	
Meeting	6,781.00	
Bank Interest	90.00	
TOTAL INCOME	11,365.00	
EXPENSE		
Postage, Copy Charges and Office Supplies	1,882.00	
Printing Volume 48	1,375.00	
Graduate Awards	200.00	
Web Page Expenditure	100.00	
Meeting Supplies	75.00	
Bank Charge	5.00	
TOTAL EXPENSE	3,637.00	
Balance on Hand: December 31, 2004		$15,829.00