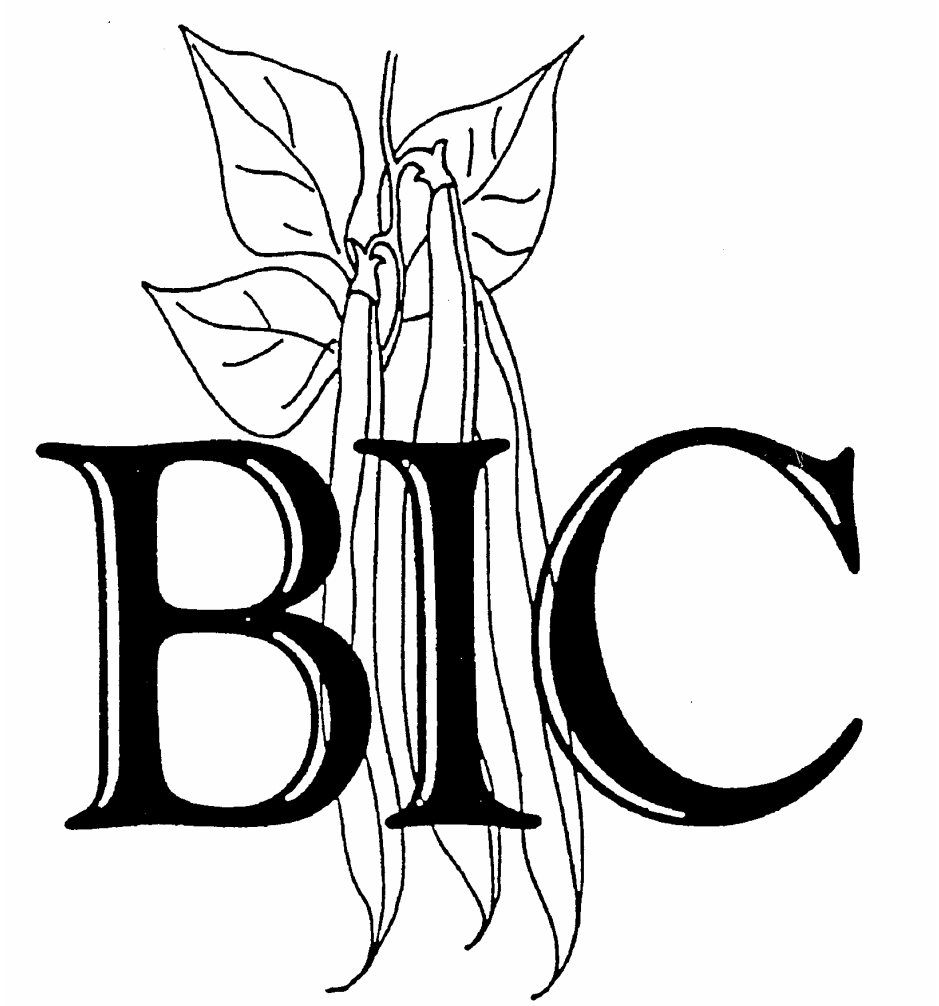
ANNUAL REPORT OF THE



BEAN IMPROVEMENT COOPERATIVE

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

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Report of The

BEAN IMPROVEMENT COOPERATIVE

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Coordinating Committee

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THE 45th ANNUAL REPORT OF THE BEAN IMPROVEMENT COOPERATIVE

The Bean Improvement Cooperative enjoyed a stimulating meeting at the 2001 Biennial Meeting in Fargo, North Dakota. The 2001 BIC meeting in Fargo had 98 registered participants. The National Dry Bean Symposium began the meeting with five talks, including the first **Frazier-Zaumeyer Distinguished Lecture**, presented by **Dr. Dermot P. Coyne**, University of Nebraska, Lincoln, NE, the first recipient of this award. The meeting had an additional 29 oral presentations and 31 poster presentations. The quality of both the oral and poster presentations was excellent.

The outstanding student oral presentation was entitled: '*Physiology of freezing resistance in the genus Phaseolus*', presented by P. Balasubramanian, University of Saskatchewan - Bert Vandenberg, advisor.

The outstanding poster presentation was entitled: '*Evaluation of seed-Zn concentration in navy bean*', presented by Shana Forster, North Dakota State University – Ken Grafton, advisor.

The meeting received excellent and generous support from the following organizations: Harris Moran Seed Company, National Dry Bean Council, Northarvest Bean Growers Association, North Dakota Dry Edible Seed Bean Growers Association, North Central Bean Dealers Association, Syngenta, Inc., Allen Canning Company, Basic American Foods, Inc., Klindworth Seed & Bean Company, Kirkeide's Northland Bean Company, North Dakota State University Departments of Plant Pathology and Plant Sciences, North Dakota Agricultural Experiment Station, North Dakota State University, Central Valley Bean Cooperative, CerexAgri, Pillsbury-Green Giant, Forest River Bean Company, and Manvel Bean Company. 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. Ken Grafton and I wish to thank the sponsors and the participants for making the meeting a success. Details of the next BIC meeting in California in 2003 are in this issue or can be found at the BIC Web page www.css.msu.edu/bic

The BIC mourns the passing of two friends and colleagues **Dr. Dermot P. Coyne** and **Dr. Donald H. Wallace**. Dermot served the bean community as geneticist, breeder, teacher and mentor for over 40 years. He was a frequent contributor to the BIC and served the BIC as President from 1967-1976, and as a member of coordinating committee, and awards committee for many years. The BIC recognized Dermot's achievements with the Meritorious Service Award in 1975 and as the first recipient of Frazier-Zaumeyer Distinguished Lectureship in 2001. Dermot's many achievements are described in the current issue of the BIC as Recipient of the Frazier-Zaumeyer Award and in Memorial by his many friends, colleagues and students. Dermot will be dearly missed and the BIC has lost an outstanding bean scientist and humanitarian.

Dr. Wallace was recognized nationally and internationally for his contributions to bean breeding and genetics research and teaching, for which he received many awards. He co-authored about 100 scientific articles, and served on over 30 graduate committees. A compilation of 45 years of research work was published in 1998 in the book he co-authored entitled "Plant Breeding and Whole-System Crop Physiology: Improving Crop Maturity, Adaptation, and Yield". Don is recognized as co-founder of the Bean/Cowpea CRSP and for his outstanding achievements in bean research, Don received the Meritorious Service Award from the BIC in 1982. He also served as a member of the BIC Coordinating Committee from 1979-1986. Don retired from Cornell University in 1992, but continued to work until a few weeks before his death.

Dr. James D. Kelly, BIC President

REPORT OF THE BIC GENETICS COMMITTEE

The Genetics Committee met in Fargo, ND on October 30, 2001 at 7 p.m. James Kelly presented data sent to him by a Brazilian bean research group (Alzate-Marin et al.) supporting a new anthracnose resistance gene locus, *Co-10*. Formal approval was given later to this new gene symbol by the reviewers of the Genetics Committee. Paul Gepts spoke about combating "biopiracy" with genetic "finger printing" procedures. There is a need for special funding to develop this capability for bean. Phil Miklas gave a status report on efforts to protect public germplasm form patents restricting future R & D. Phil Miklas and Phil McClean suggested writing a review article in Crop Science to establish the claim to conversion of all PI materials to the list of improvements used in the Nuna patent. There was further discussion of the challenge posed by the Enola patents. Existence of prior art was cited: James Kelly cited wild bean photos by Gentry (1969) of yellow beans and James Myers cited the presence of Mayocoba class bean materials in the PI collection. During 2001, the Genetics Committee also reviewed and gave mixed responses to the gene symbol *Znd* proposed by Singh and Westermann for the dominant gene controlling resistance to soil zinc deficiency in common bean.

BIC COMMITTEE MEMBERSHIP - 1957 to 2002

Coordinating Committee (approximate year of appointment):

1957 Dean, Enzie, **Frazier* (BIC Coordinator/President)**, McCabe, Zaumeyer
1960 Anderson, Atkin, Dean, Enzie, **Frazier**, McCabe, Zaumeyer
1962 Anderson, Atkin, Dean, **Frazier**, Pierce, Polzak, Zaumeyer
1968 Anderson, **Coyne**, Dean, Jorgensen, Polzak, Zaumeyer
1971 Briggs, **Coyne**, Dean, Jorgensen, Polzak, Zaumeyer
1972 Burke, **Coyne**, Dean, Jorgensen, Kiely, Polzak, Zaumeyer
1974 Ballantyne, Bravo, Burke, **Coyne**, Dickson, Emery, Evans, Kiely, Saettler, Zaumeyer
1977 Ballantyne, Bliss, Coyne, **Dickson**, Emery, Evans, Graham, Meiners, Morris, Saettler, Zaumeyer
1978 Atkin, Ballantyne, Bliss, Coyne, **Dickson**, Graham, Meiners, Morris, Saettler, Sprague
1979 Atkin, Bliss, **Dickson**, Graham, Hagedorn, Meiners, Morris, Sprague, Wallace
1980 Atkin, Bliss, **Dickson**, Hagedorn, Morris, Sprague, Steadman, Temple, Wallace
1982 Atkin, Coyne, **Dickson**, Hagedorn, Sprague, Steadman, Temple, Wallace, Wyatt
1983 Coyne, **Dickson**, Hagedorn, Saettler, Silbernagel, Steadman, Temple, Wallace, Wyatt
1985 Coyne, **Dickson**, Mok, Saettler, Silbernagel, Steadman, Temple, Wallace, Wyatt
1986 Coyne, **Dickson**, Mok, Saettler, Schoonhoven, Schwartz, Silbernagel, Steadman, Wallace
1988 Brick, Dickson, Emery, Magnuson, Roos, **Schwartz**, Singh, Steadman, Uebersax
1992 Dickson, Emery, Grafton, Magnuson, **Schwartz**, Singh, Stavely, Steadman, Uebersax
1994 Antonius, Dickson, Grafton, Magnuson, Park, **Schwartz**, Singh, Stavely, Uebersax
1996 Antonius, Grafton, Park, **Schwartz**, Singh, Stavely, Myers, Kotch, Miklas, Riley
1998 Antonius, Park, Schwartz (ex officio), Singh, Myers, Kotch, Miklas, Riley, Beaver, Vandenberg, **Kelly**
2000 Antonius, Beaver, **Kelly**, Kotch, Miklas, Myers, Park, Riley, Schwartz (ex officio), Singh, Vandenberg
2002 Antonius, Beaver, **Kelly**, Kotch, Miklas, Myers, Park, Riley, de Ron, Schwartz (ex officio), 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, Morris, 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, Stavely, Schwartz
2001 Hosfield, Magnuson, Schwartz

2001 BIC AWARD RECIPIENTS

2001 FRAZIER - ZAUMEYER DISTINGUISHED LECTURESHIP AWARD

DERMOT P. COYNE

Dr. Dermot P. Coyne was born on July 4, 1929 in Dublin, Ireland. He received B.S. at the University College Dublin, Ireland in 1953 and an M.S. at the same institution in 1954. He obtained a Ph.D. degree at Cornell University in 1958 and was awarded a D.Sc honorary degree from National University of Ireland in 1981. Dr. Coyne started his career as assistant manager in agricultural research at the Campbell Soup Co in England from 1958-1960 and joined the faculty at the University of Nebraska in 1961 where he currently is Professor of Horticulture. He served as acting chair in the Horticulture from 1974-1975 and in 1986 he was awarded the George Holmes Regents Professorship.

Dr. Coyne's accomplishments in bean breeding are numerous and well documented in his 160 journal articles, 8 book chapters, 350 abstracts/research notes and 75 other publications. His varieties and breeding lines with resistance to common bacterial blight, a serious limitation to bean production in the world, have been a major contribution to breeding programs throughout Africa and the Americas. His commitment to multiple disease resistance using both classical and molecular methods combined with a team breeding approach has resulted in advanced bean breeding lines and varieties that have benefited bean production throughout the Americas, especially when integrated with crop management practices developed in a USAID-CRSP project in the Dominican Republic in which Dermot has been the Pl. In fact, this Bean/Cowpea CRSP project has been cited a number of times for high impact throughout its 20-year existence. In addition to the successful dry bean cultivars such as pinto 'Chase' and great northern 'Weihing', released on the USA High Plains, his germplasm is found in all great northern and pinto lines bred by private industry and many public breeders.

In the course of developing multiple disease resistant bean lines and varieties he and his students developed the first RAPD molecular maps of quantitative trait loci for resistance to common blight, halo blight, web blight, white mold and rust resistance in common bean. Recently he showed the independence and epistasis of specific rust resistance and leaf pubescence in bean genotypes with adult plant rust resistance. These genes have been mapped in Dr. Coyne's lab and in fact this is the first mapping of an adult plant resistance locus in a legume. The value of multiple disease resistance has been demonstrated in many crops, including beans; however, private industry and developing countries often are unable to spend the time and resources to develop multiple disease resistance. Dr. Coyne's contribution to the use of beans as a vital source of protein in developing countries and to a strong export bean market in the USA will continue into the future as his lines are used by public and private breeders to develop new varieties.

Finally, Dr. Coyne has guided 42 graduate students, many of whom have gone onto successful careers and garner prestigious awards of their own. In the area of service he has been heavily involved in University committees, American Society for Horticultural Science (President, 1984-85), Sigma Xi and BIC where he was President/Editor from l967-1976 with 40 years of distinguished service to the bean community and numerous bean breeding and genetics accomplishments.

2001 DISTINGUISHED ACHIEVEMENT AWARD RECIPIENTS – M. BRICK, R. RILEY, J. C. ROSAS

MARK A. BRICK

Dr. Mark A. Brick was born October 6, 1947, in Green Bay, Wisconsin. He grew up on a dairy farm in eastern Wisconsin and, upon graduation from high school in 1965, operated the farm for two years. In 1967, Mark entered the U.S. Navy and served off the coast of Viet Nam in 1968. Upon completion of his military service, Mark attended the University of Wisconsin-River Falls, where he obtained the B.S. degree in Crop Science in 1972. Mark attended the University of Arizona (M.S. degree in Agronomy, 1975), then worked as a Research Station Manager for Cal/West Seed in Wisconsin for two years. Mark pursued the Ph.D. degree in alfalfa breeding and genetics at the University of Minnesota, which he obtained in 1980. He worked at Cal. State-Fresno during the 1980-81 academic years, where he taught numerous courses in plant science. Dr. Brick then accepted a position as manager of the Colorado Seed Growers Association, the official Seed Certification Agency, Colorado State University, from 1981 to 1986. In 1986, he became the leader of the dry edible bean breeding project at Colorado State University, a position he retains to this day.

Currently, his work involves teaching, research, and extension activities in the areas of plant breeding and dry bean production for the High Plains.

During his career, Dr. Brick has achieved national prominence in his work in bean cultivar development. His breeding program emphasizes the development of cultivars and germplasm suited for irrigated and non-irrigated U.S. production. He fosters a team approach with active participation from plant pathology, variety testing, agronomy, and seed production personnel and agencies throughout the state and region. Working primarily with the pinto market class, Dr. Brick has released four cultivars since 1995. These cultivars, Bill Z, Arapaho, Fisher, and Montrose have made a tremendous impact to the Colorado bean industry as well as other production regions. Virtually all of the non-irrigated, and more than 50% of the irrigated, bean production acreage in Colorado is planted to these cultivars. Many of these cultivars also are popular in other production regions. A new pinto cultivar, Grand Mesa**,** was released earlier in 2001. Grand Mesa is a high yielding cultivar with excellent seed quality traits, semi-erect growth habit, and rust resistance. In addition, Dr. Brick's program released Shiny Crow, the first shiny black cultivar released in the U.S., which may serve a niche in both foreign and domestic markets.

He has served as major advisor for 15 M.S. and 3 Ph.D. students, authored or coauthored 35 refereed and more than 100 non-refereed publications, and made more than 100 presentations to scientists, producers, and industry clientele in both the U.S. and other bean producing countries. He contributed greatly to the Extension Regional Bulletin 562A, 'Dry Bean Production and Pest Management, by serving as a technical editor and authoring or co-authoring three chapters. Dr. Brick also co-authored a chapter "Breeding Durango Race Beans" in S.P. Singh (ed.) Beans for the 21st Century.

Dr. Brick has been unselfish in providing service to the scientific community. He has chaired the W-1 50 regional research project, the W-6 technical committee, the Western Society of Crop Science, and was a member of the Phaseolus Crop Germplasm Committee. Dr. Brick also serves the Colorado producers as coordinator for research funding of the Colorado Dry Bean Administrative Committee and is on the Board of Directors of the Colorado Seed Growers Association. He was Chair of the committee to write the dry bean descriptors for PVP applications for dry and snap beans. Dr. Brick's love of bean breeding does not deter from his passion for teaching. He currently teaches Introductory Genetics, with a student enrollment of 150-170 every spring semester. In addition, Dr. Brick teaches Experimental Designs, Advanced Plant Breeding, Topics in Plant Breeding and Genetics, and Scientific Presentations. In honor of his excellent teaching skills, Dr. Brick was recently awarded the "NACTA Charles N. Shepardson Meritorious Teaching Award 2001, the highest College of Agriculture award for teaching at Colorado State.

RON RILEY

Ron Riley was born in Detroit, Michigan on November 1, 1952. He obtained the BS degree in Agronomy at Michigan State University. During his undergraduate training, Ron was fortunate to work as a student assistant to Dr. Wayne Adams. Working for Dr. Adams, Ron unknowingly began a career in bean breeding and genetics. He continued work with beans after graduation as a Research Associate at the San Juan Basin Research Center (SWCRC) at Yellow Jacket, Colorado. At the SWCRC, Ron worked with Mr. Adrian Fisher, the Superintendent of the center, and one of the most knowledgeable scientists about dryland bean production in the US. In 1985, Ron moved to Ft. Collins, CO to pursue a Master of Science degree under the supervision of Dr. Donald Wood and Mark Brick at Colorado State University. For his MS thesis project, Ron studied production of beans planted with mechanical mixtures of near isogenic lines that differed for plant architecture. During his MS program at CSU Ron developed an interest in plant cytogenetics fostered by Dr. Takumi Tsuchiya, a renowned cytogeneticist and University Distinguished Professor. This interest lead Ron to study for and complete the PhD degree in barley cytogenetics under the supervision of Dr. Tsuchiya.

In 1989, Ron returned to bean research when he accepted a position as garden bean breeder for Rogers Brothers Seed Company, now known as Syngenta in Nampa, ID. Dr. Riley has accomplished much in his short career with Rogers Brothers. Some of his accomplishments include: release of seven commercial garden bean cultivars including; three Romano, three fresh market and one Bush Blue Lake type, numerous research publications, and service to the bean community. Ron's aggressive role in the development of molecular marker facilitated selection in cooperation with prominent USDA, University, and Industry scientist throughout the US and at CIAT is unique among his peers. Ron has long been a proponent of supporting and funding public research projects having goals in common with his own program. An important PCR-based marker useful for indirect selection of the bgrn- I gene for resistance to bean golden mosaic virus was generated from collaboration between Ron and personnel at the USDA-ARS and the University of Puerto Rico. Ron has also collaborated with scientists at Cornell University, Michigan State University, Oregon State University, USDA-ARS, and others to advance marker assisted selection technologies and expanded our understanding of the genetics of resistance to anthracnose, bean golden mosaic virus, heat stress, rust, and white mold. Ron is currently

a member of the BIC Coordinating Committee and project leader for a worldwide commercial garden bean breeding program for Syngenta.

JUAN CARLOS ROSAS

Dr. Juan Carlos Rosas was born on January 12, 1945 in Unia, Peru. After obtaining his B.S. in Agronomy, National Agrarian University, Lima, Peril in 1969, Dr. Rosas began working with beans in 1975 when he joined the CIAT bean research program as a research assistant. During his stay at CIAT, Dr Rosas participated in research and co-authored several scientific articles that contributed to a better understanding of biological nitrogen fixation of beans. Interest in this area led him to the University of Wisconsin where he pursued graduate degrees 'in plant breeding and genetics under the supervision of Dr. Fred Bliss. Dr Rosas received both his M.S. and Ph.D. degrees in Plant Breeding and Genetics at the University of Wisconsin in 1983. Results from his M.S. and Ph.D. research provided insight into the importance of plant genotype and nitrogen fertilization on biological nitrogen fixation of beans. He also has authored and co-authored several scientific articles dealing with breeding strategies for improving the biological nitrogen fixation of beans.

Dr. Rosas has played an instrumental role in strengthening the bean research program at the Escuela Agricola Panamenicana (EAP) in Honduras and collaboration has been one of the hallmarks of this program. Dr. Rosas has served since 1988 as the Host Country Principal Investigator for a Bean/Cowpea CRSP project, which has developed, improved small red bean cultivars and other technologies that have benefited bean producers throughout Central America. The improved small red cultivars 'Don Victor' and 'Yeguare' were released in Honduras in 1993. The bean golden yellow mosaic virus (BGYMV) resistant and heat tolerant small red cultivar 'Tio Canela 75', formally released in 1996, is estimated to be grown by 30% of the bean producers in Honduras. This cultivar has also been formally released and is widely grown in Nicaragua and El Salvador. In 2000, the web blight and BGYMV resistant small red seeded bean cultivar 'Bribri' was released in Costa Rica. Dr. Rosas also participated in the release of the first small red germplasm that combines the recessive gene bgm-1 for BGYMV resistance with the recessive gene bc3 for bean common mosaic and bean common mosaic necrotic virus resistance. Dr. Rosas serves as the coordinator of regional performance trials for small red and black beans for PROFRIJOL. Dr. Rosas also maintains close collaboration with the CIAT bean research program in the evaluation of germplasm and breeding lines for disease resistance and tolerance to drought and low soil fertility. Dr. Rosas recently has taken the leadership in establishing a valuable link with groups interested in using participatory plant breeding techniques to improve beans. He is also involved in research and utilization of *Rhizobium* and micorrhiza inoculants.

Dr. Rosas has been very successful integrating bean research at the EAP with the primary mission of the institution, which is formal undergraduate training in agriculture. Several undergraduate students at the EAP have worked with the bean project to conduct their thesis research. Research conducted in laboratories, the greenhouse and the field are used to demonstrate the importance of beans to Central American agriculture. Dr. Rosas also has been very active in informal training of Central American and Caribbean agronomists and in sponsoring workshops for U.S., CIAT and PROFRUOL researchers dealing with important topics such as bean rust and participatory plant breeding methods.

2001 MERITORIOUS SERVICE AWARD RECIPIENTS – M. J. BASSETT & S. J. PARK

MARK JULIAN BASSETT

Dr. Mark J. Bassett was born in Washington, Indiana in 1940. He received a B.S. degree from Lake Forest College in 1963 and received his M.S. and Ph.D. degrees in Plant Breeding and Genetics from the University Maryland. Dr. Bassett has made multiple contributions in the area of seed coat genetics of the common bean. He conducted a thorough search of the literature, much of which was written in German, dealing with seed coat genetics. He was able to reconcile much of the earlier research of Prakken, Lamprecht and Kooiman in regards to seed coat color.

Dr. Bassett also has discovered numerous alleles for color or pattern of seed coats and flowers. One of his most important accomplishments has been the development of more than 75 genetic stocks with unique marker genotypes in backcross 1 to 3 into the 5-593 recurrent parent. He developed a protocol for determining seed coat color genotypes by evaluating F2 seed from test crosses with genetic tester stocks of known genotype. The availability of genetic tester stocks has permitted research dealing with flavonoids in seed coats. In collaboration with Dr. George Hosfield, the chemistry of seed coat colors was investigated for the Manteca class bean 'Prim' and the Dark Red Kidney class bean 'Montcalm'. Dr. Bassett developed or extended several linkage groups with mostly induced marker mutants and a few natural marker characters. In recent years, he has collaborated with Dr. Phil McClean in the development of RAPD and STS markers for most of the genes for seed coat pattern and color in common bean and mapping them to an RFLP map with the aid of the BAT 93 x Jalo mapping system of Paul Gepts. Marker genes for other genes of cornmon bean, *blu, arg, dgs* and y, were developed in collaboration with Dr. James Nienhuis.

Dr. Bassett has made several important contributions in the area cytogenetics of the common bean. He discovered that chromosome bridges formed in F 1 plants of P. *coccineus x P. vulgaris* crosses. He induced chromosome translocations by pollen irradiation that produced semisterility in heterozygotes. He also developed homozygous translocation lines, for which the intercrosses were analyzed cytologically. Dr. Bassett developed the five primary trisomics of common bean with distinctive plant phenotypes and developed the karyotype at diplotene for common bean chromosomes. He discovered a system of cytoplasmic male sterility with maintainer and restorer (partial) genes. He induced a male sterile (ms) mutant with full female fertility, and the *sbms* mutant for male sterility pleiotropic for a marker trait. He also developed an induced mutant do (dwarf outcrossing) for high outcrossing rates with full male and female fertility with *Fin.* Dr. Bassett supervised inheritance studies for the bean golden yellow mosaic (BGYM) resistance genes *bgm* and *bgm-2.* He has developed snap bean germplasm from an interspecific cross with superior BGYM resistance.

Dr Bassett is an active member of the *Phaseolus* genetics committee and has been responsible for publishing the list of genes in the Annual Report of the Bean Improvement Cooperative. He has made an extensive collection of illustrations of partly colored seed coat patterns and other types of seed coat patterns in electronic format.

SOON J. PARK

Dr. Soon J. Park, Research Scientist, Agriculture and Agri-Food Canada Greenhouse and Processing Crops Research Center, Harrow, Ontario, Canada was bom on January 22, 1937 and raised and educated in South Korea. He received his B. Sc. degree in agronomy (1960) and earned his M.S. degree from Seoul National University (1963). Then, Dr. Park began his research career as a rice breeder in South Korea in 1963. In 1968, he was a visiting scholar at the International Rice Research Institute (IRRI), Philippines. He received his M. Sc. degree from the University of Hawaii, Honolulu (1967) with scholarship support from the East-West Center. Dr. Park earned his doctoral degree from the North Dakota State University (Fargo) in 1973 followed by a post doctoral fellow (on haploid barley genetics and breeding) for two years at the University of Guelph (Ontario, Canada) before returning to IRRI in 1975 as Associate Rice Breeder. He was a Soybean Breeder with King Grain Ltd., Ontario, Canada from 1977 to 198 1. He has been breeding dry beans with Agriculture and Agri-Food Canada (Harrow, Ontario) since 1981.

Dr. Park developed non-nod, super-nod, and ineffective nodulation. mutants for biological nitrogen fixation genetics and breeding studies in common bean and compared their agronomic performance. This research lead collaboration with several other researchers in U. S. and Germany as well as in Canada. He has been actively breeding several market classes of dry bean cultivars for resistance to anthracnose, bean common mosaic (BCMV), common bacterial blight (CBB), root rots, and white mold, among other characteristics. Dr. Park has extensively used exotic germplasm. to broaden the genetic base of common bean cultivars for Canadian bean growing environments and this includes interspecific crosses with P. *coccineus* and *P. acutifolius* to introduce resistance to common bacterial blight, root rot and

white mold. In addition to releasing several gerinplasm. lines, Dr. Park has released 20 dry bean cultivars (in five market classes), two soybean cultivars, and one cultivar each of adzuki bean *(Vigna angularis)* and mung beans *(Vigna radiata).* He has written a book chapter and published 65 refereed and 50 non-refereed research articles.

Recently, Dr. Park's research interest is directed to application of molecular marker techniques to improve breeding efficiency of conventional bean breeding approaches. For example, his group has identified SCAR marker linked with CBB resistance and compared efficiency of marker-assisted (MAS) versus direct selection for disease resistance in common bean. Also, his group headed by Dr. K. Yu demonstrated abundant presence and usefulness of microsatelites or SSRs in common bean. As an initial application of MAS technique in bean breeding, Dr. Park pyrarmided resistant genes to BCMV, CBB, and anthracnose into navy and red kidney beans. Recently, his group has also undertaking a task to identify QTL markers for resistance to root rot and white mold. Dr. Park has been a member of the Bean Improvement Cooperative since 1982 and a member of its Coordinating Committee since 1994. He has been elected an honorary life member of Canadian Seed Growers' Association since 1998 for his active involvement in pedigree seed production system in Canada. Dr. Park has been freely exchanging germplasm with fellow researchers and is recognized nationally and internationally for his valuable contributions to bean science. He still has interest in rice and soybean, and other alternative pulses like pigeon peas *(Cajanus cajan)* though his research effort is totally devoted to dry bean breeding.

PRE-PUBLICATION OFFER / SPONSOR INVITATION FOR BIC MEMBERS

The Genus Phaseolus (Leguminosae in North America, Mexico, Central America and Panama)
By Drs. George F. Freytag and Daniel G. Debouck
BRIT/SIDA, approx. 480 pages, soft cover, 2002
Estimated Cost in the range of $ 50 – 60 + Postage/Handling

The Bean Improvement Cooperative is pleased to share this pre-publication notice about the landmark publication that our colleagues, George Freytag - retired and Daniel Debouck - CIAT, finished after their 15 year labor-of-love commitment to our international legume community. Join the BIC in congratulating and honoring these scientists for this great contribution to our science and future direction.

Their exhaustive publication has gone through its final reviews and is now in its final stages of revision and will soon be ready for prepress layout by the publisher, BRIT/SIDA (Botanical Research Institute at Texas – specializes in publishing botanical information). The authors and BRIT/SIDA (non-commercial publisher) would like to ask you to help offset up-front printing, storage and handling costs (estimated at $26,000) for this unique publication targeted to a specialized audience and libraries. Your assistance with generous contributions and pre-publication commitments will help finish this project and deliver the long-awaited publication.

Please join the Bean Improvement Cooperative and Colorado Bean Network who have already committed financial resources to help sponsor this worthy publication. If you have any questions, please feel free to call Jim Kelly (517-355-0205) or Howard Schwartz (970-491-6987).

SPONSOR INVITATION: **$ 1000 or more** (tax deductible)
Major sponsors (organization, company, individuals) will be recognized with their name (and logo if desired) opposite the title page, and will receive complimentary copies.

PRE-PUBLICATION OFFER: **$ 100 or more** (amount over cost is tax deductible)
Anyone taking advantage of this offer will also be recognized in the book with their name, and will receive a copy of the book.

Deadline for Action: July 1, 2002

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SECOND NOTICE:
4TH CANADIAN PULSE RESEARCH WORKSHOP
DELTA EDMONTON SOUTH HOTEL
EDMONTON, ALBERTA
DECEMBER 8, 9 & 10, 2002

WORKSHOP VENUE / HOTEL: A block of rooms has been booked and is being held at the **Delta Edmonton South Hotel**, at 4404 Gateway Blvd (the old 'Calgary Trail N'), under **Canadian Pulse Research Workshop**. **BOOK EARLY! Note that rooms are charged on a first-come-first-served basis, with the first 100 rooms: at $85 per regular room**; next 50 rooms: at $100; until Nov. 8; after that, standard hotel rates. These room rates apply for rooms booked to include up to three days before start and three days beyond workshop dates. Thus, those of you also attending the National Pulse Research Strategy Meeting on Dec. 11, qualify for the same rates for the extra night. Please make your own bookings by calling**: 1-800-268-1133.**

Topics for presentation may include the following categories: genetic improvement, agronomy / environmental sustainability, pest and disease management, quality and utilization, other.

Important highlights **of the attached registration and meeting information package are the following:**

Abstracts & Registration (with payment: late fees apply after this date):	due Wednesday, **Sept. 1.**
Manuscript for inclusion in 'Proceedings':	due **October 15.**
Power Point Presentations:	due for pre-loading **Dec. 2.**
Hotel reservations: Delta Edmonton South Hotel: 1-800-268-1133; local phone, 780-434-6415;	recommend by **October 1.**

On-site Registration / Reception: Sunday evening, Dec. 8
(Registration also during Breakfast Dec. 9 and Dec. 10)
Banquet, Monday evening, Dec. 9.

Hans-Henning Mündel
Agriculture & Agri-Food Canada, Lethbridge, AB
For details e-mail: muendel@em.agr.ca

2003 BIENNIAL BIC/NDBC MEETING

The 2003 BIC meeting will take place in Davis/Sacramento, California at dates to be determined towards the end of October and the beginning of November 2003. In addition to the North American Pulse Improvement Association (NAPIA) meeting, the National Dry Bean Council (NDBC) meeting, BIC, and related meetings, the program will also feature a tour of the Napa valley and of seed companies in the Davis area. Further information will be forthcoming through the Phaseolus listserver (PHASEOLUS@LISTSERV.UOGUELPH.CA), the 2003 BIC annual report, and individual mailings to the members. For further information, contact Paul Gepts at plgepts@ucdavis.edu.

IN MEMORY OF DERMOT P. COYNE

Dermot P. Coyne, 72, the George Holmes professor emeritus of horticulture at the University of Nebraska-Lincoln, died late Friday, 12 April 2002, after suffering complications from a steroid treatment of a non-viral form of hepatitis, family members said. Born in Dublin, Ireland, on July 4, 1929, Coyne arrived in the United States in 1954 at the age of 25 to embark on his life-long fascination with plant genetics, breeding and beans. Dermot had a distinguished and illustrious career as an educator, researcher, and benefactor of humankind.

Despite his retirement from UNL in June 2001, Coyne was working one-quarter time until he was hospitalized two weeks before his death. Dermot was the bean breeder at Nebraska for the last 30 years, and released many popular dry bean varieties in support of the Nebraska and High Plains Bean industry. His most recent releases included the pinto 'Chase' and great northern 'Weihing'.

Dr. Coyne achieved many professional and technical successes throughout his career, developing several varieties of pinto, pompadour and great northern beans that were resistant to bean common mosaic virus, rust, common bacterial blight and other bacterial diseases, with architectural escape from white mold. Those developments helped feed people in such countries as the Dominican Republic, which has since developed self-sufficiency in bean production thanks in part to Coyne's work. Moreover, his disease-resistant germplasm releases and varieties are parents of numerous bean varieties grown in hundreds of thousands of hectares in the Americas, Africa, Asia, and Europe.

His latest recognition by the international bean community occurred at the 2001 Bean Improvement Committee Annual Meeting in Fargo, North Dakota last November where he was the inaugural recipient of the Frazier – Zaumeyer Distinguished Lectureship. Dr. Coyne presented the keynote address entitled, "Breeding and Genetics of Great Northern and Pinto Dry Beans for Multiple Disease Resistance, Adaptation, Seed Quality, Yield, and Plant Architecture." The BIC community is very grateful that they had this opportunity to honor their dear friend who has imparted his experiences and wisdom over the past forty years.

"He was a very caring person," said his wife Ann. "His motivation for science was that he knew it would help people." Hundreds of colleagues nationwide elected Coyne the first Nebraskan to be president of the American Society for Horticultural Science in 1985.

Jim Steadman, 30-year long friend, professor and acting head of the Plant Pathology Department at UNL, said Coyne, while on his deathbed, was still worrying about getting a green card for a former graduate student and finding funding for bean projects. That was typical of Dermot," Steadman said. "He was so dedicated to people."

Ann Coyne said her husband's experience made him forever sensitive to the lives of foreign students. "He understood how confused they were--the culture shock," she said. In his spare time, Coyne loved to read such poets as W.B. Yates and tell Irish myths and legends to his children and grandchildren, but his wife said he was atypical as an Irishman.

"He wasn't a loud Irishman," she said. "He couldn't tell a joke if his life depended on it. He was into the cleverness of language...He had that dry wit."

Memorials may be sent to the University of Nebraska Foundation, Dermot P. Coyne Lectureship in Plant Breeding and Genetics, Suite 200, 1111 Lincoln Mall, Lincoln, NE 68508.

IN MEMORY OF DONALD H. WALLACE

Donald H. Wallace, 75, Emeritus Professor at Cornell University, who held joint appointments in the Departments of Plant Breeding and Vegetable Crops, passed away on the 19 April 2002. Widely recognized for his breeding program in beans and cabbage, as well as his writings on the physiology and genetics of yield, Dr. Wallace will be missed by his many colleagues, former students and friends around the world. Born in Driggs, Idaho on June 27, 1926, Don graduated from Utah State University and received his doctoral degree from Cornell University where he has been a faculty member since 1958. Don served the university well and was recognized nationally and internationally for his contributions in plant breeding research and teaching, for which he humbly received many awards. Don retired from Cornell in 1992, but continued to work until a few weeks before his death.

Dr. Wallace's expertise was in vegetable breeding and genetics. Much of his research involved fundamental studies on photoperiod responses and their interaction with temperature on adaptation and yield in beans. His work on growth systems analysis and the use of harvest index in selection for photosynthetic efficiency, resulted in the release of the first early-season light red kidney bean, 'Redkloud', followed more recently by 'Redkanner'. Previous variety releases included 'Redkote', the first halo blight resistant kidney bean. His work on temperature x photoperiod interactions on adaptation and yield in *Phaseolus vulgaris* is recognized internationally and is widely referenced in books and journal articles. Dr. Wallace co-authored about 100 scientific articles, and served on over 30 graduate committees. A compilation of 45 years of work was published in 1998 in the book he co-authored entitled "Plant Breeding and Whole-System Crop Physiology: Improving Crop Maturity, Adaptation, and Yield". Dr. Wallace served as a member of the BIC Coordinating Committee from 1979-1986, and he received the Meritorious Service Award from the BIC in recognition of his outstanding achievements to bean research in 1982.

Dr. Wallace was active in international agriculture and was one of the prime architects in creating the Bean/Cowpea Collaborative Research Support Program known as the CRSP. After receiving a Rockefeller Foundation Grant to collaborate with CIAT on bean research in 1977, Don and Wayne Adams were invited to prepare a proposal for a Bean/Cowpea CRSP planning grant. They were notified that beans and cowpeas had received high priority on BIFAD's list of strategic crops. In 1978, Don took a sabbatical leave from Cornell University to work with Wayne Adams at Michigan State University to begin the planning process that included fact-finding trips to countries in Latin America, the Caribbean and Africa, attendance at international meetings, and the posting of Requests for Proposals. In 1979, Pat Barnes-McConnell joined the planning team. Their joint efforts culminated in the presentation of a Bean/Cowpea Global Plan to Joint Research Committee in Washington in June 1980 and the approval and funding of the Bean/Cowpea CRSP in September 1980. The Bean/Cowpea CRSP community, in existence since 1980, is greatly indebted to Dr. Wallace for his vision, efforts in preparing the initial Global Plan, and for his major scientific contributions to the understanding of physiological-genetic control of yield in bean.

Memorial contributions for Dr. Wallace may be sent to the Perpetual Education Fund of the Church of Jesus Christ of Latter-day Saints, c/o Bishop Richard Park, 56 Mill Street, Ithaca, NY 14850. This fund provides supporting loans to needy individuals in less-developed countries for the purpose of higher education and technical training

MARKER-ASSISTED SELECTION FOR DISEASE RESISTANCE IN COMMON BEAN

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Marker-assisted selection can provide an effective and efficient breeding tool for detecting, tracking, retaining, combining, and pyramiding disease resistance genes (for reviews see Kelly and Miklas, 1998 and1999). For common bean, PCR-based RAPD and SCAR markers linked with more than 20 disease resistance genes have been obtained to date (Table 1).

Table 1. Resistance genes in common bean for which linked markers have been identified.

Gene	Disease	Gene	Disease	Gene	Disease
Co-1	Anthracnose	*I**	BCMV	*Ur-3*	Rust
Co-2	"	*bc-1²**	"	*Ur-4**	"
Co-4	"	*bc-3*	"	*Ur-5**	"
*Co-4²**	"	*Phg-2*	Angular leaf spot	*Ur-6*	"
Co-5	"	*Mp-1*	Macrophomina	*Ur-7*	"
Co-6	"	*Mp-2*	"	*Ur-9*	"
		*bgm-1**	BGYMV	*Ur-11*	"
				Ouro Negro	"

*genes for which MAS has been applied in our program.

Note: an in depth list of SCAR markers linked with resistance genes and QTL in common bean is available on the web at: http://www.usda.prosser.wsu.edu/Scartable3.htm

The utility of many of these linkages for marker-assisted selection of the resistance gene or QTL, however, has not been demonstrated outside of the original mapping population. There are various reasons why some of the linked markers may not be useful or have restricted utility, including: i) the linkage is not tight enough, or the linkage intensity may vary widely across different genetic backgrounds due to recombination suppression, ii) the gene is not expressed in certain genetic backgrounds (for example *bc-1²* is not expressed in a recessive *i*-gene background that lacks *bc-u*), iii) the marker is difficult to assay in certain genetic backgrounds or using different PCR protocols and equipment, which may even be true for certain SCAR markers, iv) the gene is easier to screen for using the pathogen, v) the gene may have nominal effect and not be worthwhile retaining in a breeding program, and vi) the resistance-linked markers are present in susceptible lines or susceptible-linked markers are present in resistant lines, which can occur in a gene-pool or race within gene-pool specific pattern. For instance, the A14 RAPD marker linked with *Ur-4* was found to be present in all Andean germplasm lines tested, whether they were resistant or susceptible (Miklas et al., 1993). Conversely, A14 marker was absent in all Middle American germplasm lines lacking the *Ur-4* gene; therefore, use of this RAPD marker for indirect selection is restricted to the Middle American gene pool.

Markers linked with quantitative trait loci conditioning resistance to ashy stem blight, bean golden yellow mosaic virus (BGYMV), common bacterial blight (CBB), and web blight was reviewed recently by Kelly and Miklas, (1999). Since then, additional QTL conditioning resistance to CBB (Tar'an et al., 2001), white mold (Miklas et al., 2001; Park et al., 2001; Kolkman and Kelly, 2001), fusarium root rot (Schneider et al., 2001; Chowdury et al., 2002), fusarium wilt (Fall et al., 2001), and halo blight (Ariyarathne et al., 1999) have been tagged. SCARs are available for MAS of four CBB, one white mold (Miklas et al, 2001), and one BGYMV QTL (Miklas et al., 2000). Unequivocal evidence for effective MAS of these QTL, however, has only been demonstrated for the CBB resistance QTL linked with the SU91, BC420, and SAP6 SCAR markers (Jung et al., 1999; Miklas et al., 1999 and 2000; Park et al., 1999; Yu et al., 2000; Fourie and Herselman, 2002; Mutlu et al., 2002). Some specific applications of MAS for disease resistance in bean are mentioned below. Bean rust is a hyper-variable pathogen that can rapidly overcome newly deployed resistance genes. PI 181996 was found to be resistant to 89 rust races. The resistance was conditioned by the *Ur-11* gene. This gene was quickly deployed into most common bean market types by Stavely et al. (1997). *Ur-11* is epistatic to less effective resistance genes like *Ur-4* and *Ur-5*. Linked markers are useful for retaining these defeated genes in the presence of a broadly effective gene like *Ur-11*. The A14 marker was

used to select those *Ur-11* lines which retained the *Ur-4* gene (Stavely et al., 1994). The *Ur-4* + *Ur-11* combination was later found to hold up against a newly identified race in Honduras, whereas *Ur-11* by itself was susceptible.

	Races (No.)	
Gene(s)	R	S
Ur-4	22	57
Ur-11	89	1
Ur-4 + *Ur-11*	90	0

Similarly, the hypostatic *I* gene is retained in the presence of the *bc-3* gene by MAS for the SW13 SCAR (Melloto et al., 1996; Miklas et al., 2002). This combination of a dominant and a recessive gene, likely possessing different resistance mechanisms, should provide more durable resistance to bean common mosaic virus.

Linked markers can also be used to quickly deploy a resistance gene into an adapted background. The R2 codominant RAPD marker identified by Urrea et al. (1996), and later converted to a SCAR by CIAT (S. Beebe), was used to backcross the *bgm-1* recessive resistance gene into snap bean (Stavely et al.,1997), with the pole bean cultivar Genuine a direct result of this effort. The marker is widely used by CIAT for MAS in early generations (F_1 gamete) because of the recessive inheritance of *bgm-1*, and because the disease can be difficult to screen for in field and greenhouse environments (S. Beebe, personal communication).

Backcross scheme for introgressing *bgm-1* into snap bean via MAS:

Generation	Cross
F_1	A 429 (*bgm-1*) x snap bean cultivar (*Bgm-1*)
BC_1	F_1 (*Bgm-1*//*bgm-1*) x snap bean cultivar
BC_2*	1/2 BC_1F_1 (*Bgm-1*//*bgm-1*) x snap bean cultivar
BC_3*	1/2 BC_2F_1 (*Bgm-1*//*bgm-1*) x snap bean cultivar
BC_3F_1*	1/2 BC_3F_1 (*Bgm-1*//*bgm-1*) is selfed
BC_3F_2	25% R (*bgm-1*//*bgm-1*) and 75% S (*Bgm-1*//*Bgm-1* or *bgm-1*) as expected

* denotes generation where MAS was used.

A similar MAS-backcrossing scheme was used to rapidly introgress the *Co-4*^{2} resistance gene into pinto bean to combat the emerging anthracnose disease problem in North Dakota (Miklas and Kelly, 2002). *Co-4*^{2} is the most effective anthracnose resistance gene characterized to date (Balardin and Kelly, 1998).

Use of linked markers for indirect selection of quantitative resistance traits, is more difficult because QTL generally have minor cumulative effects, and are greatly influenced by environment and genetic background. For these reasons most studies have focused on identifying markers linked with “major-effect” QTL because they offer the best opportunity for MAS. For example the SCAR markers linked with the major-effect QTL for CBB resistance have been observed to singly explain from 20 to 80% of the variability for disease resistance in segregating populations.

The utility of a SCAR marker for MAS of a major-effect QTL for BGYMV resistance was partially validated in a set of advanced lines with resistance derived from a similar source; however, direct use of SW13 for MAS of the resistance has not yet been demonstrated (Singh et al., 2000). The SCAR marker (*Phs*) linked with white mold resistance, detects a major-effect QTL that is expressed in both greenhouse (38%) and field (26%) environments, but successful MAS for the QTL has not yet been reported (Miklas et al., 2001). The RAPD marker linked with fusarium wilt resistance has not been converted to a SCAR yet, but the QTL should be amenable to MAS because it explains 63% of the variation for disease reaction in the original mapping population (Fall et al., 2001).

Once the limitations of a marker for the purpose of indirect selection have been determined, and its effective use for MAS outside the original mapping population validated, it can become an invaluable tool in disease resistance breeding as highlighted above. Integration of the resistance genes and QTL into the core map should be conducted, but if unsuccessful should not circumvent publication of important findings.

Future QTL mapping studies should attempt to use larger segregating populations to enable better resolution of minor-effect QTL and better characterization of gene clusters. Once an important QTL is found, the region should be saturated with markers using phenotype- and map-based bulked-segregant analysis in an effort to obtain tightly linked flanking markers. Further fine-mapping, using BACs for development of contigs, may eventually lead to identification and perhaps cloning of the gene responsible for the QTL.
As more resistance-linked markers are found, characterized and mapped, the power of MAS for developing more durable and multiple disease resistant cultivars will increase substantially in dry and snap bean breeding programs across the world.

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FOOD QUALITY IN DRY BEAN: MOVING FROM GENES TO PRODUCTS

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During the 20th Century consumers have been acutely interested in the chemistry of the foods they eat. Scientists, especially during the decade of the 1990's, have responded to increased consumer interest in natural chemicals called phytonutrients by examining their (phytonutrient) relationship to the health benefits that accrued from eating particular foods. The pigments that give rise to seed coat color in common bean (*Phaseolus vulgaris,* L.) all belong to a a group of phytochemicals known as flavonoids. Flavonoid pigments are a series of related water-soluble phenolic glycosides, that are formed as a result of the eight major genes that contribute to color inheritance in *P. vulgaris*: P, C, D, J, G, B, V, and Rk (Prakken 1970, 1972).

Polyphenolic compounds found in seed coats of colored seeded dry beans are known to adversely affect the nutrition of humans who eat this crop (Salunkhe et al., 1990). While there is copious literature regarding the antinutritional effects of seed coat phenolics in *P. vulgaris* (Aw and Swanson, 1995; Elias et al., 1979; Salunkhe et al., 1990) nothing has been reported on what, if any, beneficial effects are associated with these compounds. There is increasing evidence that flavonoids consumed in natural foods convey health benefits to humans by virtue of their antioxidant activity (Laughton et al., 1991; Hertog et al., 1993; Frankel et al., 1993). Dry beans are an integral part of diets in a significant portion of the world population, but the potential benefits of consuming beans from a nutriceutical standpoint have largely been overlooked. Flavonoids obtained commercially (Husain et al., 1987; Robak and Gryglewski, 1998; Sichel et al., 1991) and isolated from plant species (Gamez et al., 1998; Wang et al., 1999) are known to be effective free radical scavengers, i.e., show antioxidant activity. Recently, condensed and hyrolyzable tannins of high molecular weight also have been shown to be effective antioxidants with even greater activity than simple phenolics, e.g., flavonoid monomers (Hagerman et al., 1998).

Recently Beninger (unpublished data, 2000) in a liposome antioxidant assay (Wang et al., 1999; Arora and Strasburg, 1997) tested kaempferol glucoside and kaempferol-glucoxyloside, quercetins, anthocyanins, and condensed tannins extracted from seed coats of *P. vulgaris*. One or more of these compounds were contituents in the seed coats of only colored beans. The anthocyanins, (found only in beans with black seed coats)delphinidin 3-*O*-glucoside, petunidin 3-*O*-glucoside and the flavonol quercetin, 3-*O*-glucoside were the most active of the pure compounds tested (Beninger, unpublished data, 2000). Although the antioxidant activity of these compounds was significantly less than butylated hydroxy toluene (BHT), they still inhibited lipid destruction by over 50% relative to the iron control. The third anthocyanin, malvidin 3-*O*-glucoside was also active but had significantly less activity than BHT, delphinidin 3-*O*-glucoside, petunidin 3-*O*-glucoside, and quercetin 3-*O*-glucoside. Kaempferol 3-*O*-glucoside had the least amount of antioxidant activity of the pure compounds tested. This flavonol inhibited liposome breakdown by less than 20% relative to the control; its activity was not significantly different from the control. The tannin extracts were generally found to be as active, or slightly more active, than the pure flavonoid compounds (Beninger, unpublished data, 2000).

Recent work has shown that the most important structural feature of flavonoids for antioxidant activity is the B-ring ortho 3' 4' dihydroxy orientation (Cao et al., 1997; Dziedzic and Hudson, 1983, 1984; Husain et al., 1987) Letan, 1966; Sichel et al., 1991). The most active flavonoids studied by Beninger (unpublished data, 2000) were delphinidin 3-*O*-glucoside and quercetin 3-*O*-glucoside, which have OH groups at 3' and 4', and petunidin 3-*O*-glucoside, which has a dihydroxy group at 4' and 5'. However, malvidin 3-*O*-glucoside with both the 3' and 5' hydroxy groups methylated had significantly lower activity than the above compounds, all of which have an ortho dihydroxy substitution on the B-ring. Wang et al. (1999) tested three anthocyanins found in tart cherries, all of which had a B-ring 3', 4' substitution, and these were all found to have good antioxidant activity. Finally, kaempferol 3-*O*-glucoside, which only has a single B-ring 4' hydroxyl had no significant activity compared to the iron control. This finding is consistent with the results of the antioxidant assay of the methanol extract from 'Prim', in which no significant activity was observed. 'Prim' has no tannins, and only two kaempferol compounds (a kaempferol monoglucoside and a kaempferol diglycoside) are present in the seed coat (Beninger et al., 1998).

The J locus in bean apparently controls whether or not proanthocyanidins (condensed tannins) are produced in the seed coat. Feenstra (1960) inferred that dominant J in *P. vulgaris* probably promotes the production of proanthocyanidins in seed coats. Experimental evidence supporting Feentra's (1960) hypothesis was provided by Beninger and Hosfield (1999) and Beninger, et al. (2000) who found that only genotypes with a dominant allele at the J locus had proanthocyanidins. The J locus also has adverse effects on seed storage and food quality which makes the gene much more interesting to study than its effects on seed coat color *per se.* Feenstra (1960) found that J genotypes accumulate phlobaphenes (proanthocyanidin condensation products). The J genotypes also darken in color upon storage (aging) and become hard to cook and increasingly indigestible. All of these effects are attributable to progressive chemical changes occurring in the seed coat.

From the foregoing, there is good evidence to state that many seed coat flavonoids impart positive health benefits as antioxidants in the blood of humans and many animals (Hertog et al., 1993). Antioxidants can render cancer causing substances in the body ineffective. On the other hand, some flavonoids may cause beans to darken in color upon aging and become hard to cook and difficult to digest. Pharmaceutical companies may be interested in particular bean flavonoids because these chemicals could be extracted and marketed as nutritionally important food supplements. Resolution of the function of the genes responsible for flavonoids and tannin formation, along with the antioxidant activity of these compounds may enable breeders to select for varieties that have a range of antioxidant activities and also, perhaps, balance antioxidant activity with antinutritional effects.

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PHYSIOLOGY OF FREEZING RESISTANCE IN THE GENUS *Phaseolus*

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Introduction

Late spring and early fall frosts are a major constraint to dry bean (*Phaseolus vulgaris* L.) production on the northern prairies. Common bean is sensitive to light frost (Levitt, 1980) and is killed at the moment of ice formation (-2°C). A late spring frost results in seedling death while early fall frost results in poor seed yield and quality. Due to the risk of late spring frost, dry bean is seeded usually in late May. Delayed seeding however, increases the risk of late season drought stress.

Buhrow (1980, 1983) reported frost resistance of tertiary gene pool of common bean in the field. Balasubramanian et al. (2000) reported that both tolerance and avoidance mechanisms of freezing resistance are observed in the tertiary gene pool, although avoidance is the primary mechanism of frost survival. Ice nucleators are ubiquitous, and reliability of supercooling as a viable frost survival mechanism in common bean is not known. Acclimation of plants to low non-freezing temperatures enhances subsequent freezing tolerance. Field screening of frost resistance of *Phaseolus* species will enable breeders to decide on appropriate parents and breeding strategies to introgress this trait into bean cultivars. The ***objectives*** of this study were to investigate i) the ability of common bean and four species in its tertiary gene pool to acclimate as a means of increasing freezing tolerance, and ii) freezing resistance of *Phaseolus* species in the field.

Materials and Methods

CDC Nighthawk, two primary gene pool species *P. vulgaris* var. *mexicanus* (G11031, 2270 m – Sierra Madre) and *P. vulgaris* var. aborigineus (G23457, 2940 m; G23559, 2900 m; or G23454D, 2460 m – Andes), and four tertiary gene pool species *P. filiformis* (unknown), *P. angustissimus* (PI535272), *P. ritensis* (PI494138) and *P. acutifolius* var. *tenuifolius* (PI535248) were included. Seeds were from a uniform environment. With the exception of CDC Nighthawk, all other seeds were scarified by nicking the seed coat.

1. Acclimation and Freezing Tolerance. *Acclimation in the Natural Environment:* Accessions were grown in 4" pots filled with Rediearth at 23/18°C (8 h/16 h) with a 16 h photoperiod in growth chamber. The low temperature (18°C) overlapped both light and dark photoperiods. At 21 days after seeding, plants were in the V3 growth stage and were moved outdoors (April 28, 2000) for 14 days. Air temperature at plant height was monitored. Freezing dates were selected at 7 days intervals: 28 April (Control), 5 May and 12 May. Control plants were not moved outdoors. Freezing tolerance of plants was evaluated as follows: Four plants per accession were randomized in plastic trays. Four such trays were prepared and placed in the dark in a controlled environment chamber set at 0°C. Plants were sprayed with water kept at the chamber temperature. Air temperature was decreased to -2°C and subsequently decreased at a linear rate of 1°C h^{-1} up to -5°C. Plants were removed at hourly intervals and placed in a chamber at 4°C. After 12 h at 4°C, the $LT_{50}K$ (lethal temperature at which 50% of the population is killed) was determined as the first sub-zero temperature at which two seedlings froze. Plants were then moved to a chamber at 23/18°C for 14 days. The $LT_{50}G$ (lethal temperature at which 50% of the population re-grew) was determined as the lowest sub-zero temperature at which two seedlings showed re-growth. Plants that escaped freezing injury were not considered for $LT_{50}K$ and $LT_{50}G$ determination.

Acclimation in the Controlled Environment Chamber: Accessions/cultivars were seeded on July 5, 2000 and grown outdoors for 26 days. Accessions were in the V3 or V4 growth stage and were moved to a chamber maintained at 7/5°C (PPFD ≅ 50 μmol m^{-2} s^{-1}). The chamber was maintained at 7/5°C for 3 days and then decreased to 5/2°C and 2/0°C at three days interval. At the end of each temperature regime, freezing tolerance of plants was evaluated as described above. Control plants were subjected to 7/5°C.

Data were subjected to the analysis of variance appropriate to a split plot design with two replicates. Freezing dates were main plots and accessions/cultivar were sub plots, and both were considered as fixed effects.

2. Freezing Resistance in the Field. All accession/cultivar were seeded into Jiffy peat pellets and grown at 23/18°C (8 h/16 h) with a 16 h photoperiod for 15 days. One hundred seedlings per accession per replication were hand-transplanted in the field on 10th or 29th Aug., 2000. Two replicates per accession per transplanting date were used. Transplants were watered every day until establishment. Seedlings that survived transplant shock were counted. At the incidence of the first fall frost on Sept. 23, seedlings were in the V4 or R5 growth stage for those transplanted on 10 Aug., and were at the V3 growth stage for those transplanted on 29 Aug. Air temperature at plant height was monitored. Percentage survival on the 2nd, 7th and 14th day after the first fall frost was determined. Data were subjected to chi-square tests of independence of proportions to determine if the percentage survival/re-growth of seedlings is independent i) of its growth stage and ii) of the species.

Results and Discussion

1. Acclimation and Freezing Tolerance. Freezing dates were not significantly different for $LT_{50}K$ and $LT_{50}D$ when plants were acclimated under natural or controlled environment conditions (data not presented), indicating no significant increase in freezing tolerance (little or no acclimation). Seven days after plants were transferred outside (May 5), the $LT_{50}K$ mean decreased from -1.9°C to -3.1°C. The decrease was not significant, probably due to fewer degrees of freedom for replication x freezing date interaction (d.f. = 2), which is the appropriate error to test the significance of freezing date. In the case of

acclimation in controlled environment conditions, although the maximum and minimum air temperatures were controlled efficiently, no increase in freezing tolerance was observed across freezing dates for $LT_{50}K$ and $LT_{50}D$ (data not presented).

Phaseolus species differed significantly for $LT_{50}K$ and $LT_{50}D$ under both natural and controlled environment conditions. Three tertiary gene pool species *P. filiformis*, *P. angustissimus* and *P. ritensis*, in general were more freezing tolerant than the three primary gene pool species *P. vulgaris*, *P. vulgaris* var. *mexicanus* and *P. vulgaris* var. *aborigineus*. *P. acutifolius* var. *tenuifolius* was either intermediate in response between the primary and tertiary gene pool or responded similarly to that of the primary gene pool.

When above ground shoot froze to death, growth from the cotyledonary nodes was observed for some seedlings in *P. angustissimus* and *P. ritensis*. Both have hypogeal germination. In *P. filiformis*, cotyledonary nodes are positioned at the soil surface and re-growth was observed in few seedlings.

When plants in 4" pots were subjected to a freeze test and observed under infrared thermography, the Rediearth froze at a chamber temperature of -4°C. It is quite possible that seedling deaths in species with a lower $LT_{50}K$, and lack of re-growth from cotyledonary nodes in species with hypogeal germination were partly influenced by an earlier freezing of the Rediearth which in turn initiated nucleation of the seedlings. This however is uncommon in the field due to higher buffering capacity of soil.

2. Freezing Resistance in the Field. The first fall frost in Saskatoon in 2000 was on Sept. 23, and a subsequent frost occurred on Sept. 24, when the air temperatures at seedling height were -4.2°C and -4.9°C, respectively. Air temperature remained below -2°C for 3 h on Sept. 23 and for 5.5 h on Sept. 24.

Primary gene pool species *Phaseolus vulgaris* cv. CDC Nighthawk, *P. vulgaris* var. *mexicanus* and *P. vulgaris* var. *aborigineus* were killed by the first fall frost regardless of growth stage of the seedling. These species showed no signs of recovery or re-growth for up to 7 days after the first fall frost, although frost did not occur during the above period, and air temperatures > 20°C were recorded during the same period. This indicates the extreme susceptibility of the cultivated common bean and its primary gene pool to frost. The chi-square tests of independence of proportions indicated that among the tertiary gene pool species, with the exception of *P. filiformis* and *P. acutifolius* var. *tenuifolius*, seedling survival (on the 2nd day after frost) and/or re-growth (on the 7th day after frost) in *P. angustissimus* and *P. ritensis* were dependent on the growth stage of the species. In both *P. angustissimus* and *P. ritensis*, younger seedlings (second transplanting date) survived frost better than the relatively older seedlings (first transplanting date).

Chi-square tests of independence of proportions indicated that plant survival and re-growth on the 2nd and 7th day after the first fall frost were dependent on the *Phaseolus* species. On the 2nd day after the first fall frost, *P. angustissimus* had the highest percentage survival (74%) followed by *P. filiformis* (8%). Absence of frost between Sept. 24 and Oct. 2 coupled with air temperatures > 20°C during the same period enabled surviving plants to re-grow. In *P. angustissimus*, increased number of surviving seedlings (76%) on the 7th day compared to the 2nd day after the first fall frost was due to growth of axillary shoots in seedlings with damaged terminal buds or due to growth from seedlings in which the stem survived the frost. In *P. filiformis*, seedlings either died to the ground and then produced new shoots from the cotyledonary nodes or survived the frost intact and had 16% seedling survival on the 7th day after the first fall frost. Starting Oct. 3, air temperature dropped to -10.2°C, resulting in seedling death.

Conclusions

Little or no acclimation was observed in *Phaseolus* species in response to low temperatures. Freezing of Rediearth soilless mix ahead of seedlings may have prevented us from studying the effect of acclimation, particularly in the tertiary gene pool. Freezing resistance of *P. angustissimus* in the field is promising. Introgression of freezing resistance into common bean genotypes may enable early to mid May seeding of dry bean on the Canadian prairies. This could further expand the geographic distribution of bean crop, possibly to higher altitudes in the tropics.

Acknowledgement

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IS MECHANICAL DAMAGE OF DRY BEAN SEED GENETICALLY CONTROLLED?

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Introduction

Combine harvest and subsequent handling of dry beans cause some mechanical damage (MD) of beans. The damage has dual effects; 1) reduced market value due to split, cracked and loosened seed coats, which makes the damaged beans unacceptable to canners and 2) abnormal seedlings and reduced seedling vigour. Several factors are reported to affect the mechanical damage. Seed moisture and MD had an inverse relationship, particularly when moisture was less than 12% (Bay & Taylor, 1993). This study aims to develop a device to simulate mechanical damage (MD), to determine genetic control of MD and to select dry bean cultivar tolerant to mechanical damage.

Materials and Methods

Development of simulation devices: A drop test at a certain height (i.e. 2.6 m) and a constant moisture level (i.e. 12%) showed a good correlation with the actual MD caused by direct combining at 18% moisture level of beans. However, the drop test is time consuming slow and a tedious process. Also it may vary by how seeds are dropped. Therefore, more reliable, time saving and repeatable mechanical device with electric motor control is needed, such as pedal machine

Two recombinant inbred populations (RIP): Two crosses between white bean lines susceptible and tolerant to MD; Cross 1, Envoy/OAC Laser and Cross 2, OAC Speedvale/Vista were advanced to F_4. The F_4 plants of the two RIP were grown in plant rows at Harrow in summer of 2000. Plant type and maturity were observed and at maturity plants were pulled manually at fairly high seed moisture level (mostly close to 18%) to avoid any seed coat damage. Then, they were shelled with an Almaco thresher at 350 rpm with wide open concave to minimize seed coat damage.

Induced mechanical damage: Moisture of seed samples was adjusted to 13% and two sub-samples of 100 seed per line were prepared for MD simulation, using pedal machine set at an impact speed of 8.5 mm/sec (or 395 rpm). The cracked samples were soaked in tap water for 30-40 seconds and scored visually for damage as 0 for whole bean (no damage), 1 for hair line crack, 2 for clearly visible crack, 3 for large crack and 4 for split beans. Then, mechanical damage index (MDI) were estimated as Sum[MD score x # of seed in each score]/total # of seed x 100. Correlation between MDI and growth type and maturity of the lines was tested.

Results and Discussion

Testing device: Initially a drop test device (adapted from Dickson and Boettger, 1977) was tried to simulate MD but it was a time consuming technique. Therefore, a motorized pedal machine, was developed with two pedals to hit seed at controlled speed and seed samples were fed one by one by a magnetic feeder. The pedal machine was constructed by Agriculex Machine Shop in Guelph, Ontario and tested for its suitability with bean samples taken manually from 1999 Ontario cooperative navy bean cultivar trial grown at St. Thomas. The results were very similar to those obtained by the direct combine study in ranking of the navy bean cultivars (Gillard and Park, BIC report, 2002). This was demonstrated with the parental lines subjected to the pedal machine at a range of moisture and separation of the lines at 13% moisture level (Table 1).

Frequency distribution of MDI: Frequency distribution of MDI of cross 1, Envoy/OAC Laser is presented in Fig 1. MDI of both parental lines were 62 for the tolerant cv Envoy and 91 for susceptible cv OAC Laser. The distribution of the RIL of the cross 1 (n=131) was continuous with a slightly skewing toward the tolerant side, with a mean MDI of 75 and an MDI range of 25 to 181. The distribution of MDI of cross 2 (n=127), OAC Speedvale/Vista was very similar to that of the Cross 1. MDI of the two parental lines were 25 for tolerant OAC Speedvale and 55 for susceptible cv Vista.. Average MDI of the cross 2 was 61 with a range

of 22 to 142. The results suggested MD was under a quantitative genetic control with multiple minor genes. With the wide variation, selection for MD tolerant lines should be possible from the crosses.

Correlation between MDI and plant characteristics: Of the RI populations, MD tolerant parental lines were early maturing and short determinate growth (type I), while the two susceptible parental lines were late maturing and indeterminate growth (type II), and the characteristics were segregating in the populations. Correlation coefficients between MDI and growth type were not significant. However, MDI and maturity had significant negative correlations with r = -0.364 and r=-0.382 for cross 1 and 2, respectively. These suggested that late maturing lines tend to be more tolerant to MD than those with early maturity in contrast to the parental lines. However, this association needs to be further verified in future studies.

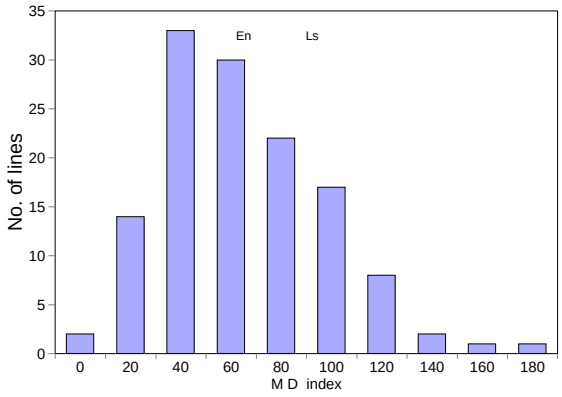
Summary: Both simulation devices, drop tester and pedal machine, induced MD of beans similar to the field combine trials. Seed moisture of 13% was appropriate to separate different degree of MD and accurate maintenance of the accurate moisture in sample was very critical. Both RIP showed continuous variation of MD and suggesting quantitative genetic control of MD, and correlation between MDI and maturity was detected. Both crosses will be re-tested at 2 locations and may be used to determine QTL markers for MD.

Table 1. Average MD* of 5 navy bean cultivars at 5 moisture levels and their response at 13% moisture level.

%Moist	%MD	Cultivar	%MD
12	45.0	OAC Speedvale	6.6
13	20.3	Envoy	8.0
14	10.7	OAC Gryphon	18.3
15	3.0	Vista	30.6
16	2.9	OAC Laser	37.6

* Mechanical damage (MD) was scored as visually as 1 (no damage), 2 (hair line crack), 3 (visible minor crack), 4 (large crack) and 5 (split bean).

Fig. 1. Frequency distribution of mechanical damage index (MDI) of cross 1 (Envoy/OAC Laser) F4 lines (n=131) at Harrow in 2000.



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PINTO BEAN STORAGE TO MAINTAIN QUALITY

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The beans were obtained from a farmer at about 20% moisture and dried to the desired moisture contents of 14%, 16% and 18% wet basis The beans were stored in covered 19 L (5 gallon) pails for 10 months at -7°C (20°F), 4°C (40°F), 16°C (60° F) and 27°C (80°F).

The change in color quality was measured with a Hunter colorimeter. The Hunter-L values for the beans stored at the specified temperatures and moisture contents are shown in Table 1. There was a dramatic decrease in the lightness or whiteness of the beans stored at the warmer treatments. The beans stored at -7°C (20°F) and at 4°C (40°F) had very little if any change in lightness for all three moisture contents. The beans stored at 27°C (80°F) and 18% moisture had considerable mold growth on the beans at the end of the storage period.

The Hunter-a values, redness, for the beans stored at the specified temperatures and moisture contents are shown in Table 2. The beans stored at -7°C (20°F) and at 4°C (40°F) had very little if any change in redness for all three moisture contents. Beans stored at the warmer temperatures increased in redness.

The pinto beans were checked for the hard-to-cook characteristic after ten months of storage, Table 3. Shorter cooking times are desired. The average cooking time prior to storage was 18.4 minutes. There was a dramatic difference in cooking times for the various treatments. After 10 months of storage cooking times of 16% and 18% moisture beans stored at –7 C (20 F) were only 1.2 times longer than before storage and at 40 F were only 1.7 times longer. The beans stored at 16 C (60 F) had cooking times about twice as long as before storage. The beans that were stored at 26.7°C (80°F) had cooking times that were 3.6 to 9.2 times longer than prior to storage.

Beans at 16% moisture content, in a box maintained at 26.7°C (80°F), were exposed to a grow light bulb representing the light spectrum of the sun to determine the affect of light on bean color. The beans darkened dramatically within weeks. The Hunter L-value decreased from 52.4 to 44.9 during three months of storage. The color darkened more quickly at the beginning of the storage period, with 77% of the darkening occurring during the first 5 weeks. The Hunter-a values increased from 5.2 to 8.1 indicating an increase in the redness of the beans, which is also deterioration in color quality. The Hunter-b value decreased from 12.5 to 10.6 indicating a decrease in the yellowness of the beans.

The measurement of the resistance to airflow of pinto and navy beans found the resistance to be similar to that of soybeans.

Acknowledgement

This project was supported by the North Harvest Bean Growers Association, Frazee, MN. The colorimeter analysis and pin cooking tests were conducted in the laboratory of K. C. Chang. Travis Lee, Matthew Loecken and Vijayakumar Shanmugasundaram completed the laboratory tests.

Table 1. Hunter-L values (whiteness) for specified storage conditions of temperature and moisture content during the second year.

Date	Time	-7°C (20°F)			4°C (40°F)			16°C (60°F)			27°C (80°F)		
	Weeks	14%	16%	18%	14%	16%	18%	14%	16%	18%	14%	16%	18%
10/11/00	0	52.5	52.4	51.6	52.5	52.4	51.6	52.5	52.4	51.6	52.5	52.4	51.6
11/1/00	3	52.7	51.7	50.8	51.9	51.2	50.7	52.2	51.2	50.4	51.5	50.3	49.4
11/21/00	6	51.0	51.1	50.9	51.1	50.5	49.5	50.5	50.1	49.7	49.6	48.4	47.1
12/11/00	9	52.0	51.3	50.8	51.4	51.4	51.2	51.3	50.5	50.0	49.3	48.0	46.6
1/2/01	12	51.2	50.7	50.9	50.9	51.3	50.0	50.5	49.9	50.1	49.1	48.0	45.8
1/23/01	15	50.5	51.6	51.1	50.6	51.2	50.6	51.0	50.4	48.9	48.6	46.9	44.2
2/20/01	19	51.9	52.3	50.8	51.7	51.9	50.9	50.0	49.7	49.4	47.7	46.0	44.3
3/20/01	23	52.1	52.0	51.5	51.5	51.6	51.2	50.3	49.4	48.9	46.8	45.0	43.6
4/24/01	28	51.5	51.5	51.6	51.7	51.6	51.3	49.9	49.2	48.5	45.9	44.3	42.3
5/23/01	32	51.9	51.7	52.1	51.4	51.4	51.2	49.5	49.2	48.6	45.5	43.6	42.3
6/22/01	36	52.0	51.5	51.9	51.4	51.6	51.7	49.5	49.0	48.0	45.3	43.1	42.3
7/31/01	41	50.9	51.6	51.1	51.2	51.0	50.4	48.6	47.5	46.7	43.6	42.1	40.8
Change		-1.6	-0.8	-0.5	-1.3	-1.4	-1.5	-3.9	-4.9	-4.9	-8.9	-10.3	-10.8

Table 2. Hunter-a values (redness) for specified storage conditions of temperature and moisture content during the second storage year.

Date	Time	-7°C (20°F)			4°C (40°F)			16°C (60°F)			27°C (80°F)		
	Weeks	14%	16%	18%	14%	16%	18%	14%	16%	18%	14%	16%	18%
10/11/00	0	5.0	5.2	5.2	5.0	5.2	5.2	5.0	5.2	5.2	5.0	5.2	5.2
11/1/00	3	5.1	5.3	5.3	5.2	5.4	5.3	5.2	5.5	5.3	5.4	5.6	5.9
11/21/00	6	5.2	5.3	5.5	5.2	5.3	5.4	5.6	5.5	5.6	5.9	6.3	6.3
12/11/00	9	5.1	5.3	5.2	5.3	5.4	5.6	5.6	5.8	6.0	6.3	6.7	7.1
1/2/01	12	5.4	5.0	5.0	5.0	5.2	5.2	5.6	5.6	5.9	6.6	6.9	7.7
1/23/01	15	5.3	5.2	5.2	5.4	5.4	5.5	5.8	5.9	6.0	6.9	7.5	8.2
2/20/01	19	5.4	5.3	5.3	5.5	5.6	5.5	6.0	6.3	6.4	7.6	8.4	8.9
3/20/01	23	5.5	5.4	5.3	5.5	5.5	5.5	6.2	6.4	6.5	8.0	8.6	9.2
4/24/01	28	5.4	5.3	5.4	5.6	5.5	5.6	6.4	6.5	6.8	8.4	9.1	9.6
5/23/01	32	5.3	5.4	5.2	5.6	5.5	5.7	6.5	6.6	6.9	8.6	9.2	9.7
6/22/01	36	5.2	5.3	5.2	5.5	5.5	5.7	6.4	6.7	7.0	8.8	9.5	9.8
7/31/01	41	5.1	5.3	5.4	5.5	5.7	5.9	6.6	6.9	7.1	8.9	9.9	9.6
Change		0.1	0.1	0.2	0.5	0.4	0.7	1.6	1.7	1.9	3.9	4.7	4.4

Table 3. Median pin cooking times for beans stored for 10 months at specified moisture contents and temperatures. Median cooking time before storage was 18.4 minutes.

Storage Temperature	Bean Moisture Content		
	14%	16%	18%
	Pin Cooking Time (minutes)		
20° F -7° C	29.6	22.6	22.8
40° F 4° C	36.0	29.7	30.6
60° F 16° C	36.0	32.7	38.0
80° F 27° C	66.2	93.0	168.5

THE ROLE OF THE EPICUTICULAR WAX LAYER IN WATER MOVEMENT ACROSS THE BEAN SEED COAT

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Water uptake influences a seed's hydration properties and its texture, palatability, and cooking time. Water uptake can also influence the leaching of pigments that occurs in color seeded and especially black beans. In black beans, leaching is a major problem because consumers expect beans to retain their color after cooking or canning. Moreover, the pigments that give black beans their color are anthocyanins, which have antioxidant properties. When anthocyanins leach out of the seed coat during cooking or canning, the antioxidant potential from eating beans is lowered compared to black beans that do not leach.

Three black bean cultivars, which differed in their leaching during cooking or canning, were chosen for this study. In addition, the cultivars differed in seed coat shininess conditioned by the Asp gene. 'Raven', has a matte seed coat (asp/asp) and exhibits heavy leaching during thermal processing. 'Black Magic' also has a matte seed coat (asp/asp) but exhibits little to moderate leaching during thermal processing. 'Shiny Crow' has a shiny seed coat (Asp/--) and exhibits little to no leaching during the cooking or canning process.

Seed of each of the three cultivars were soaked in xylene for 72 hours to remove epicuticular wax. Xylene treated seed were then compared to non-treated seed to determine the amount of water that was imbibed according to methods previously described by Bushey *et. al*. (2000) for determining the amount of water uptake in untreated bean seeds. Xylene treated and non-treated seed were examined using scanning electron microscopy (SEM)(Bushey *et. al.,* 2001).

Significant differences for the amount of water imbibed over the course of 120-minutes were detected among the three cultivars. When beans were soaked in water without xylene, 'Raven' imbibed 42% of its total weight; 'Black Magic' imbibed 23%; and 'Shiny Crow' imbibed only 0.8% of its weight in water.

Significant differences were found in the amount of water imbibed among the cultivars after being treated with xylene. 'Raven' imbibed a total of 75% its weight in water after the xylene treatment, 'Shiny Crow' and 'Black Magic' imbibed 43% of their weight in water after xylene treatment. This increase in water imbibition due to the xylene treatment compared to the non xylene treated beans was 20, 33, and 42% for 'Black Magic',' Raven' and 'Shiny Crow', respectively (Figure 1).

Scanning electron microscopy was used to determine the effects the xylene treatment had on the epicuticular wax layer of the seed coat surface to make it more permeable to water. Scanning electron micrographs of the seed coat surface taken of all three cultivars showed that the xylene was causing a gradual break down of the epicuticular wax layer. Of particular interest was the effect the xylene treatment had on 'Shiny Crow' (Figure 2). Untreated seed of 'Shiny Crow' has a smooth epicuticular wax layer that is very evenly distributed over the surface of the seed coat. After treatment with xylene, the seed coat showed regions of variability in the thickness of the wax layer much like that seen on untreated seed of 'Raven' and 'Black Magic'.

Previous studies by Bushey et. al. (2001, and unpub. data) demonstrated that the rate of water uptake in bean seeds was largely due to the structure of the epicuticular wax layer. The data obtained from the current study supports the hypothesis that it is the thickness and uniformity of deposition of the waxy epicuticular layer that influences the rate of water uptake and ultimately leaching in black bean. The xylene treatment caused physical changes to 'Shiny Crow' that mimicked the typical structure of the wax layers of

'Black Magic' and 'Raven'. Presumably, xylene removed some of the wax on the epicuticular surface of 'Shiny Crow'; thus, permitting 'Shiny Crow' to imbibe water at a rate comparable to that of 'Black Magic'.

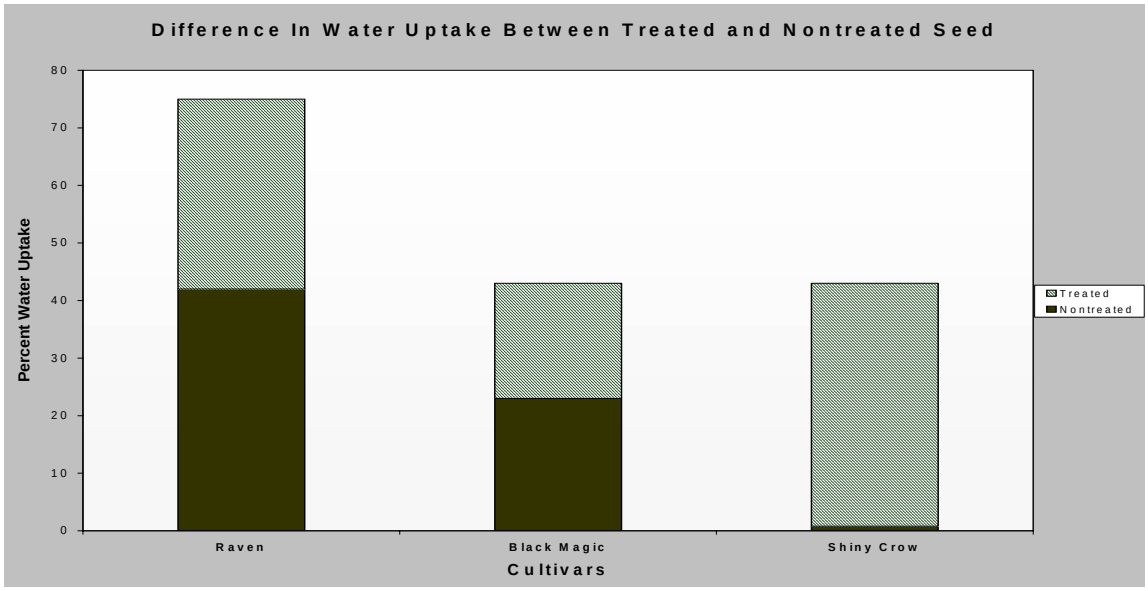

Figure 1. Differences in mean water uptake between xylene treated and non-treated seed.

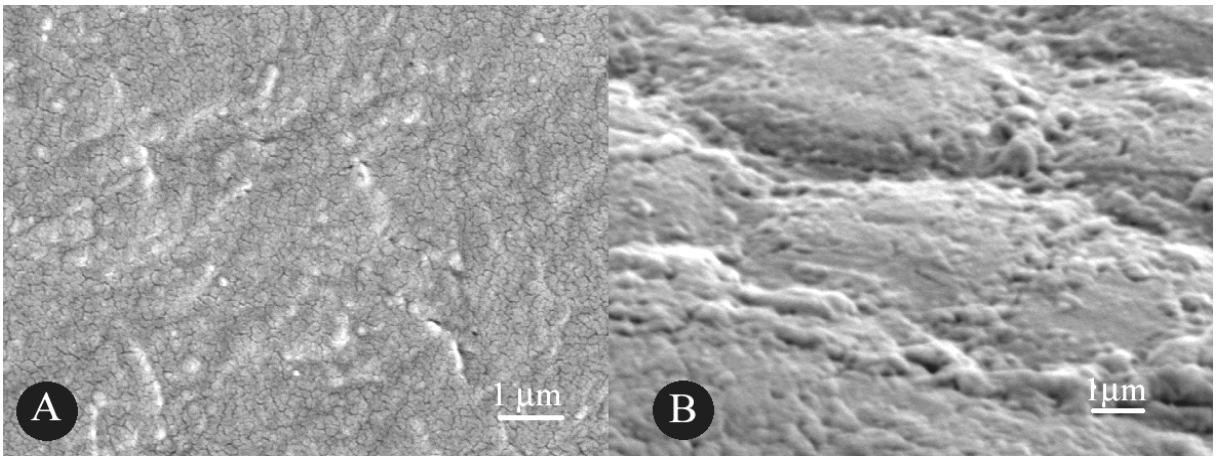

Figure 2. SEM micrographs of 'Shiny Crow' showing the surface of the bean seed coat before (A) and after (B) treatment with xylene.

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DOWNY MILDEW ON LIMA BEANS IN DELAWARE

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Lima beans (*Phaseolus lunatus*) have been an important processing vegetable in the Mid-Atlantic region for 100 years. Periodic outbreaks of Downy mildew (*Phytophthora phaseoli*) have caused significant economic loss to growers and processors. Professor Roland Thaxter of the Connecticut Agricultural Experiment Station first reported this disease in 1889.[1] Resistant varieties developed by the USDA after World War II provided control. Concurrent development of fungicides has also provided another method of control. In 1957, Dr. R.S. Hyre developed a predictive model that forecast outbreaks of the disease based on weather conditions. This system predicts the initial appearance of Downy mildew after 8 consecutive downy mildew favorable days have occurred. A day is considered favorable when the 5-day moving mean temperature is less than 79 degrees, with the minimum temperature above 45 degrees, and 7 day rainfall is 1.2 inches or above. Whenever temperatures reach 90 degrees, the cycle is broken.[2]

From 1960 to 1999, producers have enjoyed excellent control of Downy mildew (*Phytophthora phaseoli*) on lima beans (*Phaseolus lunatus*) with the use of resistant cultivars, fungicides, and the forecasting system. During this period, new races of the disease were identified as Race A, B, C, or D. Genetic resistance was maintained and developed to control these diseases. In 2000, wet, cool weather and the emergence of new races of Downy mildew caused significant losses. Seven cultivars have been evaluated for resistance to the race E and new fungicides have been tested for efficacy. Race F has also been identified and evaluations for resistance continue.

In 2000, 128 field isolates were collected from the Delaware/Maryland region. Using cultivar differentials, involving the systematic inoculation of know resistant and susceptible cultivars confirmed the presence of a new variant designated as race F. Race E was determined to be the prevalent genotype in the region.

Table 1. Results of Cultivar Differentials on selected cultivars for resistance (R) or susceptibility (S) to five races of Downy Mildew.

	Race A	Race B	Race C	Race D	Race E
Bridgeton	R	R	S	R	R
8-78	R	R	R	R	S
184-85	R	R	R	S	R
M-15	R	R	R	R	S
BG2-408	R	R	R	R	R

Table 2. Identification of variant races of Downy mildew from 128 field isolates in 2000.

Race	# of isolates	% of isolates
A	0	0
B	1	0.8
C	2	1.6
D	3	2.3
E	107	83.6
F	6	4.7
OTHER	9	7.0

Fungicide trials were conducted to evaluate the efficacy of several materials for control of Downy mildew. The variety M-15 was artificially inoculated with a sporangial suspension on September 1 and 14. Weather conditions were favorable for infection. The percent of plants and pods infected, as well as yield were measured. Fungicide applications were made on August 30, September 6, and13, and 21. Various rates and timings of Ridomil PC-GR, Quadris, Kocide, Quadris, Champ, Quadris + Kocide, Bravo Ultrex, Ridomil Gold/Copper, and RH 7281 80WP were tested. All products except tri-basic copper sulfate were significantly better than the control in reducing the percentage of plants infected.

In summary, new races of Downy mildew were not only identified, but their scope and distribution were described. Resistance to five races of selected varieties was also identified. Effective fungicides were also identified. Screening for resistance and fungicide evaluations will continue.

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MOLECULAR PHYLOGENETICS OF SNAP BEAN

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Various studies have examined molecular genetic diversity in common bean (*Phaseolus vulgaris*), but few have specifically studied snap beans. Skroch and Nienhuis (1995) used cluster analysis of RAPD (random amplified polymorphic DNA) markers to show that while snap bean accessions were predominantly of Andean origin, some were Mesoamerican, and many showed an intermediate set of markers. Beyond this study, we know little about the center of domestication derivation, and individual breeding lineages of snap beans.

Oregon Bush Blue Lake (BBL) beans were developed by breeders at OSU from 'Blue Lake Pole' bean germplasm. The Oregon BBL materials possess a unique combination of high yield, excellent pod quality, poor plant architecture, and high rogue frequency that distinguish them from other U.S. snap beans. Oregon BBL beans are difficult to recombine with non BBL snap beans; our experience has been that little of value comes from such a cross.

To better understand differences of Oregon BBL beans from other snap beans, we used RAPD markers to characterize 75 snap and dry cultivars. DNA was isolated and 110 RAPD primers were screened using standard protocols. Fifty-three markers were selected for phylogenetic analysis based on robustness and repeatability. A phaseolin specific PCR primer allowed us to distinguish "S" phaseolin from "T" and "C" phaseolin. Markers were scored as one or zero depending on whether a band was present. The computer program PAUP (Phylogenetic Analysis Using Parsimony) used UPGMA (unweighted pair group method with arithmetic averaging) to generate the tree based on genetic distances. 'Banquet' cowpea was used as an outgroup to root the tree (Fig. 1). 'Black Turtle Soup', 'UI 111' pinto, and 'Czech Dry Bean' were included to represent known races of common bean.

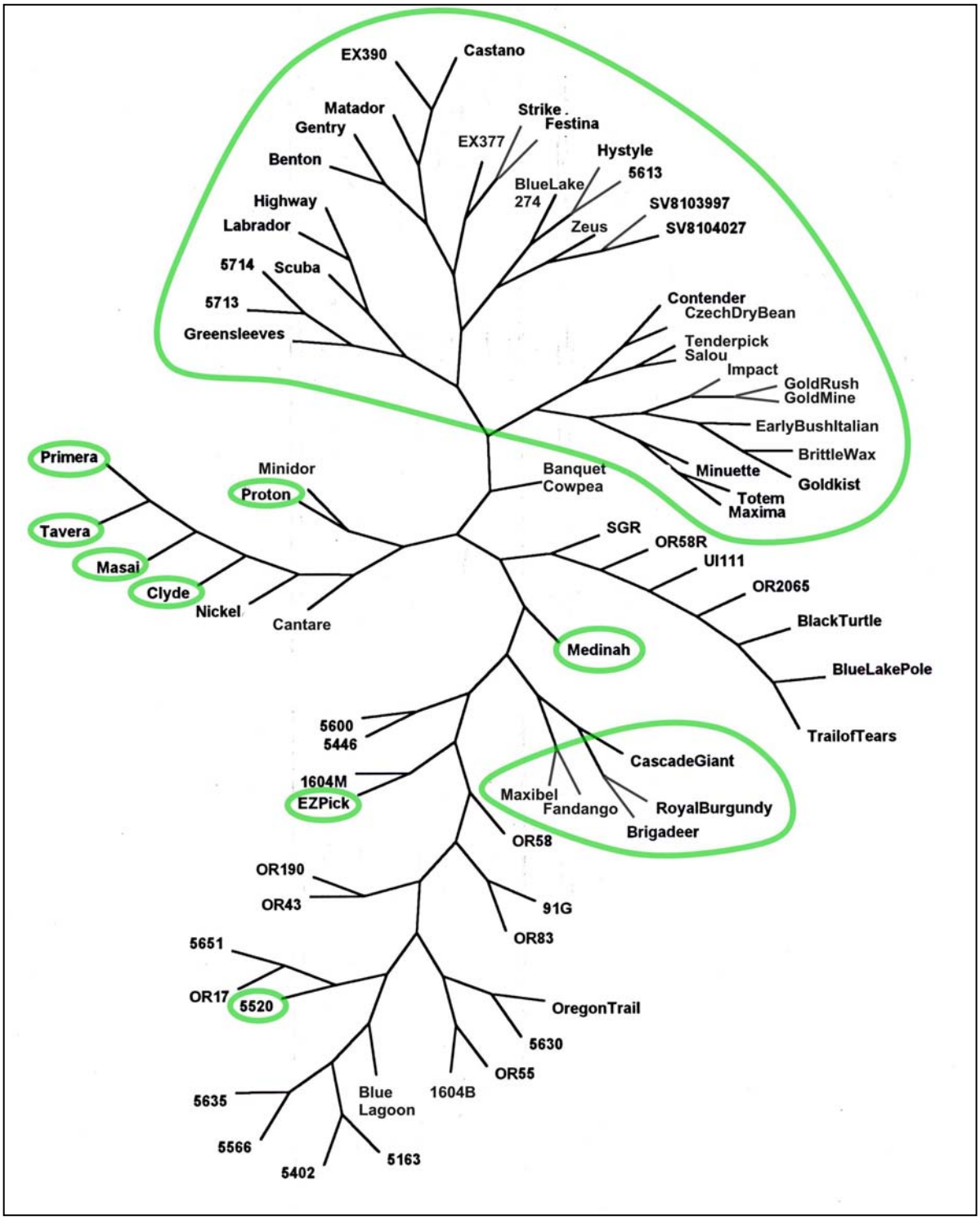
Five main branches were observed for the tree (Fig. 1). One branch included most U.S. snap beans, all of which possessed T or C phaseolin ('Contender' is the type for C phaseolin). A second branch contained European small sieve cultivars, which were a mixture of S and T (or C) phaseolin. A third branch had race Durango and Mesoamerica dry beans as well as 'Blue Lake Pole', two early OSU lines and various heirloom cultivars. All were S phaseolin. The fourth branch consisted of a mix of European small sieve and U.S. large podded pole heirloom cultivars, all with T (or C) phaseolin. The fifth branch included most OSU BBL materials, and all, with few exceptions had S phaseolin.

A major finding is that Oregon BBL cultivars are of Mesoamerican origin. This helps explain why it has been difficult to recombine Oregon BBL materials with other snap beans. Mixing among centers of domestication seems to have happened often within snap beans (European cultivars for example). U.S. fresh market and processing types do not seem to be derived from separate lineages. Clustering was observed for European small sieve types, wax beans, and Oregon blue lake materials. The Blue Lake Pole bean shows similarities to heirloom Mesoamerican dry or dual use beans. Some cultivars with apparently similar derivation can be quite different based on markers (e.g. OR 1604M and OR 1604B). While these data are based on the assumption that molecular markers are representative of underlying genetic relatedness, the phylogenetic relationships portrayed here may not necessarily correspond to pedigree relationships.

Reference

Skroch, P., J. Tivang & J. Nienhuis. 1992. Analysis of genetic relationships using RAPD marker data. pp.26-30. *In*: Applications of RAPD Technology to Plant Breeding. Joint Plant Breeding Symposia Ser; CSSA, ASHS, AGA, 1 Nov. 1992, Minneapolis, MN, U.S.A.

Figure 1. Phylogenetic tree of snap and dry bean cultivars based on 53 RAPD markers. T and C phaseolin cultivars are circled, all others are S phaseolin. 'Banquet' cowpea is the outgroup used to root the tree. Dry bean cultivars on the tree include 'Czech Dry Bean'; Andean origin, 'UI 111' pinto, race Durango; and 'Black Turtle Soup', race Mesoamerica. OSU experimental lines are designated with a four-digit number. SV or EX numbers are Seminis cultivars.



CLONING AND MAPPING OF P-LOOP (Kinase-1a) CONTAINING GENES FOR DISEASE RESISTANCE IN *Phaseolus vulgaris* L.

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Plant diseases are major production constraints, causing yield and quality losses. Breeding for resistance based on inoculation and selection for phenotypic reaction has been a slow and challenging process. However, use of molecular markers tightly linked to known plant resistance genes is expected to expedite and increase the precision of the selection process. The objective was to clone resistance genes and/or to find markers tightly linked to known resistance genes for breeding purposes. Phosphate-binding domain (P-loop) is conserved in 13 protein families, including the NBS-LRR type plant resistance gene class containing Kinase-1a domain (Traut, 1994). Degenerate primers based on conserved domains were used to pull out fragments with kinase-1a domain targeting plant resistance genes (Collins et al., 1998). Sequence analysis indicated that about 20 clones contained NBS-LRR type disease resistance signature at and around the conserved domains.

Materials and Methods

Thirty-three common bean lines/cultivars, resistant to different diseases, including the common bacterial blight (CBB) caused by *Xanthomonas campestris pv phaseoli* (*Xcp*), were inoculated with *Xcp* isolates. Then leaf samples were taken at different times. The mRNA was then converted into cDNAs and used in bulk for initial amplification of the kinase-1a containing fragments that were cloned subsequently.

Results and Discussion

RFLP mapping of these fragments using the BAT93 x JALO EEP558 RIL population (Source: P. Gepts, UCD, Davis, CA) which was used originally to create the integrated map of common bean (Freyre et al., 1998) indicated that this gene family was mostly conserved in four different chromosomes (B2, B3, B4, B7) and absent in three chromosomes (B8, B9, B10). Mapping also showed the distribution of this gene family in the genome, especially relative to known resistance genes. Detected linkages to disease resistance genes are expected to be useful in breeding for disease resistance.

Table 1. Name of the probes, length (base pairs), mapped linkage groups, and predicted proteins from whole length or motif search (NCBI database).

Probe	Length (bp)	Linkage group	Predicted protein
C3H	178	B1	PIC22, Flax rust resist
A3E	81	B2	-
B4B	303	B2	O_2 evolving-
B5G	197	B2	-
A1E	817	B2	Aminotransferase
A1F	718	B2	EDS-1 resistance
A11	370	B2	O_2 evolving-
B1A	370	B2	kinase
B6A	384	B2	M and N genes
B2E	221	B3	P.vulgaris (XZT-
A6G	556	B3	Cysteine synthase
B3C	362	B3	-
A3A	275	B4	Rubisco small
A1G	329	B4	Rubisco small
C6G	146	B4	-
B9C	253	B4	-
A5H	451	B4	-
A2E	351	B5	NADPH reductase
B4E	294	B6	NBS-LRR*
B4G	282	B7	Mammalian
B3G	290	B7	Phosphatase
A2D	569	B7	bZIP transcription
B10	220	B7	Monooxygenase
A6H	391	B7	N, PIC15
A4E	720	B7	DNA repair
B11	201	B11	-
B1D	234		-
A7H	404		RPP5
B10G	208	-	-
C8H	201	-	RPP8
C1B	186	B11	N, Pi, Mi-1.2

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SEQUENCING *P. VULGARIS* RFLP CLONES REVEALS A RICH SOURCE OF GENES AND INFORMATION ON SYNTENY WITH *Arabidopsis*

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ABSTRACT.

A set of 70 previously-mapped bean (*Phaseolus vulgaris*) genomic (Bng) clones were partially sequenced. BLAST database searches detected homologies between 53 of these clones and genes from a variety of plants, especially *Arabidopsis thaliana*. Some of the best matches in the database to the Bng clones included: S-adenosyl-L-methionine-S-methyltransferase from *Arabidopsis*, a cylophilin-type peptidyl-prolyl *cis-trans* isomerase from alfalfa, an early nodulin binding protein (ENBP1) from *Medicago truncatula,* a lon protease protein from spinach, a branched-chain amino-acid aminotransferase from *Arabidopis* and a vacuolar sorting receptor (BP-80) from *Pisum sativum*. PCR-RFLPs were developed for 6 of the clones and simple sequence repeats were identified in two Bng clones. Comparisons of the order of Bng clone sequences in the Florida *Phaseolus* linkage map with the positions of homologs in the *Arabidopsis* BAC tiling map revealed several regions of colinearity, including a 57 cM region on *P. vulgaris* linkage group G (Florida map) and *Arabidopsis* chromosome V.

INTRODUCTION.

P. vulgaris has a number of features that make it an attractive legume for genomics studies including a relatively small genome (637 Mb), simple diploid genetics (2n=2x=22), self pollinating reproduction and well developed molecular maps. Most agronomically-important grain legumes belong to the *Phaseolinae* subtribe (*Phaseolus* and *Vigna* spp.) and significant syntenic relationships exist between *P. vulgaris* and these species. However, relatively little sequence information is available for *P. vulgaris*.

RFLPs are highly reproducible and transferable making them useful as anchor loci for relating maps developed by different investigators. The first bean RFLP map was based on genomic clones (Vallejos *et al.*, 1992) produced from a bean *Pst*I genomic library (Bng) enriched for single copy sequences (Chase *et al.*, 1991). These clones have been used in several mapping studies (Gepts et al, 1999). PCR-based markers are more convenient and quicker to assay than hybridization-based markers. However, RAPD markers which have been extensively used to examine *Phaseolus* genetics are difficult to transfer from one lab to another because they are sensitive to small differences in the PCR reaction conditions. Microsatellite markers or simple sequence repeats (SSRs) offer the same robustness as RFLPs, and they are PCR-based but currently less than 50 are available for Phaseolus Yu *et al.* (2000).

PCR-RFLPs can be developed by sequencing RFLP probes and designing PCR primers to produce fragments that can be scored for polymorphisms after digestion with restriction enzymes.

In the present study 67 Bng clones were end-sequenced to allow new PCR-based markers to be produced for *P. vulgaris*. In addition, the information significantly enriched the bean DNA database and identified several bean genes of interest. These sequences, coupled with their positions on an existing linkage map, were used to identify regions of synteny between *P. vulgaris* and *Arabidopsis*.

METHODOLOGY. Sequencing was performed at University of Guelph with a capillary-based CEQ 2000 DNA Analysis System (Beckman Coulter). At the University of Florida F sequencing was performed by The DNA Sequencing Core Lab of the Interdisciplinary Center for Biotechnology Research on either a Perkin Elmer, Applied Biosystems Division (PE/ABd) 373A or 377 automated DNA sequencer, following ABd protocols. BLAST 2.0 Network client software Blastcl3 (Madden *et al.*, 1996) was used to streamline data analysis. The TBLASTX algorithm was used to search Genbank's nr, dbGSS, and dbEST databases. The information in The *Arabidopsis* Information Resource (TAIR) database (Huala *et al.*, 2001; **http://www.arabidopsis.org/)** was used to relate TBLASTX matches with *A. thaliana* BAC sequences to

chromosome position. Simulations were carried out to determine the likelihood that these groupings could occur by chance using an approach similar to that of Grant *et al.* (2000). The simulations were carried out using the Resampling Stats v5.0.2 program (Resampling Stats Inc.) and the procedures are available online (http://www.plant.uoguelph.ca/research/plantbio/SIMULATIONS.htm) **.**

RESULTS AND DISCUSSION.

The 70 *Phaseolus vulgaris* Bng (bean genomic) clone end sequences obtained in the present study ranged in length from 106 to 800 bp with an average length of 439 bp. The shortest clone Bng162 560 bp was completely sequenced. From a total of 140 sequence reads 58,851 bases pairs of high confidence single-read sequence was obtained. This information was submitted as genome survey sequence (gss) to Genbank (accessions AZ301497-AZ301506, AZ301508-AZ301510, AZ301512-AZ301559, AZ301561-AZ301568, AZ301570-AZ301616, BH011343-BH011352).

Perhaps the most intriguing outcome from the present work is the wide variety of genes that were uncovered. The BLASTX and BLASTN programs identified homologies between 78 of a possible 134 sequences using a threshold of $E=1e^{-5}$. The relatively high frequency of significant similarities between Bng sequences and ORF entries in the database can likely be attributed to the fact that these clones were enriched for inserts of single copy regions of the genome. The Bng sequences identified in our sequencencing project should provide important starting points for a variety of physiological, developmental, and molecular studies of beans (or other legumes) in the future. Most of the reported matches were to *A. thaliana* sequences, followed by soybean (*Glycine max*) and *Medicago truncatula.*

Sequencing data from the selected Bng clones not only provided important information about the previously anonymous clones, but presented the opportunity to establish them as sequence tagged sites, Sequence-specific PCR primers were designed for 15 Bng clones. These primers amplified bean genomic DNA and produced PCR products ranging in size from 0.5-1.5 kbp were obtained. Polymorphisms were detected between the cultivar Berna and the breeding line EMP419 for markers Bng42 and Bng27 by digesting the PCR products with *Mbo*I. Loci for which sequence data are available (Bng sequences generated in this project, and several known sequences) were used to produce a "gene map" that includes the most distal markers in previously maps and all of the 11 linkage groups, showing complete coverage of the mapped bean genome.

An analysis of the positions of homologs to the conceptual translations of the Bng sequences in the *Arabidopsis* BAC tiling path identified several syntenic regions between *P.vulgaris* and *Arabidopsis* (defined as homolgy between a minimum of three separate clones and an *Arabidopsis* chromosome). For example, The region on *P. vulgaris* linkage group G including Bng95-Bng94-Hsp70-Bng104 was colinear with a region on chromosome V in *Arabidopsis*. Additional regions of synteny between *Phaseolus* linkage groups and *Arabidopsis* chromosomesinclude:cM region on *P. vulgaris* Florida linkage group A and sequences on three chromosomes of *A. thaliana* and between a 54.3 cM region of linkage group D and two *Arabidopsis* chromosomes.

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CLONING FLAVONOID/ANTHOCYANIN BIOSYNTHETIC PATHWAY GENE FRAGMENTS

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The color and pattern of the seedcoat and flowers are major phenotypic characteristics that distinguish the various common bean (*Phaseolus vulgaris* L.) market classes. The underlying genetic factors controlling these phenotypes was investigated throughout the twentieth century. Prakken (12,13) elucidated the current synthesis with regards to seed coat color and described a series of "color" genes, *P* [*CR*] *J D* (now *Z* (3)) *G B V Rk*, that exhibit complex genetic interactions to produce the various seedcoat colors observed in common bean. The recent research of Bassett and collaborators (1,2,3) described a second set of "pattern" genes, *T Z L* (now *J* (1)) *Bip*, that interact to produce patterned seeds ranging from the nearly completely colored *expansa*, to the partially colored *virgarcus*, to the slightly colored weak *bipunctata*. Each pattern is characterized by the expression of color (determined by the "color" genes) on a white background. The color is restricted by the action of the "pattern" genes in a reproducible manner to specific zones of the seedcoat. Recently, Bassett and McClean (12) have worked extensively to develop and map markers linked to nearly all of the "color" and "pattern" genes. The mapping results were recently published (10).

The next logical step in these investigations is to begin experiments whose eventual goal is to clone these genes. With regards to the "color" genes, bean researchers have successfully elucidated the biochemical products associated with specific genotypes. The early experiments of Shaw and Norton (15) and Skalinska (16) indicated that both anthocyanin and flavonoid pigments are present in the seedcoats of various colored common bean seeds. Feenstra (8) extended this research and demonstrated that black and violet colored seeds were rich in specific anthocyanins, while yellow and cream-colored seeds lacked anthocyanins but instead contained flavonoid pigments. Recently, the research of Hosfield and colleagues (4,5,6,7) and others (17) has supported these conclusions. When the genotypes of the experimental materials of Feenstra and Hosfield and collegues are compared, it appears that in the presence of *C J*, a dominant *V* allele is essential for anthocyanins to appear in the seedcoat. It has also been suggested that *B* regulates the level of a common anthocyanin and flavonoid precursor (7), while dominant *J* is essential for proanthocyanin production (8).

Collectively, these results suggest that cloning the genes which encode enzymes of the flavonoid and anthocyanin biosynthetic pathways and using them as markers in populations segregating for the various color genes, may determine that one of the seedcoat colors genes encodes one of the enzymes. The first two genes in the pathway, chalcone synthase (14) and chalcone isomerase (11), have already been cloned in common bean and neither map near any "color" gene (9,10). The goal of the research reported here is to determine if three other genes from this pathway, flavonone 3-hydroxylase (F3H), dihydroflavonol 4-reductase (DFR), and anthocynanin synthase (ANS), are linked with any of the genes. We were particularly interested in the relationship between DFR and *V* because this enzyme appears to be at the branch point that may regulate the shuttle of dihydroflavonols into either flavonoids or anthocyanins. In the process of our cloning we also we able to demonstrate the efficacy of single nucleotide polymorphism technology (SNP) in common bean.

GenBank (http://www.ncbi.nlm.nih.gov/) was searched for complete sequences for F3H, DFR, and ANS. For each gene, all sequences were aligned using ClustalW (http://clustalw.genome.ad.jp/) with respect to the known exon/intron structure of *Arabidopsis* (http://mips.gsf.de/proj/thal/db/). Highly conserved nucleotide sequences were identified on each side of the second intron of F3H, the first intron of DFR, and the one ANS intron, and degenerate primers were developed. DNA of the Florida breeding line 5-593 was used for amplification, and fragments of the appropriate size were cloned and sequenced. Following sequence analysis, common bean specific primers were developed, and DNAs of 18 genotypes were amplified. Each amplified fragment was sequenced. For each gene fragment, all sequences were aligned to uncover polymorphisms. Those polymorphisms were used to develop primer combinations (Table 1) specific

to a single allele. Primers polymorphic between BAT93 x Jalo EEP558 were used to map the gene on that recombinant inbred (RI) population (9).

Fragments of appropriate size were amplified for F3H and ANS from 5-593 DNA. Sequence analysis revealed that F3H and ANS were monomorphic among the 19 genotypes studied. Amplification with DFR primers generated ≈500 and ≈700 nt from 5-593. Based on the of size intron one from other plant speices, we were surprised that the ≈700 bp DFR fragment was from that gene. That specific intron was 557 nt in size. In contrast to the other two genes, the DFR intron exhibited several polymorphisms . The pinto cultivar UI 114 represented one polymorphism, a unique 3 nt insertion. The second polymorphism, an 8 nt insertion, is represented by Jalo EEP558. Finally, a SNP polymorphism was discovered in 5-593, BAT93, and five other genotypes. That fact that Montcalm and M0056 contained fragments representing two polymorphisms suggests DFR is a small multigene family in common bean. Primers were developed that permitted the amplification of specific alleles (Table 1). The Jalo+ and 5-593 SNP primers were used in conjunction with the conserved 3' primer to score the BAT93 x Jalo EEP558 RI population. As expected the two alleles cosegregated. Furthermore, the DFR fragment did not cosegregrate with *V*. Therefore, *V* is either another structural gene in the flavonoid/anthocyanin biosynthetic pathway or a gene that regulates this pathway. Our cloning results also demonstrate the efficacy of cloning gene fragments to generate user-friendly PCR markers, including SNP primers, for common bean mapping studies.

Table 1. 5' primers used to amplify genotypic DFR intron one alleles of common bean. The corresponding 3' primer is 5'-CAGTGATAACATGAAAGTGTTAGGTTG-3'. The amplification conditions are: 45 cycles of 94^{o}C, 1 min; 55^{o}C (55 ^{o}C for 5-593 SNP), 1 min; 72^{o}C, 2 min, followed by one cylce of 72C for 5 min. Amplification of genotypes Aurora, C-20, Mayflower, Domino, and Emerson is negative for each primer.

Primer	Primer sequence	Positive amplifying genotypes
UI 114+	5'-GTATTATCATGTAGGGTCTGATGG-3'	UI 114, Fiesta
Jalo+	5'-GGTCTGGTGGATCTTTGTTGGTGC-3'	Jalo EEP558, RedKloud, CDRK 82, Montcalm
5-593 SNP	5'-TGTTTTTGGTTTTTGTATAAGTAGGTT-3'	5-593, BAT93, Sutter Pink, Coulee, ICA-Bunsi, Seafarer, M0056

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GENOTYPES FOR SEED COAT COLOR OF 'ENOLA', MAYOCOBA MARKET CLASS, AND PRAKKEN'S'WAGENAAR', WITH COMMENTARY ON THE 'ENOLA' PATENT

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The genetics of seed coat colors in common bean was summarized by Prakken (1972) in two tables. For the yellow-black series of colors, the genotype *C D* (or *d*) *J g b v* was listed as producing seed coats with "pale greenish yellow (canary) [or] 'schamois' and 'kan[arien].gelb'". Prakken (1972) in the text gave the genotype *C D J g b* v^{lae} for 'Wagenaar' and described its seed coat color as "shiny pale greenish yellow". The summary table of Prakken (1972) was obliged also to give the seed coat colors obtained by Lamprecht (1951, which summarized all his previous published works), which were schamois and canary yellow (kanarien gelb) for the same genotype. In all but one of Lamprecht's seed coat color genetic experiments, he always obtained the color schamois (chamois) for the genotype *C D J g b v*. The only exception was in his crosses with his line V0062, 'Mount Elgon' from Kenya (now PI 527719), which produced an intermediate color (canary yellow) between the yellow brown of *C D J G b v* and the chamois of *C D J g b v*. Lamprecht attributed this shift from canary to chamois to the gene *Can*, where *C D J g b v Can* produces canary yellow, but *C D J g b v can* produces chamois. Prakken (1970, see bottom p. 26) regarded *Can* as a synonym for the hilum ring color factor *D*. The canary yellow of Lamprecht is a different color with no greenish tint compared to the pale greenish yellow of 'Wagenaar'.

Our experimental hypothesis is that 'Wagenaar' carries a seed coat color gene never encountered by Lamprecht and never properly discriminated by Prakken. We obtained seed of 'Wagenaar' from the Centre for Genetic Resources, Wageningen. Seeds of 'Enola' were obtained from Mark Brick, Colorado State University, and seeds of 'Mayocoba' were obtained from an anonymous (by contractual agreement) source. Test crosses of 'Wagenaar' with the genetic tester stocks c^u BC_3 5-593 (cartridge buff tester), *j* BC_3 5-593 (margo pattern tester), and *g b v* BC_3 5-593 (chamois tester) demonstrated in F_1 progeny analysis that the stock from Wageningen had genotype *C J g b* v^{lae}, the same result as Prakken (!940, 1972) obtained.

The cross 'Wagenaar' x *g b v* BC_3 5-593 produced F_1 plants with chamois colored seeds, demonstrating that the pale greenish yellow of 'Wagenaar' is recessive to the chamois of the genetic tester. The F_2 segregated for 69 plants with chamois seeds and 14 plants with greenish yellow seeds. For the data 69 and 14, the χ^2 (3:1) = 2.928, $P = 0.09$. The seeds on the 14 plants with greenish yellow seeds were highly variable for the intensity of the color expressed and for the extent of the seed coat covered, i.e. most seeds were two-toned, pale greenish yellow and chamois. A detailed and precise description of this type of variable expressivity for seed coat color was given by Prakken (1940) and will not be repeated here. Suffice it to say that cool growing conditions result in strong greenish yellow color distributed over all the seed coat, whereas warm growing conditions lead to pale greenish yellow color expression incompletely distributed over the seed coat, with the remainder of the seed coat being chamois. The 3:1 ratio between plants with chamois vs. greenish yellow seeds, respectively, described above is consistent with the hypothesis that a single recessive gene controls greenish yellow color expression. This hypothesis was tested in F_3 from the 'Wagenaar' x *g b v* BC_3 5-593 cross. Of the 69 F_2 chamois plants, 34 were true breeding for chamois in F_3 and 28 segregated 3:1 for chamois to greenish yellow, respectively (for the data 251 and 69, the χ^2 (3:1) = 2.017, $P = 0.16$). All 14 F_2 greenish yellow parents were true breeding in F_3. For the three classes, true breeding chamois (34), segregating chamois (28), and true breeding greenish yellow (14), the χ^2 (1:2:1:) = 15.79, $P < 0.001$. The excess of true breeding chamois progenies was due to the small size of the F_3 progenies tested (mean = 8.8

plants, with the range of 1 to 27 plants). Overall, the F_3 data support the hypothesis that a single recessive gene controls greenish yellow color, and we propose the gene symbol *gy* for this gene.

Molecular genetic techniques were used to determine whether the *gy* gene was an allele at *G*, the yellow seed coat factor, or was possibly a gene not previously known to be involved with seed coat color. Three RAPD markers ($OAP7_{850}$, $OAP3_{1400}$, and $OU14_{950}$) that co-segregated with the *G* seed coat color locus were developed from the F_2 population derived from the cross *g b v* BC_3 5-593 x *G b v* BC_3 5-593. From the cross 'Wagenaar' x *g b v* BC_3 5-593, 80 F_2 plants were classified into 54 plants with chamois seed coat color and 16 plants with greenish yellow seed coat color. When the $OAP7_{850}$ marker (for the *G* locus) was applied to that population, linkage was not observed for the chamois and greenish yellow phenotypes. The RAPD marker $OAP12_{1400}$ linked to the *Gy* locus was developed from the F_2 of the cross 'Wagenaar' x *g b v* BC_3 5-593. When $OAP12_{1400}$ was applied to the F_2 from *g b v* BC_3 5-593 x *G b v* BC_3 5-593, the marker and the *G* locus segregated independently.

Using F_2 individuals that were genotypically classified using F_3 progeny tests (described above), the linkage between *Gy* and the $OAP12_{1400}$ marker was determined to be 7.5 cM. A sequence tagged site (STS) marker was developed from $OAP12_{1400}$, and this marker was mapped in the BAT93 x Jalo mapping system developed by Paul Gepts and co-workers (Nodari et al., 1992; Freyre et al., 1998). The map location of *Gy*-STS was 2.7 cM from *C* in linkage group B (McClean et al., 2001). This makes the true map position of *Gy* itself probably 4.8 cM or greater distance from *C*, based on the linkage of 7.5 cM between *Gy* and $OAP12_{1400}$. Thus, *Gy* is probably a new gene for seed coat color that is linked to *C*, but is not in the 'complex C' region described by Prakken (1974).

Two test crosses were made: 'Wagenaar' x 'Enola' and 'Wagenaar' x 'Mayocoba'. The F_1 and F_2 progenies of both crosses were true breeding for greenish yellow seed coats except for the corona and hilum ring zones. The allelism test results support the hypothesis that 'Enola', 'Mayocoba' and the entire Mayocoba market class carry the *gy* gene. All the above experimental work demonstrates that the claim of the Enola Patent (U.S. Patent No. 5,894,079) to have "invented" the greenish yellow color of 'Enola' is false (Bassett et al., 2001).

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REASSESSMENT OF THE RELATIONSHIP BETWEEN SEED WEIGHT AND SEED CALCIUM

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Introduction

Seed calcium concentration [Ca] was negatively related to seed weight (mg seed^{-1}) ($r=-0.78$, $P<0.001$) in field studies involving eight bean cultivars (2 Navy, 1 Great Northern, 1 Pinto, 1 Cranberry and 2 Red Kidney) grown at five locations in North Dakota and Minnesota (Moraghan and Grafton, 2001).

Mexican Study: As part of a study involving genetic diversity in regards to zinc concentration [Zn] in bean seed, 29 bean genotypes from the Middle American gene pool were grown to maturity in a greenhouse study at Montecillo, Mexico. Four of the genotypes in the Mexican Experiment, the relatively photoperiod insensitive 'Voyager' (Navy), 'Norstar' (Navy), 'Othello' (Pinto) and 'T39' (Black), were also included in the earlier study (Moraghan and Grafton (2001). The remaining 25 genotypes were from Mexico (23) and Brazil (2). There was no tendency for an inverse relationship between seed weight and seed [Ca] in this genetic population (Table 1). The relatively large-seeded 'Pasella Teocaltiche' (577 mg seed^{-1}) had the highest seed [Ca] (2.2 g kg^{-1}).

Table 1. Relationship between seed dry weight and seed Ca concentration in a Mexican greenhouse study.

Parameter	Seed weight (x)[1]	Seed Ca (y)[1]
	mg seed^{-1}	g kg^{-1}
$\bar{x}$	351	1.59
SD	113	0.33
Range	180-650	1.0 to 2.2

[1]Relationship between seed [Ca] and seed weight: $y=0.00104x + 1.23$ ($r = 0.35$).

Additional Seed-Class Studies: Because of the discrepancy between results of the earlier studies, seed from several North Dakota and Minnesota seed-class variety experiments conducted in 2000 were analyzed for Ca. Pertinent particulars about the experiments are given in Table 2. The linear correlation coefficient (r) between seed dry weight and seed [Ca] was -0.879 ($P<0.001$), in agreement with the earlier American field study.

Table 2. Seed weight and seed-Ca concentration in several seed class common bean experiments in 2000.

	Trial							
	Navy (Erie, n=26)[1]		**Pinto** (Erie, n=22)[1]		**Kidney and Cranberry** (Perham, n=15)[1]		**Kidney and Cranberry** (Park Rapids, n=11)[1]	
Parameter	Seed weight	Ca	Seed weight	Ca	Seed weight	Ca	Seed weight	Ca
	mg seed^{-1}	g kg^{-1}	mg seed^{-1}	g kg^{-1}	mg seed^{-1}	g kg^{-1}	mg seed^{-1}	g kg^{-1}
$\bar{x}$	181	2.28	368	1.40	515	1.08	555	0.96
SD	18	0.32	20	0.1	38	0.25	6.5	0.18
CV	10	14	5	13	7	24	12	18
Range	150-233	1.6-2.9	342-406	1.0-1.6	443-579	0.7-1.7	473-653	0.58-1.22

[1]n indicates the number of entries in the particular trial.

The large-seeded Kidney and Cranberry lines at the Park River and Perham sites had very low seed [Ca]. Were environmental rather than genetic factors responsible for this result? We do not believe that this was the case. The Kidney bean 'Montcalm' and the Navy bean 'Norstar' were both included in the earlier published study and had seed '[Ca] values of 1.1 and 2.0 g kg^{-1}, respectively (Moraghan and Grafton, 2001). 'Norstar' seed from the 2000 Erie Experiment contained 2.1 g Ca kg^{-1}. In contrast, 'Montcalm' seed from the 2000 Perham and Park Rapids Experiments contained 1.0 and 0.9 g Ca kg^{1}.

Conclusions

There is no general relationship between seed [Ca] and seed weight in Phaseolus vulgaris (L). However within American Navy, Pinto and Red Kidney seed classes, seed [Ca] is inversely related to seed weight. Data for 'market basket' samples of Navy (n=45), Pinto (n=57) and Red Kidney (n=50) seed classes in the United States also give credence to this relationship between seed [Ca] and seed weight. 'Market basket' samples of Navy, Pinto and Red Kidney seed classes had [Ca] values of 1.77, 1.36 and 0.94 mg kg^{-1}, respectively (U.S. Department of Agriculture, 2001).

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THE INHERITANCE OF PHYTIC ACID CONTENT IN NAVY BEAN SEED

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Introduction:

Phytic acid is the major phosphorus storage compound in legumes. Generally 60 to 90% of the total seed phosphorus is stored as phytic acid. This compound has been the focus of a large amount of research, especially because of its role in human and animal nutrition. Phytic acid negatively impacts human nutrition by forming a complex with zinc that is highly insoluble in digestive tissue. The resulting decrease in zinc bioavailability can cause zinc deficiency, especially in vegetarians with high phytic acid diets. A recent human nutrition study estimated that 49% of the global population is at risk for zinc deficiency based on evaluation of diets and how much zinc and source of zinc in diet (Brown et al., 2001). Reduction of the amount of seed phytic acid is one way to increase the zinc in the diet by making more zinc available for digestion. Plant breeding is one possible strategy to achieve this goal. The objective of this work was to use the technique of generation means analysis to determine how phytic acid content is inherited in navy bean seed as an indication of the best strategy to breed for reduced seed phytic acid levels.

Materials and Methods:

The inheritance study was conducted with the Navy bean cultivars Voyager and Albion. The parents were chosen based on their difference in zinc efficiency. Albion is Zn inefficient, and Voyager is Zn efficient. The zinc efficiency trait allows a plant to grow well in low zinc soils. Another consequence of this trait is higher seed zinc concentration.

Parents, along with F1, F2, BC1, and BC2 generations were field grown at Erie ND in 1999 in a completely randomized design. A sub-sample of seed from each plant was analyzed for phytic acid concentration via high performance liquid chromatography.

The generation means analysis technique was used to determine the inheritance. This analysis uses least squares regression to fit 6 generations to 6 variables. The 6 variables the model used to describe the phenotype are: midparent value (m), additive effects [d], dominance effects [h], additive x additive interactions [i], additive x dominant interactions [j], and dominance x dominance interactions [l].

Results and Discussion:

An analysis of variance for the mean seed phytic acid concentration of each generation indicated the parents were not different from each other (Table 1). Albion was different from the F1, F2, and both backcross generations. Voyager was different from the F2 and both backcross generations. The estimate of each parameter of generation means analysis indicated that the dominance and dominance x dominance interactions were significant (Table 2). Additive effects were not significant. The limited genetic variability between parents for phytic acid content decreased the usefulness of generation means analysis, which requires significant variability among parents. A breeding approach, such as mutation breeding, that does not rely on natural variation may be better suited to significantly reduce seed phytic acid levels.

These data also point to the possibility that improving zinc availability in seed for human nutrition might be achieved by increasing the seed zinc concentration. Seed zinc concentration and seed phytic acid concentration are negatively correlated (r = -0.81), so breeding for an increase in seed zinc will likely not cause an increase seed phytic acid.

Table 1. Analysis of variance for 6 generations of inheritance study

Generation	number of plants	observed mean and s.e. [phytic acid] mg/g	
Albion	5	6.05±0.375	a
Voyager	5	7.19±0.686	a,b
F1	23	7.77±0.226	b
F2	169	8.76±0.099	c
BC Albion	28	8.98±0.176	c
BC Voyager	21	10.0±0.177	d

Means not sharing a letter are significantly different by Tukey's HSD (alpha=0.05)

Table 2. **Seed [phytic acid] fit to 6 parameter model of generation means analysis**

Component	Estimate	Significance ($\alpha = 0.05$)
m	5.424±0.748	yes
[d]	-0.568±0.391	no
[h]	11.006±2.073	yes
[i]	1.195±0.673	no
[j]	0.824±0.927	no
[l]	-8.660±1.404	yes

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Brown, K.H., Wuehler, S.E., Peerson, J.P. 2001. The importance of zinc in human nutrition and estimation of the global prevalence of zinc deficiency. Food Nutr Bulletin 22:113-125

INHERITANCE OF SEED-ZN ACCUMULATION IN NAVY BEAN

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Navy bean (*Phaseolus vulgaris* L.) genotypes vary in their susceptibility to Zn deficiency. Zinc-deficiency problems have plagued commercial navy bean production since the 1960s. Zinc deficiency was first documented in 'Sanilac', a bush-type (CIAT Type I) navy bean produced from X-ray mutagenesis of 'Michelite' (Down and Andersen, 1956). Researchers have historically used foliar deficiency symptoms to determine the inheritance of tolerance to low soil Zn. Polson (1968) investigated the genetics of the Zn-efficiency trait in a population derived from a hybridization of Sanilac (Zn-inefficient) and 'Saginaw' (Zn-efficient). He found dominant genes controlled tolerance to low soil Zn and that a two-gene complimentary interaction resulted in a 9:7 ratio in the F_2. Polson eventually concluded that the 9:7 ratio was extremely close, but not absolutely correct because the F_3 was more tolerant to Zn than expected. Seed-Zn concentration has recently been identified as a means to identify Zn-efficient genotypes. Moraghan and Grafton (1999) found that Zn-efficient genotypes possessed a greater concentration of seed-Zn than Zn-inefficient genotypes. The objective of this study was to determine the inheritance of high seed-Zn accumulation in a population derived from 'Albion' (Zn-inefficient) and 'Voyager' (Zn-efficient).

Voyager (P_1) and Albion (P_2) plants and F_1, F_2, F_1BC_{P1}, and F_1BC_{P2} lines were grown under field condition at sites near Erie, ND in 1999 and 2000. Individual plants were hand-harvested from each row at maturity and seed from individual plants was bulked. Seed was counted, washed with de-ionized water containing detergent, and rinsed with de-ionized water. Samples were dried at 70C for 48 h, weighed, and ground in an agate mortar with an agate pestle to pass a 0.25-mm mesh sieve. Sub-samples of the ground seed were digested on an aluminum block with 4 mL HNO_3 and 2 mL $HClO_4$. The acid digests were analyzed for Zn by atomic absorption spectroscopy. Standard Reference Material 1572 or 1515 from the National Institute of Standards and Technology, Gaithersburg, MD, was digested and analyzed concurrently with samples.

The number of Zn-efficient genes segregating was estimated by comparing the proportion of parental Zn-efficient phenotypes observed in the F_2 generation to the proportion of Zn-inefficient phenotypes observed in the F_2 generation based on seed-Zn concentrations. Genetic models containing one or two genes were evaluated. A chi-square test was used to measure goodness of fit to a 9:7 and 3:1 ratio of distribution of F_2 lines for seed-Zn accumulation. Lines within two standard deviations above the seed-Zn concentration for Albion were classified Zn-inefficient. The genetic model with the smallest and non-significant chi-square value ($P< 0.05$) was adopted.

The DTPA-soil Zn was 2.4 and 1.0 mg kg^{-1} in 1999 and 2000, respectively. In 1999, the soil pH was 5.8, while in 2000 the soil was more calcareous having a pH of 7.2. In 1999, the seed-Zn concentration of Voyager averaged 33.2 mg kg^{-1} (Table 2). In 2000, the average seed-Zn concentration of Voyager was 21.7 mg kg^{-1} (Table 1). Albion seed-Zn concentration averaged 20.9 and 14.4 mg kg^{-1} in 1999 and 2000, respectively. The soil DTPA-Zn and pH may explain the differences in seed-Zn concentrations between years. Because of the differences in seed-Zn concentrations between parents, data from 1999 and 2000 were not combined. The distribution of F_2 lines for seed-Zn concentration was tested for a goodness of fit to a 3:1 ratio. In 1999, the distribution of 41 inefficient to 139 efficient resulted in a chi-square value of 0.47, a good fit. In 2000, the distribution of 16 inefficient to 77 efficient resulted in a chi-square value of

3.01, a good fit. F_3 progeny testing could not be conducted because of the destructive nature of seed-Zn testing.

The hypothesis of a two-gene complimentary interaction in the F_2 proposed by Polson was rejected. Based on the results in 1999 and 2000, these data collected from a segregating F_2 and backcross population indicate that one dominant gene is responsible for seed-Zn accumulation. Due to the transgressive segregation observed in the F_2, a minor gene(s) also may play a role in seed-Zn accumulation.

Table 1. Seed-Zn concentration of populations grown near Erie, ND in 2000.

		Seed Zn			
Genotype	no.	Mean	s	Range	CV
		----------mg kg^{-1}----------			
Voyager	36	21.7	1.75	19.0 - 27.8	8.06
Albion	36	14.4	0.89	13.3 - 17.1	6.18
A / V (F_1)	24	23.1	1.98	19.8 - 29.6	8.57
A / V (F_2)	93	22.6	4.43	12.4 - 34.2	19.6
A / V // A	27	16.6	2.36	13.5 - 20.9	14.2
A / V // V	21	19.8	1.67	17.2 - 23.7	8.43

Table 2. Seed-Zn concentrations of populations grown near Erie, ND in 1999.

		Seed Zn			
Genotype	no.	Mean	s	Range	CV
		----------mg kg^{-1}----------			
Voyager	7	33.2	1.5	31.0 - 35.3	4.52
Albion	7	20.9	2.54	18.1 - 23.3	12.15
A / V (F_1)	28	30.6	1.83	26.7 - 35.6	5.98
A / V (F_2)	180	28.9	5.67	15.9 - 42.7	19.62
A / V // A	27	27.3	5.17	17.8 - 37.2	18.9
A / V // V	21	33.3	2.45	28.2 - 38.4	7.36

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INHERITANCE OF RESISTANCE TO SOIL ZINC DEFICIENCY IN COMMON BEAN

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Soil zinc (Zn) deficiency is a widespread production constraint of common bean (Phaseolus *vulgaris* L.) in calcareous soils of the western states and in many other production regions of the USA and other parts of the world. A preceding crop of sugarbeet (*Beta vulgaris* L.), high manure use, or phosphate fertilizers might intensify Zn deficiency symptoms on susceptible common bean cultivars. Similarly, land leveling or deep plowing, that brings to the surface the highly calcareous subsoil may intensify Zn deficiency.

The visual symptoms of Zn deficiency may include shortening of internodes or plant stunting, interveinal chlorosis and bronzing of leaves, early seedling death, delayed flowering and maturity, and reduced biomass production and seed yield. Differences for response to soil Zn deficiency have been known since the 1960's. Some small-seeded cultivars of black and navy market classes are highly susceptible to soil Zn deficiency. Yield losses of susceptible small-seeded (< 25g/100 seed) cultivars (e.g., Mackinac, Sanilac, Seaway, and T 39) in moderately Zn deficient soils could reach up to 100%. Cultivars of the medium-seeded market classes (great northern, pink, pinto, and red) seem to possess the highest levels of resistance. These resistant genotypes should have greater fertilizer use efficiency compared to susceptible cultivars when grown in low Zn soils. Thus, resistant genotypes offer a potential to manage severely Zn-deficient soils by a combination of (1) growing Zn deficiency resistant cultivars and (2) Zn fertilization at low rates. Nonetheless, managing the soil Zn deficiency stress through fertilizer use increases production costs and reduces the competitive edge of common bean growers in global markets. Our objective was to study the inheritance of resistance to soil Zn deficiency in common bean.

Zn deficiency resistant widely adapted great northern cultivar Matterhorn was crossed with susceptible small black cultivar T 39. The Matterhorn/T39 F_1 hybrid was crossed back on to Matterhorn (BC_1) and T 39 (BC_2), and also allowed to produce F_2 seed. Matterhorn, T 39, and their F_1, F_2, BC_1, and BC_2 were evaluated in Zn deficient soil at Kimberly, Idaho in 2001. A randomized complete block design with three replicates was used. Each plot consisted of a single row, 20 ft long, spaced 22 inches apart. Within row plant spacing was approximately 3 inches. Plots were kept free from weeds and gravity irrigation was applied as necessary to assure optimum crop growth and development. However, no fertilizer was applied to any plots.

Total plant counts were made in each plot within the first week after emergence. Visual Zn deficiency symptoms if any were also scored on a 1 to 9 scale, beginning three weeks after emergence. Plants within each plot were classified into tall healthy with very mild or no visual Zn deficiency (receiving scores of ≤ 3) and short or stunted with severe foliar Zn deficiencies (receiving scores of ≥ 7). The observed frequencies of resistant and susceptible plants in segregating genotypes were compared with expected frequencies using a χ^2 test.

The seedlings as well as adult plants of Matterhorn/T39 F_1 were as normal and healthy as Matterhorn. However, Matterhorn had white flowers and seeds. All F_1 plants had purple flowers and black shiny seeds. Thus, resistance to Zn deficiency was a dominant trait.

In the F_2, both the stunted plants with typical Zn deficiency symptoms similar to T 39 (susceptible) and healthy tall plants like Matterhorn (resistant) were observed. There were 45 Zn deficiency resistant and 20 susceptible plants. These observed frequencies gave a good fit to the expected 3 resistant to 1 susceptible ratio (χ^2=1.1538, *P*=0.28). Thus, a monogenic dominant inheritance of resistance to Zn deficiency in common bean was indicated.

All plants in the BC_1 (i.e., the F_1 backcrossed to Matterhorn) were tall and healthy like Matterhorn. However, they segregated for flower and seed color, as would be expected because both white flower and seed colors are recessive traits. Since all plants of Matterhorn/T 39 F_1 were normal and healthy, and a monogenic dominant inheritance of resistance to Zn deficiency was observed in the F_2, only normal and healthy plants would be expected in the BC_1.

The F_1 backcrossed to T 39 (BC_2) had 142 healthy tall and 139 Zn deficient stunted plants. This gave a good fit to the expected 1 resistant to 1 susceptible ratio (χ^2=0.032, *P*=0.86), which once again supported the results observed in F_1, F_2, and BC_1 of a single dominant gene controlling resistance to Zn deficiency in common bean. The symbol *Znd* is proposed for the dominant allele controlling resistance to soil Zn deficiency, and *znd* for its susceptible counterpart.

The monogenic dominant control of Zn deficiency resistance in common bean should facilitate and expedite its transfer into susceptible cultivars such as T 39, Sanilac, and Mackinac. Alternative backcross, pedigree, single-seed-decent, or gamete selection methods could be used depending upon the genetic distance between parental genotypes, other objectives of the program, urgency of the project, and available resources.

UTILITY OF THE MULTIPLE-SEED PROCEDURE OF SINGLE-SEED DESCENT FOR BEAN IMPROVEMENT

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Single-seed descent permits plant breeders to maintain genetic variability while advancing generations. In Puerto Rico, as many as four generations of soybeans [*Glycine max* (L.) Merr.] can be grown within a period of 14 months, permitting the rapid development of advanced (F_6) lines. The multiple-seed procedure of single-seed descent (SSD) is used by soybean breeders to avoid high labor costs associated with the single-seed procedure. Pods containing 2 to 3 seed are harvested and bulked from soybean plants in segregating populations. Although this practice is known to reduce genetic variability, the magnitude of the reduction had not been quantified.

Macchiavelli and Beaver (2001) conducted a study to estimate the effect of number of seed bulked and population size on genetic variability when using the multiple-seed procedure of SSD. Simulations were conducted to estimate the number and proportion of F_2 families which were represented in the F_6 generation after bulking and randomly selecting seed. The effect of differences in number of seed per pod bulked and different population sizes on genetic variability was studied. Increasing the population size from 100 to 600 plants had little effect on the mean proportion of F_2 plants represented in the F_6 generation. Increased population size, however, did reduce the standard deviation and the range of the expected proportion of F_2 plants that would be represented in the F_6 generation. Number of seed bulked had a greater effect on the proportion of F_2 plants represented in the F_6 generation. At a population size of 600, the mean proportion of F_2 plants declined from 0.39 to 0.35 when the number of seed per pod bulked increased from 3 to 6.

Using the multiple-seed procedure, bean breeders could expect, on the average, that at least every third line would be derived from a different F_2 plant. Single-seed descent permits each F_2 plant to be represented only once in the F_6 generation. Unlike single-seed descent, the multiple-seed procedure would allow plant breeders to benefit from divergence in segregation patterns in lines derived from the same F_2 plant. Therefore, the multiple-seed procedure of SSD would generate considerable genetic variability for the selection of quantitatively inherited traits.

Soybean breeders have been successful in using the multiple-seed procedure of SSD to improve traits with low heritability such as seed yield (Empig and Fehr, 1971). This approach would be most appropriate for dry edible bean populations derived from crosses between elite lines within a market class. Bean breeders may consider using a larger population size to compensate, in part, for the loss in genetic variability resulting from the use of the multiple-seed procedure of SSD.

The use of the multiple-seed procedure of SSD and winter nurseries would permit bean breeders to develop F_6 lines within a two-year period. In recent years, plant breeders have placed greater emphasis on the development of bean germplasm and cultivars with specific combinations of

genes. Greater use of marker-assisted selection would be expected to accelerate this trend. Recent releases of bean germplasm, such as, BelDakMi RMR 18, combine several specific genes (*Ur-3*, *Ur-4*, *Ur-6*, *Ur-11*, *I*, and *bc3*) for disease resistance (Pastor-Corrales et al. 2001). In order to maintain these very useful combinations of genes, bean breeders may be inclined to use conservative plant breeding approaches such as backcrossing. This approach, however, may impede the improvement of quantitatively inherited traits such as seed yield and tolerance to abiotic stress. The multiple-seed procedure of SSD and winter nurseries can be used to rapidly produce inbred lines, which would increase likelihood that desired alleles would be fixed.

Single seed descent drastically reduces the minimum number of plants that need to be evaluated in order identify at least one plant with the desired genotype (Beaver and Macchiavelli. 1998). If a population were segregating for five specific genes, it would be necessary to evaluate 4,714 F_2 plants to have a 99% probability of identifying at least one plant with the desired genotype. Using single-seed descent, it would be necessary to evaluate only 171 F_6 plants to have a 99% probability to identify at least one plant with the desired genotype. Using the multiple-seed procedure, where every third plant would be derived from a different F_2 plant, it may be necessary to evaluate as many as 500 plants to have the same level of confidence of identifying the desired genotype.

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A VIRUS DISEASE COMPLEX DEVASTATING LATE SEASON SNAP BEAN PRODUCTION IN THE MIDWEST

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A virus disease complex causing widespread stunting of snap bean (*Phaseolus vugaris* L.) plants and severe pod necrosis was reported in Wisconsin during the 2000 growing season. In 2001, the disease was reported in Iowa, Illinois, Kentucky, Michigan, Minnesota, New York, Wisconsin, and Ontario, Canada. The complex has resulted in severe yield losses due to twisting of pods, pods exhibiting external and internal necrosis, and general plant decline. Many fields of late-season processing and fresh market snap beans suffered losses of up to 100%. The results of virus surveys conducted during the 2000 and 2001 growing seasons are presented.The sudden increase in virus incidence coincides with the recent introduction of the soybean aphid (*Aphis glycines*) from Asia.

Materials and Methods

Bean tissues exhibiting virus-like symptoms were collected from several growing areas in the States listed above. Samples were evaluated by ELISA for alfalfa mosaic virus (AMV), cucumber mosaic virus (CMV), bean common mosaic virus (BCMV), tobacco streak virus (TSV), white clover mosaic virus (WCMV), bean yellow mosaic virus (BYMV), and bean pod mottle virus (BPMV). All ELISA tests were conducted using standard procedures (Converse & Martin, 1990). In addition, samples were routinely tested with a potyvirus group-specific monoclonal antibody (Agdia, Elkhart, IN). Absorbance readings at A_{405} nm were recorded and samples with values greater than 3 times the absorbance of healthy plant controls were considered positive. Selected samples that were infected with two or more viruses were subjected to multiple local lesion transfers onto bean Sutter Pink, *Chenopodium quinoa* Willd., or *C. amaranticolor* Costa & Reyn to isolate the individual viruses for further evaluation.

Six selected strains of AMV isolated from infected bean plants collected from fields in Wisconsin were used to evaluate 27 bean lines included in the BCMV differential set (www.ars-grin.gov). Plants at the primary leaf stage were inoculated using infected tissue macerated in 50 mM potassium phosphate, pH 7.4, containing 10 mM sodium sulfite. Diatomaceous earth was included as an abrasive agent to aid in the inoculation. The procedure was repeated two days after the first inoculation. Plants were evaluated for severity of symptom expression.

Results and Discussion

The predominant viruses found in snap bean samples were CMV, TSV, WCMV, CYMV, AMV, and a virus that is currently unidentified. Symptoms associated with CMV included chlorotic mottle, leaf curling, blistering, and leaf malformation. Green vein banding resembling sutures or a zipper-like appearance associated with CMV was common in nearly all samples infected with this virus. Typical symptoms caused by AMV included yellow mosaic, chlorotic spots, leaf rugosity, general chlorosis, and stunting. Some bean plants exhibited necrosis of stems, petioles, or a top necrosis that resembled symptoms caused by bean common mosaic necrosis virus, although the definitive viral agent responsible for these symptoms could not be

determined. BYMV, BPMV, and BCMV were infrequently detected and do not appear to have a significant role in the virus complex affecting snap bean at the present time. Field symptoms on pods could not be attributed to a single specific virus because many of the plants were infected with more than one virus.

The variability and number of strains of AMV in snap bean was exceedingly high. For example, 15 different strains, based on host response after multiple local lesion transfers, were isolated from two snap bean plants collected in the Cambria and Belgium growing areas of Wisconsin respectively. Response on the BCMV bean differential set inoculated with six selected isolates was generally uniform. Plants were initially stunted and exhibited chlorotic and necrotic local lesions, vein necrosis, and net necrosis on primary leaves. Symptoms on secondary leaves included severe mosaic, yellow mosaic, leaf roll and twisting, and general chlorosis. Stunting was more severe when plants were inoculated with isolates designated TB-2, TB-3, and BC-4, while the three other selected isolates evaluated caused only minor stunting. Regardless of the severity of the initial symptoms, all bean lines included in the evaluations recovered from the initial stunting, after emergence of the second or third trifoliate leaves. Although the systemic chlorosis remained throughout the evaluation period, the plants in the differential set were highly tolerant and subsequent growth and vigor was normal. No adverse pod symptoms were observed. Our results suggest that early infection with AMV as the soybean aphid migrates through a field could cause a delay in crop maturity. When plants are infected with additional viruses in the complex, delay may be more significant and may adversely affect the overall plant vigor and pod fill.

In all snap bean samples evaluated from the Midwest region during 2001, fields planted later in the growing season suffered substantially higher losses compared to fields planted in the late spring. This was attributed to the explosion of soybean aphid flights observed in late July and early August when late season beans would be at their most susceptible young growth stages. Therefore, a potential strategy for snap bean production to reduce yield losses due to virus infection would be to sow seed as early as possible after the last spring frost before large aphid populations and subsequent movement occurred. This, however, compresses the harvest season and obstructs coordination of bean processing dates.

Although soybean is a host for TSV, this virus historically occurs at relatively low levels.. Thrips (*Frankliniella* sp.) are the known vectors of TSV. However, with the incidence of TSV exceeding 40% in some fields in conjunction with the increased incidence of aphid-transmitted viruses raises the question of whether soybean aphid is also a vector of TSV.

CMV is less common in soybean and although the host range is large, the predominant reservoir of this virus affecting snap bean in the Midwest is uncertain at this time. It is conceivable that CMV was introduced through planting of contaminated seed. Consequently, seed lots will be monitored for the presence of seed-borne CMV.

Studies to further characterize and identify unknown viruses in field samples are ongoing. Screening a wide array of bean germplasm for resistance to the virus complex and to individual strain components of the complex is planned.

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CHARACTERIZATION OF FUSARIUM WILT ISOLATES COLLECTED IN THE CENTRAL HIGH PLAINS

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Fusarium wilt, caused by *Fusarium oxysporum* (Fo) Schlechtend:Fr. f. sp. *phaseoli* (Kendrick and Snyder) (Fop), is a serious vascular wilt disease in common bean (*Phaseolus vulgaris* L.). Five races of the pathogen were characterized by Woo et al. (1996) based on reactions on a set of differential cultivars. The races corresponded to geographic origin with races 1, 2, 3, 4, and 5 from South Carolina, Brazil, Colombia, Colorado and Greece, respectively. Recently, two additional races from Spain have been characterized using molecular techniques (Alves-Santos et al., In Press). In the Central High Plains region of the U.S., yield losses to Fusarium wilt have been recorded as high as 30% when appropriate provoking conditions such as poor soil drainage, extremes in moisture level, temperature extremes, and soil compaction exist (Schwartz et al., 1996).

Genetic control of Fusarium wilt resistance in common bean has been shown to be race specific (Ribeiro and Hagedorn, 1979; Salgado et al., 1995). Consequently, to effectively control Fusarium wilt of common bean in the Central High Plains, the genetic diversity of Fop in the region must be assessed. The objectives of this study were to assess genetic diversity among isolates of Fop and Fo found in the Central High Plains using RAPD banding patterns, and to determine if RAPD markers could differentiate between pathogenic and nonpathogenic isolates.

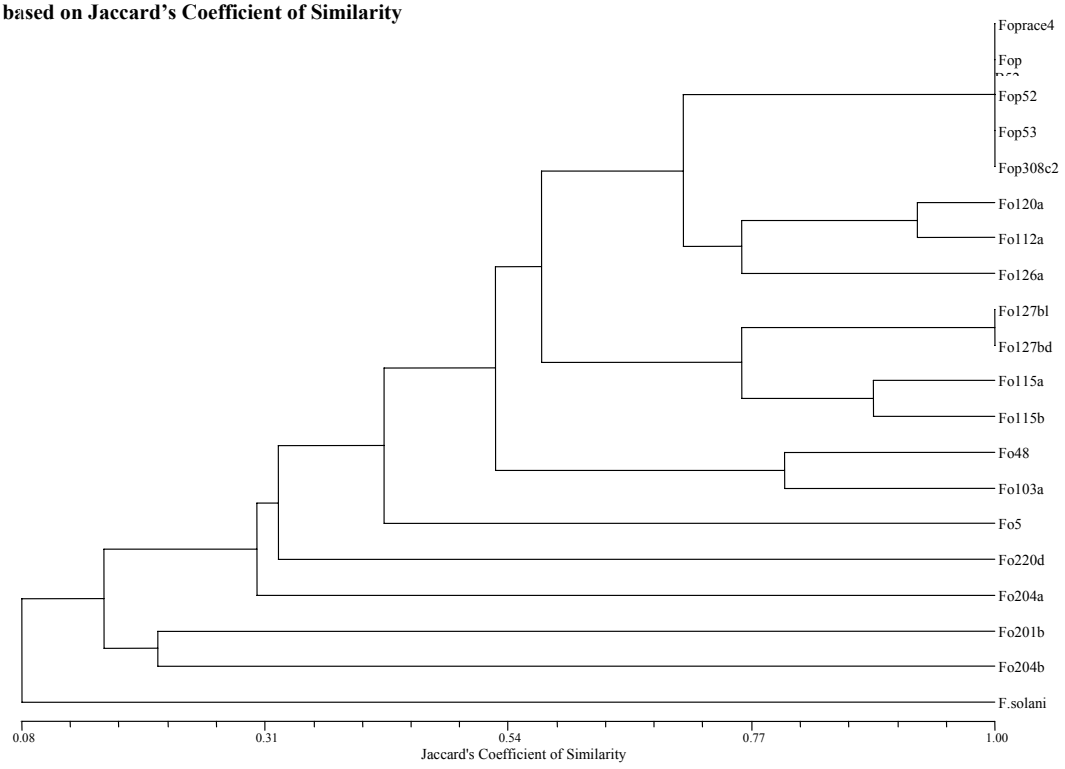
Isolates of Fo were collected from diseased common bean plants throughout the High Plains region and cultured on artificial media. Pathogenicity of the isolates was determined by reaction on the cultivar 'UI-114', a susceptible host cultivar. DNA from each isolate was extracted from and purified for the RAPD reactions. Amplified DNA was separated by agarose gel electrophoresis. Twelve RAPD primers were used to generate a binary matrix of the RAPD banding patterns. The numerical taxonomy package NTSYS 2.0 was used in all phylogenetic analyses in this study. The genetic distance matrix was subjected to the SAHN module to create a dendrogram based on an unweighted paired group method with arithmetic averages (UPGMA).

Results and Discussion

Amplification of *F. oxysporum* DNA using the RAPD technique produced clear, reproducible, polymorphic bands that differentiated pathogenic isolates (Fop) from nonpathogenic isolates (Fo). Among 19 isolates collected from diseased bean tissue, five were pathogenic and 14 were non pathogenic. All pathogenic isolates examined in this study had identical RAPD banding patterns and clustered in one phenetic group, while the nonpathogenic isolates were more genetically diverse (Figure 1). The pathogenic isolates were also compared with known races 1, 3, 4 and 6 of Fop. RAPD banding patterns were able to distinguish among races 3, 4 and 6, but not

between race 1 and 4. These results suggest that RAPD markers are an effective tool to assess genetic diversity of Fop isolates in the Central High Plains. Future work will involved a larger scale screening of isolates found in the Central High Plains, as well as the development of diagnostic tools such as scar markers to differentiate among isolates of Fo and races of Fop.

Figure 1. UPGMA Dendrogram of *Fusarium oxysporum f. sp. phaseoli* (Fop) and Non-pathogenic (Fo) isolates based on Jaccard's Coefficient of Similarity



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MANAGEMENT OF BEAN ROOT ROTS BY CULTURAL PRACTICES

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Introduction

Root rot diseases are widespread and often cause significant yield losses to beans throughout the production areas in New York State. These diseases are most damaging when soil conditions are poor as a result of inadequate drainage, poor soil structure, low organic matter, low soil fertility, and high soil compaction. General symptoms of damage by root rot pathogens to beans include poor seedling establishment, damping-off, stunting and uneven growth, chlorosis, premature defoliation, death of severely infected plants, and lower yield. Roots of infected plants are reduced in size, discolored, and exhibit various stages of decay. The tap root of severely infected plants often die, but large numbers of adventitious roots are produced from the stem above the infected areas and near the soil surface. Root diseases of beans in New York can be caused by several soilborne pathogens, singly or in various combinations, resulting often in disease complexes. The major pathogens include *Fusarium solani* f. sp. *phaseoli*, *Pythium ultimum*, *Rhizoctonia solani*, *Thielaviopsis basicola*, and *Pratylenchus* spp. (lesion nematodes). Specific damage symptoms and diagnostic lesions of distinct shapes and colors are produced on infected roots and/or stems when beans are attacked by a single pathogen (1, 3).

The involvement of multiple pathogens with diverse biology in causing root rots of beans has made it difficult to effectively control these diseases with a single and practical management option. Thus, effective management of these diseases at the present time is possible only through the use of a combination of compatible and appropriate control options (cultural, biological and/or chemical) utilizing the principals and strategies of Integrated Pest Management (SOIL-IPM). It is known that almost all crop and soil management practices have a direct or indirect impact on root disease incidence and severity. Thus, several trials were conducted over a number of years to assess the role of various production practices on bean root rot incidence and its management. These investigations were supported financially by the NYS Bean Research Associations as well as funds from other sources. The following are brief summaries of the results obtained from several tests conducted in New York dealing with the impact of cultural practices on root rots of beans (2, 4, 5).

Cover Crops Incorporated as Green Manures: The incorporation of green manures of grain crops such as oats, ryegrass, barley, wheat, sudangrass, ryegrain and others have generally resulted in reduction of root rot severity and increased bean yield. Recent results from greenhouse and field tests suggested that green manures of the cover crops included differed significantly in their effect in suppressing root rot severity and damage to beans. In one field test, a previous cover crop of ryegrain incorporated as a green manure resulted in the highest bean yield and slightly lower root rot severity ratings, whereas that of hairy vetch resulted in the lowest yield and highest root rot severity ratings. In another test, the number of lesion nematode in bean roots were lowest after a cover crop of ryegrass, rapeseed or sudangrass, but were highest in roots of beans planted after hairy vetch, alfalfa or ryegrain.

Crop Rotations: Previous results in New York and elsewhere have suggested that rotating beans with grain crops including corn, wheat, rye, barley, oats and others was effective in reducing bean root diseases and their damage. Recently, a 2-year rotation out of beans (2 consecutive years of sweet corn or 1 year of table beet and 1 year of sweet corn) significantly increased bean yield (pod weight by 25%) and slightly reduced root rot severity ratings as compared to a continuous bean production in a field with moderate infestation of root pathogens.

Composts and Soil Amendments: In fallow plots, the addition of brewery compost resulted in a significant increase in plant population and pod yield of beans as well as reduction in root rot severity

ratings. The addition of corn silage also exhibited similar effect as the brewery compost, but the differences were not significant. Interestingly, the addition of the brewery compost and the corn silage did not show clear beneficial effects in combination with the rye/vetch mixture as a cover crop, although plant population and pod yield were higher in plots receiving the two amendments. In another 4-year test, the annual application of chicken compost resulted in significant reduction of the number of lesion nematode extracted from bean roots and also increased total biomass of snap beans, but not pod weight.

Tillage Practices: Soil management practices that contribute to reducing soil compaction, increasing drainage, increasing soil temperature, and deep turning of infected crop debris will result in better root systems, higher yield and reduced damage from root pathogens. Previous results demonstrated that beans grown on raised ridges generally yielded higher and exhibited lower root rot severity ratings than those grown on flat seedbeds, especially during cool and rainy periods. Recent results showed that sub-soiling of root rot fields the previous fall resulted in significant increase in plant population and yield of beans the following season, but did not affect the root rot severity. Raised ridges and sub-soiling resulted in bigger and deeper roots systems of beans. Results from a reduced tillage experiment showed that beans grown in rototilled and chisel-plowed plots had significantly higher root rot severity and lower yield than those grown on the normally-plowed (moldboard) plots established in a heavily infested field.

Selecting Root Rot Tolerant varieties, if Available: Information on the reaction of adapted commercial varieties to root pathogens is limited. In two of the test seasons, commercial snap bean varieties were evaluated in non-treated and fumigated (methyl bromide or Vorlex) sections of the root rot field at Geneva. Results obtained suggested that all the varieties tested (> 20) were susceptible to the root rot complex in this field. Soil fumigation reduced root rot severity and greatly increased pod yield of all varieties. However, the data collected also showed that the varieties differed in their emergence, stand establishment, and yield, especially in the non-treated plots. In 1999, Summit, True Blue, Hystyle, Grenoble, Endurance and Flo were among the highest yielding in the non-treated plots. The ranking of varietal performance showed variability among seasons, which may be due to environmental adaptation or resistance to a single pathogen that becomes active that year. The availability of such data will assist in selecting the varieties to be planted in known infested fields.

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APPARENT VULNERABILITY OF CERTAIN OF RUST-RESISTANCE GENE COMBINATIONS IN COMMON BEAN FOR THE MANAGEMENT OF
Uromyces appendiculatus

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The rust disease of the common bean is economically very important in many bean-producing regions of the world including the United States. It is particularly important in South Africa, the Caribbean and other regions of Central America and Mexico. The bean rust fungus has abundant virulence diversity and many races have been reported in the United States and other parts of the world. The fungus is macrocyclic and genetic recombination occurs in this pathogen. In addition to its extensive virulence diversity, the virulence composition of the pathogen in one location may shift from one season or year to another.

The broad and ostensibly shifty diversity of the rust pathogen complicates the development of bean cultivars with durable rust resistance, particularly in areas, such as Central America, where the pathogen exhibits vast diversity. To mange effectively bean rust the resistance used should be broad spectrum and should also limit the capacity of the pathogen to adapt to the resistance deployed. Using bean cultivars with single rust resistance genes to control bean rust has often resulted in failures. Similarly, cultivars with two genes for resistances to a narrow spectrum of the pathogen races have not been effective. The pinto bean cultivar Olathe was resistant to rust when it was released in Colorado but currently it is susceptible to several races of the pathogen present in Nebraska, Colorado, and other states. The failure of single genes to manage plant diseases caused by virulence-variable pathogens, prompted scientists to use strategies, such as gene pyramiding that result in the accumulation of multiple disease resistance genes in a variety. Gene pyramiding has been used successfully to achieve durable resistance to leaf and stem and leaf rust of wheat (1). Several dry and snap bean cultivars released recently have several rust resistance genes (2). Wheat cultivars with combinations of effective stem rust resistance genes have been more resistant over a long period of time than closely related cultivars that have fewer of the same resistance genes. However, there are factors other than gene number that are also associated with durability of resistance. Certain gene combinations appear to be more durable than others. In beans, combining two or three rust resistance genes may not be sufficient to control the broad virulence diversity exhibited by the rust pathogen in some locations.

All the rust resistance genes identified to date in beans are dominant. These genes vary in the number of races of the rust pathogen that they control (Table 1). Most, including Ur-3, *Ur-5*, *Ur-11*, and others, are from beans of Middle American origin, but two genes, *Ur-4* and *Ur-6*, utilized extensively in snap and dry beans, are from beans of Andean origin. The genes of Middle American origin have resistance to a much larger number of races than the Andean genes. *Ur-11* is resistant to all but one race of the 90 races of the rust pathogen maintained at Beltsville but it is susceptible to race 108. Race 108 is controlled by *Ur-3* and *Ur-4* and other sources of resistance. Thus, a combination of *Ur-11* with *Ur-3* or with *Ur-4*, provides resistance to all 90 races maintained at Beltsville. Another gene of Middle American origin, from CNN, is susceptible to only 7 of the 90 races maintained at Beltsville but it also is susceptible to race 108.

Some gene combinations in beans appear to be vulnerable even though they contain several rust resistance genes. For example, the combination of *Ur-3*, *Ur-4*, *Ur-5*, and *Ur-6* is susceptible to 7 races (races 58, 67, 93, 96, 100, and 107) of the 90 maintained in Beltsville. However, if *Ur-3* is replaced with Mexico 235, the combination is susceptible to only one of those races and if *Ur-6* is replaced with Olathe, the combination is resistant to all 90 races. A combination of *Ur-3*, *Ur-4* and *Ur-6*, which is similar to that of BelDakMi-RMR-18 minus the *Ur-11* gene, is susceptible to 14 of the 90 races maintained at Beltsville. Again, if *Ur-3* and *Ur-6* are replaced by Mexico 235 and Olathe, this combination is resistant to all 90 races. The *Ur-4* and *Ur-11* combination, which is present in many snap and dry beans released, provides resistance to all 90 races maintained at Beltsville. However *Ur-4* is susceptible to 57 of the 90 races maintained at Beltsville and to many races throughout the world. Thus, when Ur-11 is combined with *Ur-4*, the resistance in this combination is many locations is provided only by *Ur-11*. To avoid the exposure of *Ur-11* and prevent its demise, it should be combined with other genes that provide broader resistance than *Ur-4*. Moreover, there are some locations in Honduras and perhaps other parts of the world where *Ur-11* is susceptible and Ur-4 does not provide

resistance, rendering this rust resistance gene combination totally susceptible. Alternatively, combining the resistance of *Ur-11* with that of PI 260 418, and Andean bean with resistant to race 108 and most races of the rust pathogen, is more likely to provide broad and durable rust resistance in most locations of the world.

Reaction of selected bean differential cultivars with dominant rust resistance genes to 90 races of the rust pathogen, *Uromyces appendiculatus*

Bean Cultivar	Resistance gene	Reaction				Reaction to race 108
		Immune	Highly Resistant	Resistant + MR	Susceptible	
Aurora	***Ur-3***	0	44	7 + 1	**38**	Resistant
Mexico 235	***Ur-3*+**	3	50	20 +6	11	Resistant
Early Gallatin	***Ur-4***	0	31	1+1	57	**Resistant**
Mexico 309	***Ur-5***	0	22	48	20	Susceptible
G. G. Wax	***Ur-6***	3	22	2 + 1	**62**	Susceptible
Olathe	***Ur-6* +**	2	22	31 + 2	33	Resistant
CNN		4	10	67 + 2	7	Susceptible
PI 181996	***Ur-11***	0	0	89	1	Susceptible

Immune: No visible symptoms. Highly resistant: Necrotic spots without sporulation. Resistant: Small Sporulating pustules predominantly less than 0.3 mm diameter but also with some pustules 0.3-0.5 mm in diameter. Susceptible: Large sporulating pustules larger than 0.5 mm in diameter.

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GENETIC VARIABILITY AMONG *Colletotrichum lindemuthianum* RACES USING RAPD MARKERS

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Introduction:

Common bean anthracnose caused by the *Colletotrichum lindemuthianum* fungus is one of the most important diseases of the crop found mainly in regions where cool temperatures and high air humidity are favourable its development (Pastor-Corrales and Tu, 1989). In recent studies using the binary nomenclature, Balardin (1997) in Rio Grande do Sul state enabled the identification races, namely 5, 65, 31, 453, 17, 23, 73, and 55. In Paraná state, Thomazella et al. (2000) identified races 7, 31, 65, 69, 73, 81, 87, 89. Presently, genetic divergence studies on *C. lindemuthianum* have been carried out by associating the data obtained from the 12 differential cultivars reaction with the data obtained from molecular analysis at the pathogen DNA level. The objective of this study was to investigate the genetic variability of *C. lindemuthianum* races using RAPD molecular markers.

Material and Methods:

A total of 15 primers was screened to select polymorphic primers for races 7, 31, 65, 69, 73, 81, 87, 89, 95, and 2047. Execpt race 2047, these races were chosen because of their ocurrence in Parana State. Genomic DNA was obtained essentially by the method of Raeder and Broda (1985). RAPD reactions were carried out with following primers selected (OPA 05, OPA 13, OPK 12, OPM 04, OPF 07, OPF 06, OPF 05, OPD 11, OPC 08, OPF 16, OPG 05, OPE 18, OPI 03, OPC 12, OPG 02), which consistently generated major polymorphic amplicons in all races. RAPD bands were scored as present (1) or absent (0) for each race. The genetic distance between races was estimated by using a binary matrix combining all the data pooled from the 15 RAPD primers for the 10 races. The races were clustered by Tocher and Single Linkage methods, based on matrix binary, using the Genes computer program.

Results and Discussion:

The DNA amplification products of the *C. lindemuthianum* races reproduced a total of 138 polymorphic bands using 15 primers. Figure 1 shows the amplification obtained by the OPA 13 primer, where the presence of markers can be visualized. The data analysis using the arithmetic complement of the Jaccard index showed that the greatest distance (94.33%) was between races 7 and 2047. Races 73 and 81 showed the smallest genetic distance with magnitude of 21.66%. It is pointed out that these two races showed virulence only for the cultivars of Mesoamerican origin (Thomazella et al. 2000). According to Pastor-Corrales (1996), *C. lindemuthianum* can be divided into two groups according to virulence, one group specialized in hosts from the Mesoamerican gene pool and the other specialized in hosts from the Andean gene pool.

Grouping analysis: The Tocher Cluster analysis on RAPD data formed four groups. Group I contained races 73, 81, 87, 31, 69, 89 and 65; group II, group III and group IV included only one race each, respectively, races 95, 7 and 2047. Using the same RAPD technique, Balardin et al. (1997) who obtained one group containing races 81, 89, 31 and 73, and an isolated group that included race 2047 only. Figure 2 shows that races clustered into four groups by Single Linkage method. The results obtained by the Single Linkage method were similar to those by the Tocher method. The binary data obtained by RAPD markers of various isolates of the 7, 31, 81 and 87 races were multivariate analyses. The results showed the presence of molecular variability within the races (7, 31 and 81). Similar results

were obtained by Balardin and Kelly (1998), who suggested that races 7, 17, 31 and 73 present intra-race molecular variability.

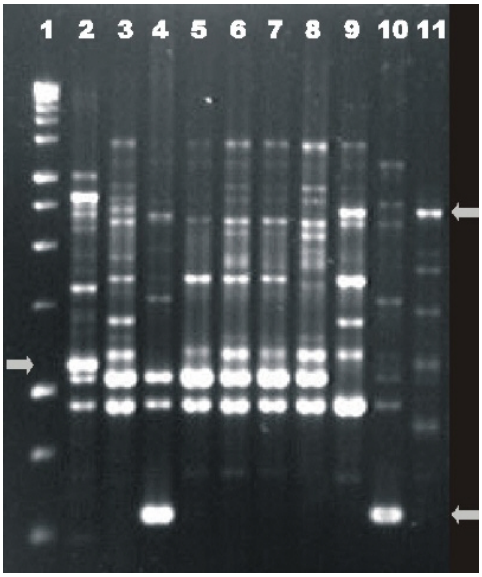

Figure 1. Electrophoretic analysis of amplification genomic DNA from *Colletotrichum lindemuthianum* races, using OPA 13 RAPD marker. Lane 1, molecular weight marker; lanes 2 - 11, respectively races 7, 31, 65, 69, 73, 81, 87, 89, 95, and 2047.

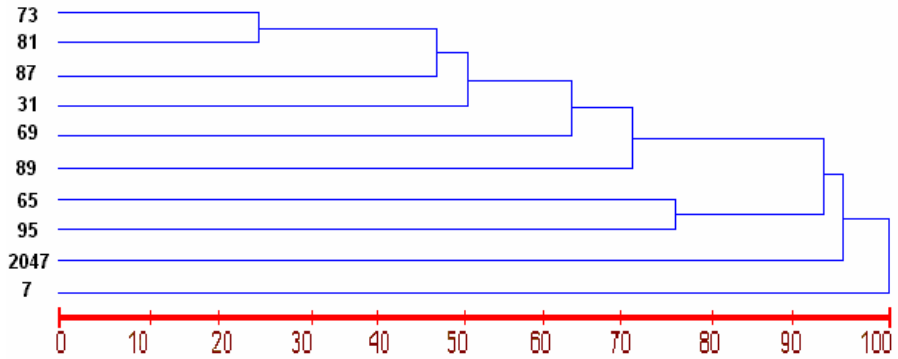

Figure 2. Genetic dissimilarity among 10 *Colletotrichum lindemuthianum* races, by the Single Linkage method based on 138 polymorphic fragments using the Arithmetic Complement of the Jaccard Index.

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Suppression of White Mold in Dry Bean with Lactofen Application

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White mold [*Sclerotinia sclerotiorum* (Lib.) deBary] is capable of infecting hundreds of plant species including dry bean (*Phaseolus vulgaris* L.). Favorable disease conditions can result in severe yield losses in dry bean (Schwartz and Steadman, 1989). Adequate genetic resistance to white mold is not available in commercial cultivars; therefore, North Dakota dry bean growers rely on fungicide applications as a primary control method (Lamey et al., 2000), in spite of the $60/ha expense for fungicide application. Interactions between herbicides and plant pathogens can result in decreased or increased disease severity (Altman and Campbell, 1977). Dann et al. (1999) found an interaction between white mold and lactofen [(±)-2-ethoxy-1-methyl-2-oxoethyl 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoate] application in soybean (*Glycine max* L.). They reported a 40 – 60 % reduction in disease severity from lactofen treatment under high white mold pressure. Seed yield, however, was not significantly increased due to white mold suppression. Lactofen is a post-emergence herbicide labeled for annual weed control in soybean with a grower cost of approximately $0.06/g a.i.. Lactofen causes leaf necrosis in susceptible species via cell membrane disruption. We evaluated white mold reaction in dry bean after lactofen application to determine its feasibility as an alternative white mold control.

The interaction of lactofen application and white mold reaction in dry bean was evaluated in three environments. Four-row plots of 'Othello' pinto and 'Norstar' navy were planted in 76-cm wide rows at Erie, ND, 2000 and 2001, and Hatton, ND, in 2001. Treatments were replicated three times and included untreated control, and 35 and 53 g a.i. ha^{-1} of the lactofen formulation Cobra® (Valent U.S.A., Corp.) in all environments. In 2001, a fungicide treatment (benomyl) and two rates (35 and 53 g a.i. ha^{-1}) of another lactofen formulation, Phoenix® (Valent U.S.A., Corp.), were included. All herbicide treatments were applied at pre-bloom and fungicide treatment at 20% bloom. Ascospores were sprayed at peak bloom in the plant canopy at a rate of 2.5×10^5 spores/plot to supplement natural white mold infection. Plant height (PH) was recorded 7 days after treatment (DAT). Phytoxicity (percent injury compared to untreated) was recorded at 7 and 14 DAT in both environments. White mold was evaluated using a disease index (DI) calculated by summing the mean 0 – 6 score of 20 samples/plot where 0 = no infection and 6 = plant death, plus 0.5 for a sample plant with main stem infection, plus 1.0 for a dead sample plant, plus a 0 – 6 rating on a whole plot basis. White mold evaluation was conducted at approximately R6 and R9 growth stage. Yield and 100 seed weights were calculated based on seed harvested from 3.7 m of each of the two center rows of each plot.

Adequate white mold was obtained only in Othello at Erie 2000 and in both cultivars at Hatton 2001. Conditions at Hatton 2001 were excellent for crop growth and white mold potential. In Othello at Erie 2000, DI scores for Cobra 35 and Cobra 53 were 4.1 and 3.4, significantly less than the untreated with a DI of 6.1. Seed yield was higher in both Cobra treatments but was not significant. Disease index was significantly reduced and yield significantly increased with benomyl application in both cultivars at Hatton 2001 (Table 1). In general, lactofen application tended to decrease DI and increase yield in both cultivars at Hatton 2001. In Othello at Hatton 2001, all four lactofen treatments had DI's less than the untreated, but only Cobra 35 was significant (Table 1). For seed yield, Phoenix 35 was the only lactofen treatment showing a significant increase; however, all lactofen treatments had higher yields than the untreated check. In Norstar at Hatton 2001, all lactofen treatments significantly reduced DI compared to untreated, however seed yield was not significantly higher for any lactofen treatment and Phoenix 53 yielded less than the untreated check (Table 1). Plant height and phytotoxicity ratings at Erie 2000 and 2001, were similar to Hatton 2001 (Table 1). Lactofen application reduced plant height and caused leaf necrosis; however, approximately 2 to 3 weeks after application, injury symptoms were less evident, especially under optimum growing conditions observed at Hatton 2001. Plant height was significantly reduced in lactofen treatments compared to untreated in both cultivars (Table 1). The higher rate of lactofen was more injurious to both cultivars for both formulations while Cobra 35 caused the least amount of injury in both cultivars (Table 1).

Table 1. Response of two dry bean cultivars to lactofen application at Hatton, ND, 2001

Treatment		Plant Height	Average Phytotoxicity	Disease Index	Yield	
		--cm--	--%--			kg ha^{-1}
Othello						
	Untreated	41.0	0.0	11.7	2620	
	Benomyl	43.3	0.0	5.5	3570	
	Cobra 35	38.0	19.2	7.1	2740	
	Cobra 53	36.3	24.2	8.8	2700	
	Phoenix 35	37.3	20.8	7.8	3110	
	Phoenix 53	35.7	25.8	7.6	2900	
	LSD (0.05)	2.5	3.9	4.4	460	
Norstar						
	Untreated	39.3	0.0	9.6	3060	
	Benomyl	39.0	0.0	4.2	3570	
	Cobra 35	35.3	18.3	6.8	3260	
	Cobra 53	34.3	22.5	5.5	3320	
	Phoenix 35	35.3	20.8	4.6	2970	
	Phoenix 53	36.0	22.5	6.7	3320	
	LSD (0.05)	2.6	2.7	1.9	500	

Summary

Based on these data, lactofen application decreased white mold and generally increased seed yield. It appears that larger differences are observed under high white mold pressure. Lactofen did not control white mold equal to benomyl fungicide, but the response observed from lactofen would be cost effective from a grower perspective. Therefore, further investigation of the interaction between lactofen and white mold is justifiable. Evaluation of phytoalexin accumulation after lactofen application could confirm a relationship between the herbicide and plant defense response. Also, manipulation of the lactofen treatments may lead to more disease reduction or a reduction of the phytotoxic effects observed in dry bean.

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SCREENING FOR AND IDENTIFYING SOURCES OF RESISTANCE TO *SCLEROTINIA SCLEROTIORUM* IN COMMON BEAN

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Introduction

White mold of common bean, incited by *Sclerotinia sclerotiorum*, causes yield losses to both dry and succulent bean crops throughout the world. Difficulties in managing this disease result in part from persistence of sclerotia inoculum in soil, wide host range of the pathogen and lack of highly resistant germplasm. Developing resistant cultivars is the most economical disease management strategy for the grower. The objective of the study was to identify broadly resistant bean genotypes by testing putative sources of partial resistance developed by bean breeders with laboratory, greenhouse and field methods in different locations.

Materials and Methods

Field tests consisted of two rows of each entry and a common susceptible genotype resulting in a three-row plot 4.6m (15 ft) long replicated three times in a randomized complete block design. The laboratory and greenhouse tests were detached leaf (Steadman *et al.*, 1997), straw (Petzoldt & Dickson, 1996), oxalate (Kolkman & Kelly, 2000), and modified limited term (Pennypacker & Hartley, 1995). In addition to the data of the authors Kolkman and Steadman, data were generated by J. Costa (Brazil), K. Grafton (North Dakota), J. Kelly (Michigan), K. Kmiecik (Wisconsin), J. Myers (Oregon) and P. Miklas (Washington). Location and tests were as follows: oxalate - Brazil, North Dakota, Michigan; field - Michigan, Washington, Wisconsin; straw - Oregon, Washington, Wisconsin; detached leaf - Nebraska; and limited term - Brazil. Because of the differences in data sets, e.g., field disease severity, lesion size, length of stem affected and number of nodes affected, the entries were ranked from most resistant (1) to most susceptible (12) in each test. A Spearman's rank correlation was used to compare entry rankings in each test.

Results and Discussion

In general, there were significant ($p< 0.05$) positive correlations among Michigan field, Oregon straw, Washington field and straw, and Wisconsin field and straw tests. The three most highly associated tests were between Michigan field and Washington field (r=0.746, p=0.005); Michigan field and Wisconsin field (r=0.795, p=0.002) and Washington field and Wisconsin straw (r=0.762, p=0.004). Oxalate tests were not correlated significantly with each other nor were they correlated with other tests. Both environmental variation between tests and variation in methodologies may have contributed to the lack of correlations between oxalate tests. The detached leaf test was similar to the oxalate test in its lack of correlation with other tests. Since only one detached leaf test and one modified limited term test were compared, it was not possible to evaluate consistency in different locations. However, the straw and field tests from Washington, Michigan, Wisconsin and Oregon produced similar rankings even though different *S. sclerotiorum* isolates were involved at each state location.

When an ANOVA was used on ranks, with each test as a block and bean genotype as a treatment, there were significant differences (p=0.004) among genotypes (Table 1). B7354 (J. Myers) and I9365-25 (P. Miklas) had the best mean rank, but L192 and MO162 (J. Myers), G122, PC-50 and NY6020-5 (M. Dickson) all were significantly ranked lower than the susceptible control great northern Beryl.

Table 1. Mean ranking of bean lines for reaction to *Sclerotinia sclerotiorum* from 11 field and in vitro tests.

Entry	Ranking	T Grouping
Beryl	9.723	A
N97774	8.273	AB
Prosperity	8.000	ABC
ND8915146-02	7.818	ABC
ExRico (Bunsi)	7.727	ABC
NY6020-5	6.273	BCD
PC-50	6.091	BCD
M0162	5.455	BCD
G122	5.273	CD
L192	4.818	D
I9365-25	4.455	D
B7354	4.455	D

Means with the same letter are not significantly different LSD (0.05) = 2.832.

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BREEDING FOR COMMON BLIGHT RESISTANCE IN DRY BEANS IN SOUTH AFRICA

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Introduction

Common bacterial blight is a major constraint limiting South African dry bean production. The disease is widespread and occurs in all the major commercial production areas. Improvement of cultivars, by introducing stable resistance, is therefore the main objective of the bacterial research program at the Agricultural Research Council-Grain Crops Institute (ARC-GCI), South Africa.

Material and Methods

XAN 159 and Wilk 2 were selected for backcross breeding to improve the resistance of Kranskop (speckled sugar bean) and Teebus (small white canning bean). These cultivars were selected on the basis of their commercial value. Crosses were made between the resistant donor (pollen) parents, (XAN 159 and Wilk2) and the recurrent susceptible parents (Kranskop and Teebus respectively). First trifoliate leaves of plants from the F1-generation were inoculated with a bacterial suspension containing approximately 10^8 CFU/ml water, using the multiple needle puncture method (Andrus, 1948). Leaves were rated for infection 14 days after inoculation on a 1 to 9 scale with 1 being highly resistant and 9 being highly susceptible. Susceptible plants were discarded (plants rated 4-9) and resistant plants (rated 1-3) selected for backcrossing. Major emphasis was placed on testing leaf blight resistance. The process of backcrossing was continued until a total of five generations of backcrossing and testing was completed.

Segregating populations from backcrosses were evaluated in field trials at Potchefstroom during the growing season and in KwaZulu/Natal during winter. Teebus were planted throughout the plot as susceptible check and also served as spreader rows. First or second trifoliate leaves of each plant were inoculated using the multiple needle method and this was followed by spray inoculating plants weekly using a backpack sprayer. Each plant was rated separately and single plant selections made. Spray inoculated canopies were evaluated periodically from when first symptoms appeared on the susceptible checks until the crop matured. Single plant progeny rows were inoculated and rated similarly. Single plant selections were made until single rows with uniform high levels of resistance could be selected. After extensive field evaluation homozygous lines were handed to the breeder and evaluated for yield and other agronomic traits.

In addition to phenotypic disease reaction, molecular markers were used during the latter stage of the study to confirm transfer of resistance to local cultivars. The markers, SU91 and BC420 (XAN 159 derived), were tested on resistant lines from segregating populations. SCAR marker SAP6, derived from a resistant line GN Nebr. Sel#27, was also included.

Results

Improvement of small white canning beans

Resistance from XAN 159 and Wilk 2 were successfully transferred to the cv. Teebus in two separate backcross programs. Both programs have progressed to the completion of BC5. Approximately 98% of Teebus has been recovered with the addition of CBB resistance genes. Final selections were made from the F5-progeny rows. Field selections of Teebus backcrosses

judged to be homozygous for important properties were tested for canning quality. The best lines will be evaluated in yield trials in four different localities for 2 consecutive years. Successful lines will be entered in the National Cultivar Trials and seed be made available to farmers.

Improvement of large seeded red speckled sugar beans

Progress in improvement of CBB resistance in Kranskop, has had limited success. Resistance was successfully transferred from both XAN 159 and Wilk2. Only moderate levels of resistance were, however, observed in lines with acceptable seed color. Crosses were made between Kranskop and Vax 4. High levels of resistance were identified in F1 progeny and first backcrosses were completed. Some of the progeny from BC_1 to BC_3 that have been selected in the field as offshoots of the backcrossing program exhibit excellent desirable properties such as high yield and disease resistance, but are not suitable for use as such, having a seed color not acceptable for the market. Some of these lines might be acceptable in other African countries where a greater variety of seed types are planted. These lines were distributed to the SABRN and ECABREN networks for evaluation.

Both markers SU91 and BC420 (XAN 159 derived) as well as marker SAP 6 (GN Nebr. Sel. # 27) were successfully used to confirm resistance in selected lines. Marker SAP6 was present in Teebus and Kranskop and could have been introduced by parents used in developing these cultivars. Advanced Teebus lines developed through backcross breeding with XAN 159 had both SU91 and BC420 markers. Greenhouse results indicated that these lines had higher levels of resistance than XAN 159. This can be explained by the combined resistance from GN Nebr. #1 sel. 27 and XAN 159, present in these lines.

Both SU91 and BC420 markers are present in Wilk 2, 4 and 6 which indicates that resistance from XAN 159 (or the same source) was used in developing these lines. PCR studies from BC 5 lines derived from backcrosses between Wilk 2 and Teebus as recurrent parent indicated that both markers SU91 and BC420 (XAN 159 derived) were transferred to these lines.

XAN 159 derived Kranskop-lines only had moderate levels of resistance when tested in the greenhouse. PCR studies indicated that the BC420 marker was absent in these lines. This marker is near the *V* locus conditioning purple flower color (Miklas *et al*., 2000) resulting in resistant plants exhibiting seed unacceptable for the market. Presence of marker BC420 seems important to obtain high levels of resistance.

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A MAJOR QTL FOR COMMON BACTERIAL BLIGHT RESISTANCE IN GN#1 SEL 27 DERIVES FROM THE GREAT NORTHERN LANDRACE MONTANA NO. 5, NOT TEPARY BEAN

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Common bacterial blight [CBB, caused by *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye] is a major disease of common bean (*Phaseolus vulgaris* L.) worldwide. Only low levels of resistance are found in common and scarlet runner (*P. coccineus*) beans (Singh and Muñoz, 1999). The tepary bean (*P. acutifolius* A. Gray), however, possesses the highest level of resistance. Honma (1956) is credited with the first interspecific cross between common and tepary bean. Embryo rescue was used to recover four self-fertile F_1 plants from the great northern common bean cultivar Montana No.5/Tepary 4 cross, which in turn produced a few F_2 seeds. The F_2 plants were selfed to produce the F_3 that was used to study segregation of CBB resistance, seed size, leaf length and width, and other plant traits. Segregation of plant traits was obviously skewed toward Montana No.5, the *P. vulgaris* parent.

Trait	Montana No.5	F_3 mean (range)	Tepary No.4
Seed size (g)	0.34	0.32 (0.20-0.47)	0.13
1^0 leaf length (mm)	72.6	75.7 (66-96)	46.3
1^0 leaf width (mm)	52.8	48.3 (41-63)	30.3

CBB score was normally distributed for 206 F_3 plants, but parental reactions were not reported. Eventually the Great Northern Nebraska No. 1 (GN#1) cultivar, selected for CBB resistance and good agronomic characteristics, was derived from this interspecific cross in 1961. A later maturing common blight resistant rogue plant found in GN#1 by Coyne et al.(1963) was subsequently named Great Northern Nebraska #1 Selection 27 (GN#1 Sel 27). The GN#1 Sel 27 cultivar is the source of common blight resistance in great northern cultivars Tara, Jules, Valley, Harris, Star, and Starlight among others. This source of common blight resistance has also been extensively used in the tropics and subtropics of Latin America and elsewhere (Singh and Muñoz, 1999).

The belief that the common bacterial blight resistance in GN#1 Sel 27 derived from tepary bean played a crucial role in the subsequent searches for higher levels of CBB resistance in tepary bean. Moreover, additional interspecific crosses between common and tepary bean were made (Haghighi and Ascher, 1988; Mejía-Jiénez et al., 1994; Thomas and Waines, 1984) with higher levels of common blight resistance transferred into common bean (McElroy, 1985; Scott and Michaels, 1992).

Evidence that the primary source of CBB resistance in GN#1 Sel 27 might actually derive from Montana No.5 came from two different sources simultaneously. Field trials in North Dakota in 1999 revealed that Montana No.5 exhibited a level of CBB resistance comparable to resistant cultivars with GN#1 Sel 27 derived resistance, which was supported by additional field and greenhouse tests (Table 1). In the Prosser laboratory, the $SAP6_{820}$ SCAR marker, tightly linked with a major QTL for CBB resistance derived from GN#1 Sel 27 (Miklas et al., 1996;1999), did not amplify in tepary bean (Table 2) like the other resistance-linked markers (Jung et al., 1997; Bai et al., 1997; Pedraza et al., 1997; Yu et al., 2000) which have permitted researchers to trace back the resistance genes to their respective donor tepary accessions (Miklas et al., 1999; unpublished data). However, SAP6 did amplify in Montana No. 5.

To examine the relationship of the SAP6 marker in Montana No.5 with CBB resistance, 50 individual F_2 plants from the cross Montana No.5/Othello were assayed for SAP6 marker and inoculated with CBB. Regression analysis revealed that SAP6 did cosegregate (35%) with CBB resistance, confirming that the major-effect QTL in GN#1 Sel 27 derives from Montana No.5 not tepary bean. However, the consistent observation of a higher level of resistance in GN#1 Sel 27 suggests that it likely possesses additional minor genes for CBB resistance not present in Montana No.5. Analysis of CBB reaction in the cross GN sel 27/Montana No. 5 revealed a few transgressive segregants in the F_2 (later confirmed by F_3 progeny tests), confirming presence of minor gene differences between the two cultivars.

Table 1. CBB reaction in field and greenhouse for some common bean genotypes.

	Field			Greenhouse
	Erie, ND		N. Platte, NE	Fargo, ND
Line				
	1999			
2000	**2000**			
	(1-9)		(%)	(1-9)
Montana No.5	6		7	5
Harris	6		14	3
Weihing	8	5	6	
Othello (Susc, check)	9		-	7
GN#1	-		10	5
GN#1 Sel 27	-		4	1
Matterhorn (Susc. check)	-	75	8	

Table 2. Presence (+) or absence (-) of SCAR markers linked with CBB resistance, for some tepary and common bean genotypes.

	SAP6		R7313	SU91	BC420
Tepary bean (many R lines)	-	+	+	+	
*XAN 159	-		+	+	+
Montana 5	+	-	-	-	
GN NE No.1	+		-	-	-
GN NE No.1 sel 27	+	-	-	-	
Harris	+		-	-	-
Othello (S check)	-		-	-	-
Matterhorn (S check)	+		-	-	-

* SAP6 derives from GN#1 Sel 27 via XAN 176, R7313 derives from tepary PI 440795 via OAC 88-1, SU91 and BC420 derive from tepary PI 319433 via XAN 159.

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NODULATION, SEED YIELD AND DINITROGEN FIXATION IN DETERMINATE AND INDETERMINATE COMMON BEAN CULTIVARS

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Legumes form a symbiosis with nodule-forming *Rhizobium* bacteria and are able to fix their own nitrogen. Legume crops with effective nitrogen fixation can be grown with reduced input of nitrogen fertilizers. Common bean is generally regarded to be a poor nitrogen fixer and application of N fertilizers has been recommended in bean production. The success of the inoculation process may depend on a number of factors including environmental variables and the host cultivar. Substantial genotypic variability exists within common bean cultivars for nitrogen fixation (Hardarson et al. 1993; Pena-Cabriales et al. 1993). Cultivars are classified into four main groupings based on growth habit (Kelly et al. 1987) and nitrogen fixation may vary widely depending on growth habit of the genotype (Graham 1981). The objectives of the present study were 1) to compare twelve common bean cultivars for nodulation and nitrogen fixation and 2) to determine the relationship between nitrogen fixation and seed yield among growth habit types.

The study was conducted at two locations in Saskatchewan: Saskatchewan Pulse Growers (SPG) farm (Dark Brown Chernozemic soil) and Seager Wheeler Farm near Rosthern (Black Chernozemic soil) in 2000 and 2001. In both years, the sites chosen for the study had low (less than 20 kg/ha) available N in the surface 60 cm. Twelve bean cultivars representing three commercial classes and three growth habits (Table 1) were used in the study. The treatments consisted of a control with no inoculant application and an application of granular inoculant placed in the furrow. Triple superphosphate (0-45-0) at the rate of 20 kg/ha P_2O_5 was applied in the furrow at planting. No nitrogen fertilizer was applied. The study was arranged in a randomized complete block design with four replications. Data were collected on nodule dry mass and shoot dry mass at mid- podfill, and seed yield and amount on nitrogen derived from fixation at final harvest. Nitrogen fixation was determined by the natural abundance method (Bremer and van Kessel 1990).

The results report nodule dry mass and shoot dry mass data averaged over two locations and two years of experimentation. Seed yield and nitrogen fixation are based on the first year data. Nodule dry mass was significantly higher for the inoculated plots compared to control plots for all bean cultivars (Figure 1). Type II and Type III (indeterminate) cultivars had increased nodule dry mass compared to Type I (determinate) cultivars. Nodule dry mass varied little within each growth habit group. Inoculant had no effect on shoot dry mass for all cultivars (data not shown). Applying granular inoculant increased seed yield significantly over the control. This increase in seed yield was higher for Type III cultivars (30%) compared to Type I (18%) or Type II (19%) cultivars (Figure 2). The response to inoculant application varied within Type I and Type III cultivars but was the same within Type II cultivars. Granular inoculant application increased the amount of N fixed by bean plants significantly compared to control plots (Figure 4). Type III cultivars fixed significantly greater amounts of N compared to Type I or Type II cultivars. The amount of N fixed in inoculated plots ranged from 21 to 36 kg ha^{-1}. Type III cultivars were at the upper range whereas Type II cultivars were at the lower range. Results of this study show significant differences in nodulation and N_2 fixation among common bean cultivars. The differences were associated with growth habit.

Table 1. Characteristics of bean cultivars used in the study

Name of cultivar	Commercial class	Growth habit[z]
CDC Camino	Pinto	I
CDC Pintium	Pinto	I
CDC Pinnacle	Pinto	III
CDC Altiro	Pinto	III
CDC Bianca	Great Northern	I
AC Polaris	Great Northern	II
CDC Crocus	Great Northern	III
US 1140	Great Northern	III
CDC Expresso	Black	I
AC BlackDiamond	Black	II
CDC Nighthawk	Black	II
UI 906	Black	II

[z]Growth habit: I = determinate bush; II = indeterminate upright short vine; III = indeterminate postrate long vine.

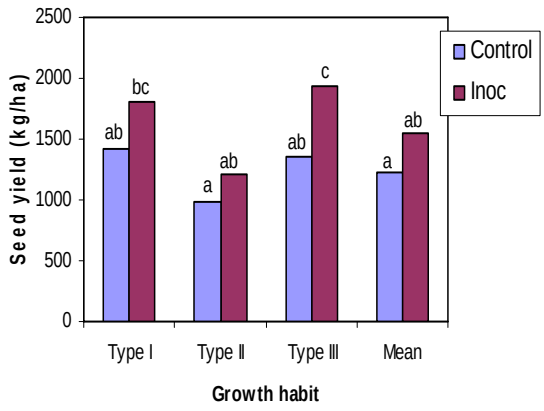


Figure 2. Effect of bean growth habit and inoculant on seed yield

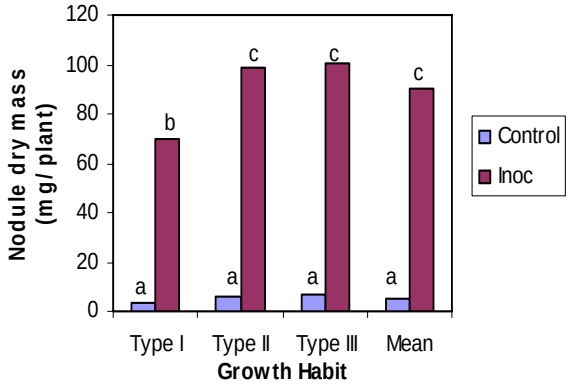
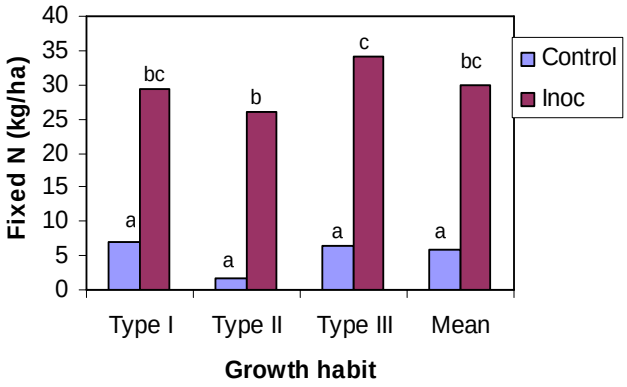


Figure 1. Effect of growth habit and inoculant on nodule dry mass



inoculant on amount of N_2 fixed

Figure 3. Effect of bean growth habit and inoculant on amount of N_2 fixed

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Drought Resistance of Black Bean Evaluated in a Lowland Tropical Environment

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Introduction. At least 60% of dry bean production in Latin America is limited by moisture stress (Singh, 1995). The lowland tropical areas of Central America are predisposed to terminal drought. In Honduras, terminal drought occurs during the second production season, la Postrera, which is characterized by less rainfall and diminishing soil moisture. Bean production area increases three-fold during la Postrera despite an average yield reduction of 50% (Cotty et al., 2001). Since adequate irrigation schemes are unrealistic due to socio-economic constraints, genetic improvement for drought resistance offers a long-lasting means of improvement.

A breeding strategy based on selection for geometric mean (GM) followed by yield under drought stress was proposed as the most efficient way to breed for drought resistance in bean (Schneider et al., 1997). Two black bean recombinant inbred line (RIL) populations combining drought resistance with tropical adaptation were developed in Michigan. An experiment involving moisture stress and non-stress treatments was conducted at Zamorano, Honduras during the dry, summer season (January-April). RILs, selected for high and low GM in Honduras, were evaluated in Michigan in 2001. The objective of this study was to confirm the drought resistance of lines selected in Honduras and identify drought resistant material suitable for black bean breeding in the Latin American/Caribbean region.

Materials and Methods. MSU drought resistant black bean line, B98311, was crossed to the tropical lines TLP 19 and VAX 5 to create two RIL populations, L88 and L91. TLP 19 and VAX 5 also offer resistance to *Macrophomina phaseolina* and *Xanthomonas campestris*, respectively. Single seed descent was used to increase 150 RILs from the two black bean populations to the $F_{3:6}$ generation in Michigan.

A completely randomized design including the 150 RILs, three parents and seven checks was planted on January 23, 2001 at the Escuela Agrícola Panamericana (EAP) in Zamorano, Honduras. Two treatments, moisture stressed and non-stressed, with three replications each, were used to assess the genotypic differences on a loamy soil. Furrow irrigation, overhead sprayers, and rainfall accounted for approximately 300 mm of water in the drought plots and approximately 500 mm of water in the irrigated plots. Yield under stress and non-stress were recorded to calculate geometric mean.

The resistant (top 10%) and susceptible (bottom 10%) RILs that were selected based on GM in Honduras were field tested in Michigan to confirm the results in 2001. A 6X6 lattice design including two moisture treatments with three replications each was used to plant 31 RILs, three parents and two checks on a McBride sandy loam in Montcalm county, Michigan. Irrigated plots received 390 mm of water, while the drought plots received 350 mm of water. Statistical analysis was performed with SAS to calculate significance of field results at $p>0.05$ (SAS Institute, 1999).

Results. A five-fold difference in mean yield was recorded between the irrigated (1957 kg/ha) and drought (269 kg/ha) treatments in Honduras. The drought intensity index (DII=0.86) was severe in which 1.0 indicates a total loss of production due to drought. A high coefficient of variation (CV) of 77% in the drought treatment resulted from the high drought stress and the incidence of *Macrophomina phaseolina*. Some RILs out-yielded parental, local, and drought resistant cultivars grouped within and outside the black bean seed class (Table 1).

Due to weather patterns, drought stress did not develop in Michigan as shown by a DII of 0.02. Low CV and LSD values in Michigan allowed the separation of lines such that 12.5% of the selected resistant lines were significantly different when compared to the highest yielding susceptible line. Yields of drought resistant and susceptible lines exhibited a similar trend compared to Honduras results. GM was significantly correlated between locations ($r = 0.63$, $p<0.001$). These results indicate that drought resistant lines identified in Honduras exhibited significantly higher yields in the Michigan environment when compared to the susceptible lines. Further testing will be conducted at the EAP and CIAT bean breeding programs in order to confirm these findings.

Table 1. Geometric mean (GM), yield under drought (Yd), and yield potential (Yp) compared between selected lines grown in Honduras and Michigan in 2001.

	Honduras			Michigan		
Line	GM	Yd	Yp	GM	Yd	Yp
	kg•ha^{-1}			kg•ha^{-1}		
L88-63	1473 (1)†	842 (1)	2576 (6)	3603 (3)	3006 (16)	4318 (2)
L88-30	1328 (3)	779 (2)	2263 (30)	3404 (9)	3454 (8)	3353 (12)
L88-69	1286 (4)	680 (4)	2432 (18)	3844 (2)	4004 (1)	3690 (5)
L88-18	368 (146)	90 (144)	1501 (148)	1996 (34)	2052 (35)	1940 (33)
TLP 19 (p)	637 (95)	169 (118)	2399 (22)	3532 (6)	3477 (7)	3589 (6)
B98311 (p)	951 (22)	375 (33)	2411 (20)	3495 (7)	3903 (2)	3129 (15)
L91-30	1073 (9)	599 (6)	1922 (83)	3483 (8)	3073 (15)	3948 (3)
L91-3	1023 (14)	435 (20)	2406 (21)	3232 (16)	3398 (11)	3073 (18)
L91-10	1004 (16)	448 (19)	2250 (31)	4050 (1)	3780 (3)	4340 (1)
L91-69	60 (160)	2 (160)	1534 (142)	1795 (36)	1929 (36)	1671 (36)
VAX 5 (p)	663 (90)	249 (82)	1765 (110)	2238 (32)	2097 (34)	2389 (31)
SEA 5 ‡	893 (30)	524 (11)	1521 (145)	-	-	-
Rio Tibagi	886 (31)	372 (34)	2108 (51)	-	-	-
Bat 477	784 (54)	400 (27)	1536 (141)	-	-	-
Tío Canela-75	602 (105)	218 (95)	1657 (129)	-	-	-
Phantom	-	-	-	3589 (4)	3611 (4)	3567 (7)
T-39	-	-	-	3538 (5)	3544 (5)	3533 (9)
Mean		269	1957		2961	3006
LSD (0.05)		333	933		819	953
CV		77	30		17	19

† Rank shown in parenthesis, (p) designates parent.

‡ Drought resistant and local checks are included in this section.

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POTENTIAL NEW SOURCES OF RESISTANCE TO WHITE MOLD IN THE *Phaseolus* CORE COLLECTIONS

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White mold, caused by *Sclerotinia sclerotiorum* (Lib.) deBary, is a serious disease on bean (*Phaseolus vulgaris* L.) in temperate regions of the world. A survey of bean growers in North Dakota and Minnesota ranked white mold as the most serious production constraint (Lamey et al., 1996). Schwartz and Steadman (1989) indicated that Nebraska dry bean crop losses averaged 30%, with individual field losses as high as 92%, because of white mold. Genetic resistance and disease avoidance due to plant structure have both been identified as possible mechanisms to reduce white mold damage in dry bean. Complete resistance is unknown and unique sources of resistance are limited, which has hampered developing resistant cultivars. Miklas et al. (1998) evaluated a portion (89 of 182 entries) of the Central and South American (CASA) core of the USDA *Phaseolus* Plant Introduction (PI) collection in an attempt to identify new sources of putative resistance. We expanded their initial evaluation to include all entries of both the Mexico (MEX) and the CASA cores by evaluating entries using the straw test (Petzoldt and Dickson, 1996) and the detached leaf assay (Steadman, 1997).

The two core collections were evaluated separately in Fargo, ND, and in Lincoln, NE, in 1998, 1999, and 2001, with different assays performed at each location. At Fargo, USDA PI's composing each of the core collections were evaluated for reaction to white mold with the straw test (Petzoldt and Dickson, 1996). Ten plants, each plant representing a replicate in a randomized blocks design, of each PI were grown in 15-cm diameter clay pots in a greenhouse maintained at 22-34°C with a 14-hr photoperiod. Plants were inoculated 28 days after planting using agar plugs on which *S. sclerotiorum* mycelium was actively growing. Plants were evaluated 10-12 d post inoculation using a 1 – 9 scoring system, where 1 = no disease symptoms and 9 = total plant collapse.

The cores were grown in a greenhouse in Lincoln for evaluation using the detached leaf assay as outlined by Steadman et al. (1997). The youngest, fully expanded trifoliolate leaf of each plant was selected for testing, transferred to the lab with petioles under water, and placed in rubber-stoppered orchid tubes filled with sterile distilled water. The leaves (with petioles remaining in the tubes) were placed in aluminum pans lined with wet paper toweling. One agar plug from the advancing mycelial margin of a culture of *S. sclerotiorum* was placed near the center, but not on the mid-vein of the middle leaflet. The pans were then covered with plastic food wrap to create a humid environment, and incubated at 22°C for 48 hr. Lesion size (cm^2) was calculated from lesion dimensions measured with a ruler. 'Othello' pinto was used as a susceptible check in all tests.

Both core collections, tested for white mold reaction using the straw test, had frequency distributions approaching a normal curve. A high number of lines scored < 5, indicating a number of lines with resistance; however, the relatively warm temperatures in the greenhouse during these test were sometimes above the maximum limit for white mold development. Othello, usually very susceptible, exhibits plant death in the straw test. However, it exhibited a less susceptible reaction in these tests, indicating that the relatively warm environment decreased disease development. Also, since these lines are heterogeneous, there was variability within many PI lines for white mold reaction. Disease reaction of the eight best PI lines from the MEX core to white mold is presented in Table 1. Eight lines were selected using results of the straw test, with comparative data from the excised leaf test. In all cases, these PI lines had scores indicating a high level of resistance, with the disease progressing to, but not beyond, the first node. However, these lines scored similar to, or slightly better than, the susceptible check Othello when tested using the excised leaf test. These discrepancies may occur because the two methods may be testing different components (leaves vs. stems) of physiological resistance to white mold. Also, eight PI lines from the MEX core were selected on the basis of performance in the excised leaf test. Again, scores were much lower than the susceptible check; in some cases, notably PI 318695 and PI 201354, scores were lower than the check in both tests. Further evaluation of these lines is warranted.

Table 1. Performance of MEX core lines to white mold reaction from two screening procedures

Entry	Straw Test Score	Excised Leaf Test Lesion Size cm^2
Straw Test		
PI 313348	2.4	8.1
PI 263596	2.5	11.4
PI 313425	2.5	9.1
PI 312018	2.7	12.7
PI 201354	2.8	8.2
PI 311974	2.8	8.9
PI 319683	3.0	12.7
PI 325653	3.0	11.4
Excised Leaf Test		
PI 417782	5.3	3.8
PI 325691	5.0	6.9
PI 318695	3.3	7.2
PI 417721	5.8	7.1
PI 325685	5.0	7.9
PI 313254	5.3	8.0
PI 313348	2.4	8.1
PI 201354	2.8	8.2
Othello	8.0	11.9
G122	3.4	12.2
LSD (0.05)	2.05	4.02

We were able to identify several lines that were classified as resistant in white mold tests. These lines may be used as parents in breeding for improved physiological resistance to this important pathogen. The value of the core collection is that it offers the possibility of testing the variability that exists in the entire PI collection while keeping entry numbers to a manageable level. Evaluating the two core collections may provide some insight as to the existence of new sources of resistance to white mold. As we identify and verify new sources of resistance, we can then expand the search to PI lines that were collected in the same geographical region.

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PRELIMINARY EVALUATION OF SCARLET BEAN LANDRACES FROM SPAIN

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Scarlet bean (*Phaseolus coccineus*) is a frequent crop in small farms and gardens on the North of Spain and Portugal. In spite of the fact of its reduced contribution to the overall production of bean in these countries, some landraces have importance in these areas as a traditional crop for self consumption or sale in local markets (Campion and Servetti, 1991; Zeven, 1993). This species deserves also special attention due to the possibility of being a germplasm source to transfer to common bean (*Phaseolus vulgaris*) resistance to diseases (Vanderborgt, 1983) as well as tolerance to some abiotic stresses. In addition, selection in a *P. vulgaris* x *P. coccineus* progeny for increased outcrossing could be an interesting objective of breeding.

At the Misión Biológica de Galicia (CSIC, Pontevedra, Spain) there is a germplasm collection, founded in 1987 (Ron et al., 1997), that includes some accessions of *P. coccineus*, being 24 landraces from Spain studied in the present paper. The evaluation of these landraces was focused on agronomic performance, as well as on quality traits of seed. The traits evaluated were the following ones (IBPGR, 1983): beginning of flowering BF (days), period of flowering PF (days), pods/plant PP, 100 seeds weight SW (g), seed length SL (mm), water absorption WA (%), shell proportion SP (%), seed yield SY (g). Additionally some organoleptic traits were evaluated in laboratory tests performed by a specialized team to set up the Global Quality (GQ) of grain . These traits, were determined after 15 hours soaking and 15 minutes boiling under pressure, according to a scale 0 to 5, were the following ones: grain integrity GI, coat surface smoothness SS, coat hardness CH, albumen hardness AH, albumen buttery AB, albumen granular AG, albumen floury AF, taste T and stripping S. Table 1 shows the results of the morpho-agronomic evaluation and GQ is shown in table 2, together with the scale for its interpretation.

According with this results it could be pointed out some landraces with appropriate attributes to be produced in this area which could be selected for variety development and common bean improvement. These landraces are:

PHA-0163, PHA-1020, PHA-1023, PHA-1024: earliness, and very good GQ
PHA-0409, PHA-1024, PHA-1025, PHA-1027: large seed
PHA-0127, PHA-0282, PHA-0469, PHA-1022, PHA-1027: very good GQ
PHA-0163, PHA-0322, PHA-0352, PHA-1019, PHA-1021: high yield

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Table 1. Results of the morpho-agronomical evaluation of 24 Spanish landraces of *Phaseolus coccineus*

LANDRACE	BF (days)	PF (days)	PP	SW (g)	SL (mm)	WA (%)	SP (%)	SY (g)
PHA-0127	45.3	74.0	31.6	103.3	18.0	117.5	0.073	36.7
PHA-0163	43.5	76.0	51.3	111.1	18.1	111.4	0.078	112.2
PHA-0166	44.0	75.5	35.8	98.6	17.5	109.5	0.078	74.9
PHA-0175	45.0	73.3	30.3	87.9	16.7	110.6	0.075	62.6
PHA-0282	47.0	71.5	34.6	126.5	19.1	109.7	0.090	88.2
PHA-0311	49.5	69.3	29.0	111.1	17.9	111.4	0.088	56.2
PHA-0322	49.5	76.8	37.5	116.9	18.7	116.2	0.083	128.7
PHA-0344	46.5	72.3	23.2	111.8	19.2	109.0	0.090	57.7
PHA-0352	49.5	79.5	57.1	111.9	17.5	95.3	0.105	123.4
PHA-0406	50.5	70.0	26.4	105.4	17.3	112.0	0.100	77.0
PHA-0409	58.0	71.5	24.5	195.0	22.4	112.9	0.170	77.9
PHA-0456	50.0	72.5	13.4	132.7	20.0	107.6	0.143	34.6
PHA-0469	47.5	74.5	31.8	117.0	18.6	110.6	0.083	89.6
PHA-1005	47.5	74.8	42.5	100.1	18.0	120.3	0.090	98.8
PHA-1015	62.3	63.3	29.3	96.0	18.0	115.2	0.075	29.1
PHA-1018	46.0	77.3	39.0	95.7	17.2	113.2	0.080	98.7
PHA-1019	46.8	74.3	37.9	133.6	19.7	108.3	0.108	100.0
PHA-1020	43.5	77.5	30.3	130.4	18.4	107.7	0.095	77.2
PHA-1021	43.3	77.5	32.4	136.7	19.4	108.2	0.118	110.2
PHA-1022	45.5	78.0	27.0	121.9	19.2	114.6	0.113	84.3
PHA-1023	40.8	78.0	31.2	103.9	18.9	108.0	0.093	63.7
PHA-1024	43.8	88.7	27.4	177.4	22.3	117.6	0.095	80.7
PHA-1025	49.5	82.3	20.3	169.0	22.6	122.7	0.130	59.3
PHA-1027	46.3	83.7	21.9	209.0	22.9	107.3	0.147	85.0

Table 2. Global Quality (GQ) of 19 *P. coccineus landraces* from Spain

GQ: 2.6G – 2.25SS – 2.6CH – 2.8AH – 2.4AB + 2.8 AG – 2.5AF + 2.4T + 2.6S			
SCORE			
54 to 25 excellent	25 to –4.5 very good	-4.5 to –34 fair	-34 to –63 poor
NONE	PHA-0127, PHA-0163, PHA-0282, PHA-0469, PHA-1020, PHA-1022, PHA-1023, PHA-1024, PHA-1027	PHA-0166, PHA-0311, PHA-0322, PHA-0344, PHA-0406, PHA-1005, PHA-1018, PHA-1019, PHA-1021, PHA-1025,	**NONE**

MARKER-ASSISTED SELECTION FOR WHITE MOLD RESISTANCE IN COMMON BEAN

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Introduction

White mold, caused by *Sclerotinia sclerotiorum*, is one of the most devastating diseases of common bean (*Phaseolus vulgaris*). Resistance to white mold in bean is a quantitative trait, complexly inherited, and highly influenced by the environment, making selection for resistance difficult. There is no complete field resistance to white mold, but there are genotypes that exhibit partial resistance, that includes physiological resistance and morphological avoidance mechanisms. Markers linked to white mold resistance have recently been reported (Park et al., 2001; Miklas et al., 2001; Kelly and Kolkman, 2001) and would be useful for MAS (marker-assisted selection). The goal of this study was to evaluate the potential of selection based on RAPD and AFLP markers linked to white mold, previously identified in the navy bean cultivar, Bunsi (Kelly and Kolkman, 2001).

Material and Methods

MAS was based on RAPD and AFLP markers identified in a F_3-derived family population, developed from a cross between two navy bean cultivars, Bunsi and Newport (Kolkman, 2000). Bunsi with an indeterminate (type II) growth habit has physiological resistance and a porous canopy for avoidance to white mold. Newport is a susceptible cultivar with a determinate (type I) growth habit. MAS was conducted on a 96 $F_{4:7}$ recombinant inbred line (RIL) population developed from a cross between Bunsi and Midland, a susceptible cultivar, using markers previously identified by Kolkman (2000). The 96 RILs were scored based on one RAPD marker (BC20.1800) mapped on linkage group B2 and two AFLP markers ($E_{AAC}M_{CTT}130$ and $E_{AGG}M_{CTT}85$) on B7. Two groups of 10 RILs were selected, one with and the other without the three markers linked to the QTL conferring resistance to white mold. To investigate the association of growth habit with resistance to white mold, five lines selected in each group were determinate and five lines were indeterminate. The 20 RILs, including Bunsi and Midland were planted in Montcalm, MI, in June 2001. Plot rows were 6 m in length, with 0.5 m row spacing. The two center rows of each plot were planted with the RIL, while the two border rows were planted with the highly susceptible cultivar, Midland. Plots were rated for disease prior to harvest, when the plants had reached the physiological maturity. Thirty plants per plot were rated from 0 to 4, where 0 = no disease present, 1 = 1 to 25 %; 2 = 26 to 50 %; 3 = 51 to 75 %; and 4 = 76% to 100 % of the plant with white mold symptoms. Disease incidence (DI) and disease severity index (DSI) were calculated for each plot. Days to flower were recorded as the number of days following planting, when 50% of the plants had at least one open flower. All plots were harvested at maturity and seed yield and weight of 100 seeds, adjusted to 18 % moisture content was recorded. The experiment was analyzed as a mixed model, considering the groups (presence or absence of markers) and growth habit (determinate and indeterminate) as fixed factors, and the genotypes as a random factor. In addition, the 20 RILs and the parents were screened in the greenhouse based on resistance to oxalate (ROX), an indirect assay to determine

physiological resistance to white mold (Kolkman and Kelly, 2000).

Results and Discussion

The potential of MAS, based on three molecular markers linked to white mold resistance, identified previously in the cultivar Bunsi, was not confirmed in this first year of field evaluation of the Bunsi x Midland RILs, at 0.05 of significance (Table 1). However, disease incidence was lower in the group of RILs selected based on the presence of the markers, at the level of 0.075 of probability. The combination of the three markers in the original population (Bunsi x Newport) explained 27 to 30% of the phenotypic variation for DI, and DSI, respectively. The lines with the three markers were more resistant to oxalate than the lines without the markers used for MAS (Table 1). Growth habit was not associated with the resistance to white mold in both groups, with or without the markers. White mold is complexly inherited and highly influenced by the environment; therefore, it is necessary to evaluate the RILs in more environments and/or years to determine the potential of MAS for resistance to white mold using the combination of these three markers.

Table 1. Disease severity index (DSI), disease incidence (DI), resistance to oxalate (ROX), yield, seed size and days to flower of the two groups of RILs selected based on the presence or absence of markers linked to QTL for resistance to white mold.

MAS[1]	DSI (%)	DI (%)	ROX	Yield (kg/ha)	Seed size (g.100 seed^{-1})	Days to flower
Presence	35.9	61.6	2.5	3511	20.0	41
Absence	42.2	72.3	3.4	3227	18.7	43
P-value	*0.172*	*0.075*	*0.001*	*0.240*	*0.061*	*0.104*
Mean	39.1	66.9	2.9	3368	19.3	42.0
CV(%)	32.8	21.5	18.3	15.7	4.0	1.8
Midland	51.4	80.0	3.9	3794	18.0	40
Bunsi	23.9	45.6	1.4	4144	22.3	41

[1] Marker assisted selection based on the RAPD (BC20.1800) and AFLP ($E_{AAC}M_{CTT}130$; $E_{AGG}M_{CTT}85$) markers.

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COMPLETION OF TESTING OF *Phaseolus coccineus* PLANT INTRODUCTIONS (PIs) FOR WHITE MOLD, *Sclerotinia sclerotiorum,* RESISTANCE

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White mold, causal pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary, results in widespread yield and quality loss of snap and dry beans. In favorable white mold environments 100% of the crop may be lost. In the United States, the annual yield loss to white mold is about 3.5% for dry beans and 2% for snap beans. Furthermore, processing plants may reject snap bean lots if pod infection rate exceeds 2% [3].

Resistance in common bean has taken the form of temporal avoidance, architectural avoidance and physiological resistance mechanisms. Studies have shown that these forms of resistance are inherited quantitatively and have low heritability [3]. One test for physiological resistance of beans to white mold is the straw test developed by Petzoldt and Dickson [4]. *P. coccineus* is known to possess greater resistance to this disease than does *P. vulgaris* [1]. We used the straw test method to examine all available accessions of the *P. coccineus* collection held by the National Plant Germplasm System that we had not previously tested in 1999. The number of accessions tested in 2001 was 334, including 50 lines that had high levels of resistance in our 1999 test.

We inoculated seedlings 23 to 25 days after planting, and read them 8 days later. Plants that appeared resistant at time of reading were re-inoculated after 13 to 15 days, and were read again 8 days later. The controls OR 91G, a susceptible snap bean, and MO162, a fairly resistant dry bean, were inoculated at 31 days because extra time was needed to obtain sufficient growth for testing. On a scale of 1-9, a rating of 3 or less implies a high degree of resistance while a score of 7 or greater indicates a high degree of susceptibility. A plant was scored as 4 when white mold mycelial growth stopped at the first node. Average white mold ratings for OR 91G and MO162 were 5.5 and 4.5, respectively. Overall average white mold scores for the three species in the *P. coccineus* collection were: *P. coccineus* = 4.01, *P. polyanthus* = 4.78 and *P. vulgaris* = 4.44. Table 1 shows the 40 most resistant accessions identified in our screen.

The seeds of each accession were examined and separated by species and also by seed color. Seed color was used as a category because *P. coccineus* and *P. polyanthus* (previously *P. coccineus* spp. *Darwinianus* [2]) may have considerable out-crossing. Several accessions were a mix of two or three species. The average number of seeds tested per accession was 14.

In our studies many plants that appeared resistant at the first straw test reading slowed, but did not stop the white mold's advance. To further characterize resistance, plants were observed, usually for about three more weeks, to see if the plant's defenses fully halted the mycelium's growth. If any plant in a pot allowed the mold to advance past the first node, the line received an asterisk (Table 1). Multiple asterisks were assigned when an accession had different species, color categories or multiple pots.

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Table 1. Evaluation of white mold resistance of the Phaseolus coccineus collection[z]

PI #	Seed color	Origin	Em[y]	Species	No. seeds	1st test AV	CM[x]	2nd test AV
165421	Gray	Mexico	H	P. coc	14	3.4		4.0
193045	Black	Guatemala	H	P. coc	12	3.4	*	4.5
196413	Black	Guatemala	E	P. vulg	14	4.1		5.0
201297	Color mixture[w]	Mexico	H	P. coc	13	3.6	*	3.7
201304	Color mixture[w]	Mexico	H	P. coc	21	3.0		3.4
201320	Red	Mexico	H	P. coc	15	3.7	*	2.5
201366	Color mixture[w]	Mexico	H	P. coc	13	3.8		3.4
209669	Color mixture[w]	Mexico	H	P. coc	11	3.5	*	3.9
311194	Small Red[v]	Guatemala	E	P. vulg	13	3.6	*	3.7
311217	Brick Red	Guatemala	E	P. poly	10	4.3	*	4.0
311819	Color mixture[w]	Guatemala	H	P. coc	13	3.7		3.7
311859	Black	Guatemala	E	P. vulg	15	3.7		4.3
311985	Dark Pink	Mexico	H	P. coc	13	3.5		3.3
313310	Tan	Mexico	E	P. poly	17	3.9		4.7
317551	Tan	Guatemala	H	P. coc	13	2.9	*	3.6
317575	Red	Guatemala	E	P. poly	12	4.3		5.0
317576	Color mixture[w]	Guatemala	E	P. poly	16	4.4	*	4.0
325599	Color mixture[w]	Mexico	H	P. coc	15	3.7		4.0
361371	Mottled Tan	Bhutan	H	P. coc	15	3.5	*	4.3
361510	Color mixture[w]	India	H	P. coc	11	3.6	*	4.2
433236	White	Guatemala	H	P. coc	12	2.7	*	3.8
433237	Color mixture[w]	Guatemala	H	P. coc	13	3.5	*	3.4
433242	Color mixture[w]	Guatemala	H	P. coc	14	3.3	**	3.3
433246	Pink	Guatemala	H	P. coc	14	2.4		2.3
433247	Color mixture[w]	Guatemala	H	P. coc	13	3.5	**	3.0
433250	Tan	Guatemala	H	P. coc	13	3.7	*	2.8
433251	Color mixture[w]	Guatemala	H	P. coc	12	3.3	*	3.6
439534	Mottled Pink	Netherlands	H	P. coc	14	3.1	*	3.4
451863	Color mixture[w]	Guatemala	H	P. coc	12	3.0		4.1
451868	White	Guatemala	E	P. vulg	14	3.6	*	4.3
451873	Pink[v]	Guatemala	E	P. vulg	6	3.8	*	3.5
475745	White	Netherlands	H	P. coc	13	3.0		3.8
583554	White	Japan	H	P. coc	12	3.1	*	3.9
311184	Red	Guatemala	E	P. poly	14	3.9	**	3.6
189023	Color mixture[uw]	Guatemala	H	P. coc	12	2.7		3.2
311210	Black[u]	Guatemala	H	P. coc	13	3.2	*	3.9
317550	Color mixture[uw]	Guatemala	E	P. poly	14	4.1	**	3.7
407387	White[u]	China	H	P. coc	10	3.9		3.8
433253	Color mixture[uw]	Guatemala	H	P. coc	15	3.3	**	3.7
313417	Color mixture[uw]	Mexico	H	P. coc	22	3.1		4.0

[z]Included are accessions classified as *P. coccineus* that contained *P. polyanthus* and *P. vulgaris.*

[y]Em = emergence, either hypogeal or epigeal.

[x]CM refers to continuation of the white mold down the stem of the plant after the initial eight day reading.

[w]Color mixture refers to accessions that had multiple seed colors, but all colors appeared to be about equal in their resistance rating.

[v]One species in a mixed accession that had greater resistance.

[u]Accessions that had been tested in 1999, and then were retested in 2001.

DEVELOPMENT OF *Sclerotinia sclerotiorum Inoculum* FOR FIELD STUDIES

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Introduction

At Colorado State University, the establishment of a *Sclerotinia sclerotiorum* nursery was essential to consistently screen breeding lines and identify effective chemical and biopesticide treatments to manage white mold of dry beans. Large, unmarketable zucchini squash were collected in late summer, syringe-inoculated with a mycelial suspension of the fungus, and incubated on a grassy surface until infected fruit decomposed with large masses of sclerotia the following spring. Sclerotia were removed by hand from plant debris and redistributed throughout a field nursery for secondary increase of inoculum on sunflower and future studies with dry beans.

Materials and Methods

- August 2000 - Zucchini was chosen as the vessel to increase sclerotia. Large unmarketable zucchini were collected.
- August 2000 - The zucchini were transported and unloaded onto a well drained, sloped area.
- September 2000 - The zucchini were inoculated with a mycelial suspension. The fruits were pierced with a narrow screwdriver in 4 - 5 equidistant sites. A needle and syringe were used to inject 1 - 2 ml at each site.

Results

- By October 2000 the tissue of infected zucchini was breaking down and sclerotia began to form.
- Hard, black sclerotia formed on both the outside and inside surfaces of the zucchini by early November 2000. The zucchini were left intact and allowed to dry in the field throughout the winter.
- In early May 2001, the dried material was crushed by hand to provide a uniform inoculum to spread in the field.
- Sclerotia were spread in an area of 80’ x 120’, and a confectionary sunflower variety was planted June 2001 in 15” rows and watered daily by sprinklers to increase sclerotia (< 5 % plant infection) and reduce weed seed banks for next year.
- Dry beans will be planted in the infested nursery for germplasm and fungicide evaluations in 2002, and part of the nursery will be rotated annually with white mold – susceptible sunflower or soybean to manage weeds while maintaining disease inoculum.

THE USE OF MAS TO DEVELOP PINTO BEAN GERMPLASM POSSESSING *Co-4²* GENE FOR ANTHRACNOSE RESISTANCE

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Anthracnose, caused by *Colletrotrichum lindemuthianum* (Sacc. & Magnus), is a major seed-borne disease of beans in Michigan, New York and Ontario. In 2000, anthracnose was observed in pinto bean fields in Manitoba, and this outbreak moved into neighboring North Dakota in 2001. Herein we describe the development of pinto bean lines with *Co-4²* resistance to anthrancose disease using marker-assisted selection (MAS). A RAPD marker ($AS13_{950}$) tightly linked with *Co-4²* was identified and converted to a SCAR by Young et al. (1998). This marker has been extensively studied by Melotto and Kelly (2001) and subsequently, additional SCAR markers linked with *Co-4²* were developed by Awale and Kelly (2001).

Co-4², with a Resistance Index of 97 (resistant to 33 of 34 races), is the most effective anthracnose resistance gene characterized to date (Balardin and Kelly, 1998. The tropical black-seeded line SEL 1308 from CIAT (Young et al., 1998) was the donor parent in this study. The *Co-4²* gene ultimately derives from the differential line G2333. The gene was moved into pinto bean using a MAS "pseudo" backcrossing scheme. "Pseudo", because a different pinto parent was used for each backcross (Fig. 1). The primers for the $SAS13_{950}$ SCAR are as follows: forward= CAC GGA CCG AAT AAG CCA CCA ACA and reverse = CAC GGA CCG AGG ATA CAG TGA AAG. The thermal cycling profile = 34 cycles of denaturing for 10s at 94°C, annealing for 144s at 72°C, and a final extension cycle of 5 min at 72°C (Young et al., 1998).

Donor **Adapted pinto**

SEL 1308 X Othello
F_1 X Maverick
BC_1F_1* X (Buster/OT9743-586-1)
†BC_2F_1* X USPT-CBB-1 or Buster/USPT-CBB-3)
BC_3F_1*
BC_3F_2 selected in field for adaptation
$BC_3F_{2:3}$ progenies were tested for homozygous reaction to anthracnose races 73 and 1545 in GH inoculation tests

Figure 1. Depiction of the MAS "pseudo" backcrossing scheme used to develop anthracnose resistant pinto bean germplasm lines. (*) indicates generation where MAS for the $SAS13_{950}$ SCAR marker (Young et al., 1998) tightly linked with the *Co-4²* gene (0.39 cM recombination; Melotto and Kelly, 2001) was conducted. (†) a residual BC_2F_2 selfed progeny, from a BC_2F_1 plant with the marker, segregated 3 resistant to 1 susceptible as expected.

Actual phenotypic selection (disease screening) for resistance to anthracnose was not conducted until the $BC_3F_{2:3}$ generation. The identification of true breeding anthracnose resistant $BC_3F_{2:3}$ progenies indicated that MAS-backcrossing successfully introgressed *Co-4*^{2} from the unadapted SEL 1308 breeding line into adapted pinto bean. Adapted pinto bean lines with *Co-4*^{2} resistance to anthracnose, developed by MAS, are in the process of being officially released by USDA-ARS in cooperation with the Michigan Agricultural Experiment Station.

Pedigrees of the proposed releases:

USPT-ANT-1 = (USPT-CBB-1/4/Buster/OT9743-586-1/3/Maverick//Othello/SEL1308)
USPT-ANT-2 = (Buster/USPT-CBB-3/4/Buster/OT9743-586-1/3/Maverick//Othello/SEL1308)

In summary: i) anthracnose resistant cultivars can be rapidly developed in the absence of pathogen screening, which is beneficial for breeding programs without mist-chambers and other facilities necessary for conducting pathogen tests, ii) potential release of highly virulent non-endemic races (ie. 1545) for confirming presence of extremely effective genes like *Co-4*^{2} is minimized, iii) breeding programs in areas with a quarantine against anthracnose can still effectively breed for resistance if they have a MAS program, and iv) given the pathogenic diversity of *C. lindemuthianum*, MAS offers the only effective method to pyramid diverse anthracnose resistance genes into single resistant cultivars.

The enhanced lines described above will be most useful for protecting the 400,000 acres of susceptible pinto bean production in the Red River Valley from emerging anthracnose disease. Until seed increases of the potential releases are completed, for now, only small 5 seed samples are available upon written request to the senior author.

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PROGRESS IN BACKCROSS BREEDING WITH RAPD (SCAR) MOLECULAR MARKERS TO PYRAMID QTLS FOR RESISTANCE TO COMMON BACTERIAL BLIGHT IN PINTO AND GREAT NORTHERN BEANS

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The common bacterial blight (CBB) disease in common bean (*Phaseolus vulgaris* L.) (P.v.), caused by *Xanthomonas campestris pv. phaseoli* (*Xcp*), reduces bean yield and seed quality. CBB resistance is inherited quantitatively with low to moderately low heritability estimates (reviewed by Singh and Munoz, 1999) thus reducing selection efficiency. Pinto >Chase= (Coyne et al., 1994) is a high yielding, rust resistant variety with moderate resistance to *Xcp* derived from great northern (GN) Nebraska #1 selection 27. XAN-159 (CIAT) is a black mottled, small seeded breeding line with genes for high resistance to *Xcp* that are different from those in GN Nebraska #1 sel 27, and are derived from a different tepary source PI 319443 (Jung et al., 1997). G. N. 'Weihing' (Coyne et al., 2000) is another variety resistant to halo blight, brown spot, rust (Ur-3 and b), BCMV (I gene), and moderately resistant to CBB. There is a need to pyramid different genes for resistance to CBB derived from different germplasm sources into elite bean germplasm and cultivars in order to increase the level of resistance to CBB and to provide for more durable resistance.

Materials and Methods

Backcross procedure: BC1, BC2, BC3, BC4 and BC5 populations were generated using Pinto 'Chase' as the recurrent parent. Also the most resistant BC2F3 lines of this cross were backcrossed to GN 'Weihing' to incorporate the *Xcp* resistance. The BC plants were screened for phenotypic reactions to *Xcp* with multiple needle method (Andrus, 1948) and resistance was confirmed by the presence of RAPD –(SCAR) markers.

Result and Discussion

Pinto Breeding: Resistance to *Xcp* was confirmed in BC5F1 plants and successive selfed lines up to BC5F5 of P. 'Chase' x XAN-159 cross via both scar marker $BC420_{900}$ (Yu et al., 2000) and *V* locus as well as phenotypic selection with two *Xcp* isolates (DR-7, EK-11). Resistant BC5F3 and BC4F4 lines were identified and single plant selections were also made within those lines under natural infection in the field in 2000 in NE, and subsequently they were increased in Puerto Rico for field trials in NE (2001). In greenhouse grown BC5F1, only two resistant plants possessed the SCAR marker $BC420_{900}$ but lacked the dominant *V* locus. The SCAR marker is closer to *V* locus than to QTL for resistance to *Xcp*. The presence of the *V* locus is always associated with dark seed coat color and purple flowers. It was crucial to break the linkage between *V* locus and resistance to *Xcp* in order to recover desired Pinto seed color pattern. Replicated field trials in Summer 2001 showed that yield and seed size, resistance to *Xcp* and small seed size were also linked in many lines (Park et al., 2000). However a number of lines were similar to P.'Chase' for seed size, shape and pinto color, indicating this linkage was broken now. Some lines appear to have better seed coat color than 'Chase'.

Great Northern Breeding: Resistance to *Xcp* was also confirmed up to BC3F1generations of the recurrent parent GN. 'Weihing' variety. Resistant recombinants from BC2F3 of the Pinto 'Chase' x XAN-159 cross were used as donor parents, because they did not cause hybrid lethality as occurred in the cross GN 'Weihing' x XAN-159, and because they possessed suitable seed size, shape, and resistance to CBB. Enhanced GN lines are expected to combine the major QTL from

XAN-159 and one additional minor QTL from P. ‘Chase’ derived from G.N.Nebraska #1 sel 27, which GN. Weihing lacked (SCAR AP6).

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Reaction of dry bean cultivars of the Northern Plains to anthracnose

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Bean anthracnose, caused by the fungus *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams.-Scrib., is a devastating disease prevalent in cool humid areas. Accidental introductions of this disease to North Dakota in 1983 (4) and 1992 (1) ended quickly with the destruction of infected materials. The recent detection of anthracnose in Manitoba, Canada, has markedly increased the risk of accidental reintroduction of this disease to North Dakota. Anticipating this situation thirty of the most popular dry bean varieties planted in this region were screened for their reaction to races 7, 73, and 89. These three races are present in Michigan (1), and Ontario (3).

Materials and Methods

Cultivars were evaluated using a Completely Randomized Design with three replications and 10 plants per replication. Seedlings were grown in 6 x 6 cm plastic square pots containing Sunshine Soil Mix # 1 (Sun-Gro Horticulture, Canada) for 14 days prior to inoculation. Isolates of anthracnose races 7, 73, and 89, obtained from J. Kelly (Michigan State Univ.), were cultured on bean juice agar or Mathur's medium for 7-14 days. Spores were suspended in distilled water with 0.01% Tween 80 (v/v), and adjusted to a concentration of 1 x 10^6 spores/ml. Inoculum was delivered to the abaxial side of primary leaves until runoff using a Paasche airbrush with 137 kPa of pressure. Inoculated plants were incubated for five days at >90% RH and 20ºC with 14 hours of fluorescent light daily, then moved to a greenhouse at 22ºC with 14 hours light daily. Disease reaction was measured three days later, as the proportion of leaf tissue visibly affected.

Results and discussion

Most kidney beans were resistant to anthracnose races 79 and 83, but susceptible to race 7. 'Isles' was the only kidney bean resistant to all three races (Table 1). Cranberry beans were susceptible to races 7 and 89. 'Taylor Hort' was resistant to race 73 (Table 1). All pinto beans were susceptible to all three races (Table 2). Most navy beans were resistant to race 7 (Table 3), but five of them were susceptible to races 73 and 89. 'Newport' and 'Envoy' were resistant to all three races, while 'Norstar' was susceptible. The most popular cultivars planted in the Northern Plains, 'Montcalm', 'Maverick', and 'Norstar' differed in their reaction to anthracnose. 'Montcalm' was resistant to races 73 and 89, but susceptible to race 7; while the latter two were susceptible to races 73 and 89. 'Maverick was resistant to race 7.

Over 90% of the beans planted in North Dakota are pinto beans, and 45% of the beans planted in Minnesota are navy beans (2), and most of them are highly susceptible to anthracnose. The incorporation of germplasm with resistance against these races into the breeding program is necessary and should be conducted promptly.

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Table 1. Reaction of kidney and cranberry beans to anthracnose races 7, 73, and 89

	Races		
	7	73	89
Cultivar	% tissue infected		
Kidney bean			
Sacramento	100	0	0
California Dark	98	0	0
California Early	95	0	0
Foxfire	85	23	34
Montcalm	83	0	0
Drake	11	0	0
Isles	5	0	0
Cranberry bean			
Taylor Hort	100	10	51
Cran-09	88	46	100

Table 2. Reaction of pinto bean cultivars to bean anthracnose races 7, 73, and 89

	Races		
	7	73	89
Cultivar	% tissue infected		
Olathe	62	94	100
Hatton	42	91	100
Winchester	42	88	100
Fiesta	40	87	100
Topaz	35	17	98
Fargo	19	100	99
Focus	19	86	100
Othello	17	100	100
Chase	9	54	77
Maverick	1	39	76

Table 3. Reaction of navy bean cultivars to bean anthracnose races 7, 73, and 89

	Races		
	7	73	89
Cultivar	% tissue infected		
Norstar	41	100	100
Upland	22	0	---
Fleetwood	19	0	0
Albion	7	77	96
Schooner	5	21	86
Envoy	4	3	0
Mayflower	2	98	84
Aspen	1	100	95
Navigator	1	99	78
Newport	1	1	0
Vista	0	98	0

GENETIC RESISTANCE TO *Colletotrichum lindemuthianum* RACE 2047 In G 2333

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Introduction

Anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. et Magn.) Scrib., is one of the most important diseases of the common bean (*Phaseolus vulgaris* L.). The use of resistant cultivars is considered to be the most efficient and economic control method for the disease. Pastor-Corrales et al. (1994) showed that only the G 2333 line was resistant to 380 isolates of *C. lindemuthianum*. This line was resistant to all the Brazilian isolates and all the European and North American races (Pastor-Corrales and Tu, 1989; Balardin and Pastor-Corrales, 1990; Balardin et al., 1990). Resistance in G 2333 is controlled by two independent dominant genes with equivalent effects (Pastor-Corrales et al. 1994). In addition, Young et al., 1998, detected in G 2333, three different dominant resistance genes, *Co-4*^{2}, *Co-5* and *Co-7*, the *Co-4*^{2} allele, at the *Co-4* locus.

In the present study, the inheritance of resistance in G 2333 to race 2047 of *C. lindemuthianum*, is described.

Material and Methods

Genetic Plant Material: Parental genotypes, G 2333 (resistant to race 2047) and TU (susceptible the race) were crossed to study the genetic characterization of the resistance. Race 2047 was used to determine the inheritance of resistance in G 2333, in the cross G 2333 x TU. One F_2 population derived from the cross between G 2333 x TU consisted of 151 plants and backcrosses were obtained.

Inoculation and incubation: Parental, F_1, F_2 and backcross plants were inoculated with a spore suspension (1.2 x 10^6 spores ml^{-1}) of the pathogen. The protocol for spore inoculation was as follows: 14 to 18-day-old parental, F_1, F_2 and backcross plants with their first trifoliate leaf completely developed were inoculated by the use of a paint brush previously moistened in a spore suspension, from an adaptation of the method used by Cárdenas et al. (1964).

After inoculation, the seedlings were kept in a humid chamber for 96 hours at 20ºC ± 2°C, controlled light (12 hours with 680 lux illumination alternated with 12 hours of darkness) and approximately 100% relative humidity.

Results and Discussion

Table 1 shows the parental (G 2333 and TU), F_1, F_2 and backcross phenotypes in their reaction to race 2047.

Characterization of the genetic resistance in G 2333 to race 2047 was determined after analyzing the data observed segregating ratios obtained from the disease phenotypic of the F_2 population and backcross generations (BC resistant and BC susceptible). Chi-square values revealed that fit was obtained for the segregation in the F_2 at a ratio of 3:1, the expected ratio of resistant to susceptible plants (R-:rr), respectively. Segregating in the 1R:1S when the F_1 was backcrossed to TU, and 1R:0S when the F_1 was backcrossed to G 2333, suggesting that a single dominant gene conferred resistance to race 2047. This result indicated that the dominant gene in G 2333 is located at the different locus as the *Co-5* gene in TU and in G 2333. According by Young and Kelly (1996), none of the races tested (7, 23, 64, 73, 1545, and 2047) were pathogenic on G 2333 and its derived

line SEL 1308. In addition, according Young et al. (1998), the SEL 1308 carries a single dominant gene, named *Co-4*^{2} that confers resistance to races 73, 64, 521, and 1545. Since SEL 1308 was derived from G 2333 and both are resistant to race 2047 (Young and Kelly, 1996), in this study, it is assumed that the *Co-4*^{2} allele, at the *Co-4* locus, confers resistance to race 2047.

Table 1. Segregation for resistance to race 2047 of *Colletotrichum lindemuthianum* (*) in common bean G 2333 cultivar

Pedigree	Generation	Reaction	Nº of Plants				
			Observed		Expected ratio	X^2	P value
			R	S	R:S		
TU	P_1	S	0	32	-	-	-
G 2333	P_2	R	20	0	-	-	-
G 2333 x TU	F_1	(R x S)	32	0	-	-	-
G 2333 x TU	F_2	(R x S)	115	36	3:1	0.10	0.74
F_1 x TU	B_{CS}	(R x S)	29	28	1:1	0.02	0,89
F_1 x G 2333	B_{CR}	(R x R)	52	0	All R	-	1

* R = Resistant S = Susceptible.

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BREEDING FOR CBB RESISTANCE IN CRANBERRY BEANS USING MARKER ASSISTED SELECTION

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Common bacterial blight (CBB), the most important bacterial disease of common bean, is caused by the soil pathogen *Xanthomonas axonopodis* pv. *phaseoli* (Xap). OAC Rex is a CBB resistant white bean developed at the University of Guelph. The resistance in OAC Rex originates from plant accession PI 440 795, a *Phaseolus acutifolius* line (Scott and Michaels, 1992). A previous study found one major gene and two minor genes for CBB resistance in OAC Rex (Tar'an, 2000). In addition, three molecular markers for CBB resistance in OAC Rex were developed including a microsatellite marker J045551 that explains 42.2%, and two RFLP clones (BNG 71 and BNG 21) that explain 36.3% and 10.2%, respectively, of the variation in disease resistance scores (Tar'an, 2000).

Cranberry beans comprise 10% of the total dry beans grown in Canada. SVM Taylor Hort is an early, high yielding bush type cranberry bean that is susceptible to CBB.The objective of the current work was to introgress CBB resistance into cranberry bean using marker-assisted selection (MAS).Crosses between OAC Rex and SVM Taylor Hort were made and F_1s were selfed in a growth room at the University of Guelph. F_4 lines were created by single seed descent. At the F_4 stage, 25 seeds per row were planted at the Elora research station in a 14 x 14 simple lattice design with two replications. All plants were inoculated at the 3-4 trifoliate stage with Xap using a high pressure sprayer (180 psi). At 15, 22 and 36 days post inoculation all lines were rated visually on a scale of 0-4 (0= no symptoms, 4= necrotic spots on most leaves). A plant with a score of 2 or less is deemed resistant.

One trifoliate was taken from each line at the F_2 and F_4 generations and genomic DNA was extracted using the CTAB method (Doyle and Doyle, 1990). PCR was used to test each line with marker J045551 (Forward 5' GAGGGTGTTTCACTATTGTCACTGC '3 Reverse 5' TTCATGGATGGTGGAGGAACAG '3).

F_4 lines had CBB scores between 0.67 and 3.08 with a mean value of 2.05. The average score for OAC Rex was 1.14 and 2.58 for SVM Taylor Hort.. XAN 159 was used as a control and had an average score of 2.03. Marker J045551 was polymorphic between parents and segregated in the F_2 population. BNG clones 21 and 71 were end sequenced and sequence specific primers were designed (BNG 21 Forward 5'ACCCAACTTACAGAGCTGTTTG 3' Reverse 5' TTCGATTTGGAACATTGGCTG 3', BNG 71 Forward 5' GTTGCTGTTAAGATATCAAACG 3' Reverse 5' GGGACATGTTAT TCATATGGTT 3'). The PCR products for BNG 21 and 71 were produced from OAC Rex and SVM Taylor Hort. template DNA and had expected sizes of 1500 bp and 1600 bp, respectively. Digestion of BNG 21 PCR products with Taq I and BNG 71 PCR products with Alu I produced polymorphisms. This result creates PCR-RFLP markers that are useful for high throughput screening.

F_4 lines with a variety of seed coat colours were obtained. 18 F_4 lines had cranberry type seed coat. Of these, seven were found to have resistance to CBB.

In summary, a population segregating for seed coat colour and CBB resistance was created from a cross. The population was selfed to the F_4 stage and tested in the field for CBB resistance. A

microsatellite marker linked to resistance was shown to segregate in the population and two PCR-RFLP markers were created from RFLP markers that are also linked to resistance.

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USING SPECIFIC RACES OF THE COMMON BEAN RUST PATHOGEN TO DETECT RESISTANCE GENES IN *Phaseolus vulgaris*

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Host resistance is the most effective method of managing the rust disease of dry and snap beans (8). Some cultural practices and fungicides provide supplementary but less effective means of bean rust management (3). However, accomplishing effective and durable resistance to the rust pathogen in common bean is difficult. The causal agent of bean rust, *Uromyces appendiculatus*, is known to have abundant diversity for virulence (1). A multitude of races of the rust fungus have been identified in many bean-producing regions of the world (2, 4, 6, 8). Ninety races of the bean rust fungus have been isolated, identified and maintained in storage at Beltsville since 1980 by J. R. Stavely (7). Many of these races are from the United States but some are also from Africa, Asia, Europe, Central America and the Caribbean. Races of *U. appendiculatus* are differentiated by the characteristic symptoms or reaction grade that they elicit on each of a set of 19 differential cultivars. Correspondingly, several rust resistance genes, including *Ur-3*, *Ur-4*, *Ur-5*, *Ur-6*, *Ur-7*, and other uncharacterized and unnamed genes, have been identified in common bean. More recently, *Ur-11*, which provides resistance to 89 of the 90 races maintained at Beltsville, was identified and incorporated alongside other rust resistance in dry and snap bean cultivars with multiple rust resistance genes (7).

Molecular markers and specific races of the rust pathogen can be utilized to identify bean plants with one or more rust resistance genes. This is particularly important when developing beans with multiple rust resistance genes. While the prospective value of molecular markers in marker assisted selection of beans with rust resistance genes is immense, at present some of the molecular markers available are not fully reliable. Using specific races of the bean rust pathogen in multiple individual race inoculations provides a reliable means of detecting rust resistance genes. Stavely selected races of *U. appendiculatus* that produced proven reactions in the presence of the rust resistance gene of choice. Race 47 was used for detecting *Ur*-6, race 53 for *Ur-3*, race 67 for identifying *Ur-11*, and race 49 to detect presence of *Ur-4* with *Ur-11*. Additionally, races 41, 44, 73, and 108, were used for confirmation (Table 1). It is important to take into account that *Ur-3* and *Ur-6* are epistatic to Ur-11 for all the races controlled by them and *Ur-11*.

The presence of *Ur-3* results in a grade 2 type of reaction when inoculated with race 53 and with most races that *Ur-3* controls. This reaction is epistatic to the grade 3,2 produced by bean plants with *Ur-11* when these are inoculated with race 53 or with the other races controlled by *Ur-3*. Thus, race 53 elicits a different type of resistant reaction in bean plants with *Ur-3* than in plants with *Ur-11*. Since race 53 is not controlled by Ur-3 or Ur-6, this race can be used for detecting *Ur-3* (Table 1). Likewise, the resistant reaction elicited by race 47 in plants with *Ur-6* is epistatic to the resistant reaction produced by plants with *Ur-11*. Additionally, race 47 is not controlled by *Ur-3* or *Ur-4*; thus, race 47 can used to detect Ur-6. Race 67, which is controlled only by Ur-11 but not by *Ur-3*, *-4*, or *–6*, can be used to detect *Ur-11*. Ur-4 produces a type 2 of reaction to race 49 in absence of other resistance genes. Plants with Ur-11 without Ur-4 produce a 3,2 reaction. If Ur-4 and Ur-11 are present a faint chlorotic reaction results. Thus race 49 can used to detect Ur-4 with Ur-11.

Table 1. Using selected, specific, races of the bean rust fungus in multiple, individual inoculations of bean cultivars to identify presence of certain rust resistance genes

Bean Cultivar	**Resistance Gene**	**Reaction[a] to *Uromyces appendiculatus* Race**							
		41	**44**	**47**	**49**	**53**	**67**	**73**	**108**
Aurora	***Ur-3***	**2,2+**	**5,4**	**4,5**	**5,4**	**2,2+**	**4,5**	**5,6**	**2,2+**
E. Gallatin	***Ur-4***	**4,5**	**2+, 2**	**4,3**	**2+, 2**	**4,5**	**4,5**	**2,2+**	**2,2+**
G. G. Wax	***Ur-6***	**2+,2++**	**2+, 2**	**2,2+**	**4,5**	**4,5**	**4,5**	**2**	**5,4**
PI 181990	***Ur-11***	**3,2**	**3,2**	**3,2**	**3,2**	**3,2**	**3,2**	**3,2**	**5,6,4**
Ur-4 & Ur-11					**f 2**				
Gene identified →				***Ur-6***	***Ur-4 & Ur-11***	***Ur-3***	***Ur-11***		

[a]Standard bean rust grading scale: 1 = Immune, no visible symptoms. 2, 2+, 2 ++, 2 +++ = Highly Resistant; Necrotic Spots without sporulation and less than 0.3 mm, 0.3 – 1,0 mm, 1.0 – 3.0mm, and greater than 3.0 mm in diameter, respectively. 3 = Resistant; Uredinia - Sporulating lesions - less than 0.3mm in diameter. 4 = Moderately Resistant; Uredinia 0.3-0.5mm in diameter. 5 = Moderately Susceptible; uredinia 0.5-0.8 mm in diameter. 6 = Susceptible; Uredinia larger than 0.8mm in diameter.

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INHERITANCE AND HERITABILITY OF LEAFHOPPER RESISTANCE IN COMMON BEANS (*Phaseolus vulgaris* L.)

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The potato leafhopper *Empoasca fabae* Harris has been identified as the most important *Empoasca* species attacking dry beans in North America. The related species *E. kraemeri* Ross and Moore is considered the main insect pest attacking dry bean in Latin America (Schoonhoven et al., 1978). Yield losses of about 20% were reported on susceptible bean varieties attacked by *E. fabae* in North Platte, NE (Lindgren and Coyne, 1995). In some field trials *E. kraemeri* caused about 64% losses in yield (Schoonhoven et al., 1985). The wide range of host plants for *E. fabae* and *E. kraemeri,* along with the many generations that develop during a growing season, contributes to the economic importance of this species in comparison with other *Empoasca* relatives. Some mechanisms of plant resistance to leafhopper injury such as antixenosis and antibiosis have been suggested. Antixenosis constitutes a well-studied mechanism of resistance in dry bean to leafhopper. Ovipositional antixenosis makes the plant less preferred as a host for oviposition than susceptible or tolerant lines (Kornegay and Temple, 1986). Non-preference tests suggest that there is no clear preference of *E. kraemeri* for bean plant leaves. Quantitative inheritance of leafhopper injury (E. kraemeri) in common bean has been reported (Galvey and Evans, 1982; Kornegay and Temple, 1986; Schoonhoven et al., 1985). No heritability estimates for leafhopper *E. faba* injury in dry beans were reported previously (Singh, 1999).

The objectives of this experiment were: 1) to determine inheritance of potato leafhopper (*E. fabae* Harris) injury in dry beans, 2) to determine the narrow sense heritability (h^2) of leafhopper injury in populations derived from resistant x susceptible parents to leafhopper injury.

Dry bean varieties Tacarigua and pinto (P) Sierra (resistant to leafhopper injury), and great northern (GN) Starlight (susceptible to leafhopper injury) were used as parents in crosses. C crosses were: Tacarigua (black,res) x GN Starlight (sus), and P. Sierra (res) x GN Starlight (sus). F1 generations were grown under greenhouse conditions to produce F2 seed. Parents, F1, and F2 progenies from both crosses were grown in 1999 under field conditions at WCREC, North Platte, NE. ·Parents and F3 families were planted at WCREC, North Platte, NE in 2000 under a square lattice design. Visual scores (1=no injury to 5=severe injury) were used to rate leafhopper injury. SAS statistical procedures were used to analyze the data. A quantitative inheritance pattern of response to leafhopper injury was observed in both crosses, with partial dominance for susceptibility. Narrow sense heritability (NSH) values (20%) were estimated by regressing F3 progeny mean ratings on individual F2 plants. Low NSH estimates indicated that selection on a single plant basis in early generations would not be efficient. Testing of advanced lines in replicated trials is recommended to detect superior resistant lines. Current work involves the detection of RAPD molecular markers linked to QTL for resistance to leafhopper injury in the above populations as well as in two RIL populations. Molecular markers linked to QTL for resistance should enhance the efficiency of selection because of the low h^2 values for the phenotypic leafhopper injury ratings.

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EVALUATION OF SEED-Zn CONCENTRATION IN NAVY BEAN

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Navy bean (*Phaseolus vulgaris* L.) cultivars vary in their susceptibility to Zn deficiency. Zinc-deficiency problems affect common bean production worldwide, particularly on calcareous soils. The majority of common bean production in North Dakota occurs on high pH soils that are frequently low in available Zn. A 1999 Northarvest Bean Growers Survey indicated that Zn fertilizer was applied to 54% of North Dakota respondents' acres (Lamey et al., 2000). According to Graham (1984), a Zn-efficient cultivar or species has the ability to grow and yield well in soils that are Zn-deficient for a standard cultivar. Jolley and Brown (1991) found that the Fe stress response in Zn-inefficient genotypes caused an increase in Fe uptake and also enhanced Zn-deficiency symptoms. Even though Zn-inefficient genotypes were identified over 40 years ago, navy bean cultivars susceptible to Zn deficiency are still released. Zinc-inefficient lines grown in soils high in available Zn accumulate less seed Zn than Zn-efficient genotypes (Moraghan and Grafton, 1999). Using seed-Zn concentration as a method to determine Zn-efficiency status is useful because genotypes can be evaluated in the absence of foliar Zn-deficiency symptoms. The objective of this study was to use seed-Zn analysis to evaluate the Zn-efficiency status of selected navy bean genotypes from various common bean improvement programs. These lines had not previously been classified as Zn-efficient or Zn-inefficient based on seed-Zn concentration. The same gene(s) may control both Zn-efficiency and seed-Zn accumulation in navy bean.

Nineteen genotypes (Table 1) were evaluated in 2000 at sites near Erie and Hatton, ND. The experimental design was a Randomized Complete Block with four replicates. Thirty mature pods were randomly collected from each plot and analyzed. Seed from each plot was bulked, counted, washed with de-ionized water containing detergent, and rinsed with de-ionized water. Samples were dried at 70C for 48h, weighed, and ground in an agate mortar with an agate pestle to pass a 0.25-mm mesh sieve. Sub-samples of the ground seed were digested on an aluminum block with 4mL HNO_3 and 2mL $HClO_4$ and analyzed for Zn and Fe by atomic adsorption spectroscopy. Standard Reference Material 1572 or 1515 from the National Institute of Standards and Technology, Gaithersburg, MD, was digested and analyzed with samples to provide an indication of accuracy.

A Bartlett's X^2 test for homogeneity of variance was conducted to determine if a combined analysis across environments was possible. Combined analyses of variance across environments were conducted for the seed Zn. Means were separated using an F-protected LSD (P=0.05).

Significant differences (P=0.05) among seed-Zn and seed-Fe concentrations were observed. Mean seed-Zn concentrations ranged from 18.2 to 28.1 and from 14.7 to 28.6 mg kg^{-1} at Hatton and Erie sites, respectively. Mean seed-Fe concentration ranged from 53.3 to 74.0 and 50.9 to 73.4 mg kg^{-1} at the Hatton and Erie sites, respectively. Lines ISB 1252, ISB 1256, and ND 93-105-01-05 were the most Zn efficient of lines evaluates. ISB 3156, 'Vista', 'McHale', and 'Mackinac', were low seed-Zn accumulators and considered Zn inefficient, containing less than 19 mg kg^{-1} of seed Zn. No relationship between seed-Zn and seed-Fe accumulation was observed. No relationship between seed-Zn and yield was observed.

Table 1. Mean seed-Zn and seed-Fe concentrations and yield of navy bean lines grown at Erie and Hatton, ND in 2000.

	Location				
	Erie		Hatton		Combined
Genotype	Zn	Fe	Zn	Fe	seed yield
	mg kg^{-1}				kg ha^{-1}
ISB 1256	28.6	66.5	27.7	74.0	2326
ND 93-105-01-05	27.6	66.8	28.1	72.8	2358
ISB 1252	27.3	66.7	27.7	72.8	2470
Mayflower	25.9	59.4	26.5	65.8	2435
O510	25.9	56.3	25.6	65.8	2340
OAC Laser	23.8	53.3	25.5	62.3	2221
Norstar	24.1	55.7	25.1	62.8	2021
Aspen	24.9	56.7	23.8	55.0	1839
Stingray	23.3	57.8	25.2	61.5	2350
OAC Thunder	23.5	50.9	24.8	58.8	2366
AC Trident	23.5	57.2	24.4	68.5	2566
Arthur	23.5	57.0	23.6	59.3	2339
OAC Gryphon	24.3	55.0	22.1	59.3	2101
Mast	23.2	57.2	21.4	60.3	2428
AC Compass	23.1	58.7	20.3	53.3	2533
ISB 3156	17.7	73.4	19.6	66.0	2342
Vista	16.5	71.5	20.2	68.3	2381
McHale	16.3	63.1	17.9	69.5	2176
Mackinac	14.7	63.8	18.2	63.0	2188
CV (%)	8.8	6.9	8.9	7.1	11.0
LSD (0.05)	2.8	6.1	3.0	6.4	250

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SEEDING COMMON BEAN UNDER SUB-OPTIMAL SEEDBED TEMPERATURE

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Introduction

Chilling (-2 to 15°C) and freezing (≤-2°C) temperatures are detrimental to common bean (*Phaseolus vulgaris* L.) plants at all stages of growth (Dickson and Petzoldt, 1987). Sub-optimal seedbed temperatures inhibit and/or delay dry bean emergence resulting in poor and non-uniform seedling stand. Hence, dry bean seeding on the northern prairies is delayed until late-May, however, makes the crop more vulnerable to early fall frost resulting in poor seed yield and quality. Preliminary sub-optimal temperature seeding evaluations under controlled conditions identified dry bean cultivars/lines that possessed superior emergence under controlled sub-optimal temperature regimes. Cumulative effects of sub-optimal temperature during germination and emergence on subsequent bean phenology and yield have not been studied. The ***objective*** of this study was to compare selected dry bean cultivars seeded under sub-optimum and optimum (>20°C) seedbed temperatures in the field for their phenology and yield.

In the above experiment, early seeded (mid-May) dry bean cultivars had higher seed yield and quality compared to those seeded late May in years with early fall frost. Relatively poor seedling stand was a concern in early seeded bean cultivars. Wild tomato accessions from higher altitudes were superior to those from lower altitudes for their germination at <15°C (Patterson et al., 1978). The ***objective*** of this study was to evaluate common bean accessions, and their wild, weedy or escapes from the high altitudes of the Sierra Madre and the Andes for their emergence under sub-optimal temperature in the field.

Materials and Methods

1. Seeding Date Effects on Emergence and Yield of Dry Bean

Navy bean cultivars AC Skipper and CDC Whistler, and black bean cultivars UI 906 and CDC Nighthawk were grown. Source seed was from yield trials grown in Outlook, SK. Seeds were treated with Apron FL @ 0.046 L per 100 kg of seed. Seeding dates were 17 May in 1999 and 2000 (correspond to mid May, seedbed temperature < 15°C), and 5 June in 1999 and 30 May in 2000 (correspond to recommended seeding date, seedbed temperature > 20°C). Seeds were planted at approximately 5 cm depth at the rate of 60 seeds per m^{-2}. Soil (5 cm) and air (1 m) temperatures were monitored using a datalogger.

Traits evaluated: i) percent emergence at 20 and 30 days after seeding, ii) cumulative thermal units to anthesis and 50% physiological maturity, iii) number of pods per plant and number of seeds per pod, iv) yield on a dry weight basis, and v) the 100-seed weight and percent frost damaged seed at maturity.

Experimental design was a RCBD in a split-plot layout with four replicates. Planting dates (main plot) and cultivars (sub plot) were considered as fixed effects. Separate analyses were performed for each year, since seeding date effects were not consistent over years for percent emergence at 20 and 30 days after seeding, percent frost damaged seed and seed yield. Anthesis, maturity and yield traits were subjected to analysis of covariance with number of plants harvested as covariate.

2. Response of Diverse Bean Accessions to Sub-optimal Seedbed Temperature

Bean accessions from CIAT were increased in the phytotron. One hundred and eighty accessions including cultivated common bean, and their wild, weedy or escapes from the high altitudes (≥ 2000 m) of the Sierra Madre and the Andes were used in this study. CDC Whistler, CDC Nighthawk and UI 906 were included as check cultivars. Seeds of wild and weedy accessions of common bean were scarified by nicking the seed coat. Seeds were treated with Apron and planted on May 3, 2000. Soil and air temperatures were monitored. The experiment was laid out as an augmented design with six blocks.

Traits evaluated: i) percent emergence at 20, 30, 40 and 50 days after seeding, ii) cumulative thermal units to 50% anthesis and 50% physiological maturity, iii) yield, and iv) the 100-seed weight and percent frost damaged seed at maturity.

Confirming field results in 2001: Five accessions with the highest seedling emergence at 20 days after seeding (G9345, G8823, G8090, G9430, G7551), five accessions with no emergence at 20 days after seeding (G5024, G991, G19504, G19899, G746) and three check cultivars were seeded on May 3, 2001 to confirm previous year results. Experimental design was a RCBD with two replicates. Fifty Apron FL treated seeds per accession per replicate were seeded.

Results and Discussion

1. Seeding Date Effects on Emergence and Yield of Dry Bean

May of 1999 had 71 mm above average precipitation causing a delay of late May seeding until June 5. Seedbed temperature ranged between 6 and 18°C in 1999, and 6 and 17°C in 2000 during the first week of mid May seeding. Seeding date effects were significantly different for emergence at 20 and 30 days after seeding in both 1999 and 2000. Seeding date effects were also significant for cumulative thermal units to 50% maturity, number of pods per plant,

number of seeds per pod, yield per m^{-2} and percent frost damaged seed in 1999. Cultivars were significantly different for most traits in both years.

Percent emergence at 20 and 30 days after seeding was significantly lower for the mid May seeding compared to the late May seeding in both 1999 (Table 1) and 2000 (data not presented), primarily due to sub-optimal seedbed temperatures at seeding. Several etiolated plants with slender stems were observed at the time of harvest of plots, indicating late emergence of seedlings from viable seeds. In 1999, Saskatoon had two successive frost on Sept. 13 and 14 (-1.6°C and -2.6°C, respectively). CDC Whistler was relatively late maturing in 1999 compared to AC Skipper and CDC Nighthawk. Cultivars seeded in late May yielded half that of mid May seeded crop in 1999, and had a higher percent frost damaged seed (Table 2). In 2000 however, the first fall frost was on Sept. 23 (-3.8°C) by which time, cultivars in both seeding dates have attained physiological maturity. Hence, no difference in yield or frost damaged seed was observed among seeding date (data not presented)

Table 1. Means for percent emergence at 20 and 30 days after seeding (DAS) for navy and black bean cultivars in Saskatoon, 1999.

	% Emergence 20 DAS		% Emergence 30 DAS	
Cultivar	mid May	late May	mid May	late May
AC Skipper	61.3	93.0	68.1	94.3
CDC Whistler	62.5	93.7	69.0	93.5
UI 906	65.3	96.6	76.9	96.6
CDC Nighthawk	70.8	96.2	83.3	96.0
Mean†	**65.0b**	**94.9a**	**74.3b**	**95.1a**

†Means followed by the same letter are not significantly different at 0.05 level.

Table 2. Means for yield and percent frost affected seed for navy and black bean cultivars in Saskatoon, 1999.

	Yield (g/m^{-2})		% frost affected seed	
Cultivar	mid May	late May	mid May	late May
AC Skipper	371	205	1.0	28.0
CDC Whistler	396	184	15.8	74.3
UI 906	363	202	1.5	39.8
CDC Nighthawk	351	185	0.0	18.0
Mean†	**370a**	**194b**	**4.6b**	**40.0a**

†Means followed by the same letter are not different significantly at 0.05 level.

2. Response of Diverse Bean Accessions to Sub-optimal Seedbed Temperature

Seedbed temperatures were colder in 2000 compared to 2001, enabling efficient identification of lines with the ability to emerge under sub-optimal temperatures. Seedbed temperature for the two weeks after seeding ranged between 1 and 18°C in 2000, and 5 and 17°C in 2001. Appearance of hypocotyl hook at the soil surface was observed on or after 15 days from seeding. At 20 days after seeding (May 23, 2000), two accessions, G9345 (USA) and G8823 (The Netherlands) had a significantly higher percent emergence (35%) than check cultivars. These two accessions and CDC Nighthawk had the highest emergence (> 70%) at 30 days after seeding (June 2, 2000). Similar trends were observed in 2001 except for G9345 at 20 days after seeding. At 50 days after seeding (June 22, 2000), all accessions except G8855 had emerged. Most bean accessions with < 50% emergence were wild, weedy or escapes from the high altitudes of the Sierra Madre and the Andes. Poor percent emergence of these accessions may partly be due to the imbibitional chilling injury by nicking of the seed coat.

UI 906 had less than 8% of seedlings emerged by May 23, the traditional date for seeding. Even at 30 days after seeding (June 3), emergence of UI 906 was less than 35% in both years. With increasing soil temperature during early-June, emergence of UI 906 increased to about 90%. Delayed emergence however, resulted in delayed maturity in both years, and lower yield in 2000 compared to CDC Nighthawk (data not presented). Delay in maturity even by few days severely reduce yield in years with early fall frost. Early emergence response of G9345 and G8823 can be attributed to a lower temperature maximum required for germination and emergence to proceed, compared to UI 906. Common bean genotypes with emergence response similar to that of G9345, G8823 and CDC Nighthawk can be considered chilling tolerant while that of UI 906 are chilling sensitive.

Conclusions

Development of bean cultivars with frost resistance would enable early seeding of dry bean on the prairies, but will require a long-term research effort. Conventional breeding can be used to breed bean cultivars with emergence pattern similar to UI 906, possibly enabling earlier seeding dates on the northern prairies. G8823 can be used as a parent to develop elite bean cultivars with the ability to emerge under sub-optimal temperature for regions with a low risk of frost at the bean seedling stage. Wild *Phaseolus* accessions from high altitudes does not possess the ability to emerge under sub-optimal temperatures.

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Evaluation of a Recombinant-Inbred-Line Population for Reaction to White Mold

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North Dakota dry bean (*Phaseolus vulgaris* L.) producers have rated white mold [*Sclerotinia sclerotiorum* (Lib.) deBary] as the leading disease problem every year since 1995 (Lamey et al., 2000). Losses to white mold occur annually, but the magnitude varies depending on environment and other factors. Plant architecture plays a critical role in disease development because of its influence on the microclimate within the plant canopy (Saindon et al., 1995). Genotypes with upright growth avoid white mold by allowing air movement between rows compared to genotypes with prostrate growth habit. Sources of genetic resistance to white mold are limited. Resistance has been identified in germplasm collections (Miklas et al., 1999), but is not adapted to North Dakota. Developing adapted white-mold-resistant cultivars has proven difficult because of lack of readily available and adapted sources of resistance. Green stem is observed in some dry bean genotypes including 'Bunsi', which has partial resistance to white mold. Green stem refers to the inability of the plant to mature uniformly, resulting in plants with mature pods that retain leaves and have actively growing green stems. Green stem is a harvest problem in commercial production due to non-uniform dry-down of the plant, resulting in stained seed and difficulty combining.

The primary objective of this research was to evaluate a recombinant-inbred-line (RIL) population for reaction to white mold and identify resistant lines. Evaluation of green stem characteristic, which has not been previously conducted in dry bean, was a secondary objective of this research. Evaluation of the green stem characteristic and a potential relationship with white mold reaction may lead to a better understanding of the type of white mold resistance displayed by Bunsi.

A RIL population comprised of 119 $_{F2:12}$ lines developed from hybridization of Bunsi x D76125 navy bean by Miklas and Grafton (1992) was evaluated. Bunsi is resistant to white mold, late maturing, has an upright growth habit, and displays green stem characteristic while D76125 is susceptible to white mold, early maturing, has a prostrate growth habit and uniform dry-down. Three replicates of the population, parents, and check navy cultivars 'Huron' and 'Midland' were planted in a randomized complete block design (RCBD) at eight locations from 1997 to 2000. To increase the chance of white mold infection the following procedures were implemented: Sprinkler irrigation, spreader rows, narrow-row spacing (45 cm), wind barriers, and artificial inoculation of each plot with 1.25×10^6 ascospores at peak bloom. White mold and green stem were visually evaluated on a whole plot basis as plants were near harvest maturity. White mold was evaluated using a 1-9 scale, where 1 = no infection and 9 = severe infection or plant death and green stem evaluation used a 1-5 scale, where 1 = no green stem and 5 = severe green stem. Combined analysis of all environments was performed using PROC ANOVA in Statistical Analysis System (SAS Institute, 1992). Pearson's correlation coefficients were calculated based on treatment means from individual environment analysis.

Parental means were significantly different at the 95% level of confidence with Bunsi and D76125 scoring 2.4 and 5.4 and 4.2 and 2.2 for white mold reaction and green stem, respectively. Population distribution for white mold reaction approached a normal distribution based on treatment means, suggesting polygenic control of white mold resistance. Two lines, ND1107 and ND1108, had white mold means lower than the resistant parent, Bunsi, suggesting transgressive segregation for white mold resistance. The same two lines had green stem scores less than Bunsi, but were not significantly different. White mold x plant architecture measurements (plant height, plant width, and plant density) had positive, but low correlations (Table 1). White mold x plant density was equally correlated at each of the Carrington environments ($r = 0.27$). White mold x days to maturity and white mold x days to maturity both were negatively correlated (Table 1). White mold x days to

maturity at Carrington 1999 had the highest correlation of any interaction (r = -0.60). These data suggest negative relationships between white mold and green stem and white mold and days to maturity.

Table 1. Correlation coefficients of variables measured at two environments in 'Bunsi' x D76125 RIL population.

Correlation	Carrington 1999	Carrington 2000	Pooled†
		r	
White mold x plant height		0.20*	0.16	0.18
White mold x plant width		0.27**	0.21*	0.24
White mold x plant density‡		0.27**	0.27**	0.27
White mold x green stem		-0.50**	-0.20*	-0.36
White mold x days to maturity		-0.60**	-0.17	----

† Pooled correlation estimates calculated only after Chi-square homogeneity test
‡ Plant density = plant height x plant width

Summary

Two lines, ND1107 and ND1108, were identified that had lower white mold scores than the resistant parent, Bunsi. White mold means from three environments approached a normal distribution suggesting that white mold resistance in this population is quantitatively controlled. Late maturity and green stem are undesirable characteristics for commercial cultivars. Correlations between white mold x days to maturity and white mold x green stem generally were negative. Therefore, lines from this population with resistance to white mold will be later maturing and exhibit green stem, which are undesirable linkages for commercial cultivars. If linkage exists between white mold resistance, green stem, and/or late maturity, breakage of linkage may be difficult based on the apparent quantitative nature of white mold resistance. This research confirms the need for continued work to identify more sources of white mold resistance, as current sources are limited and often have unfavorable linkage.

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EFFECTIVENESS OF F_1-SELECTION FOR SIMULTANEOUS IMPROVEMENT OF RESISTANCE TO BACTERIAL BLIGHTS

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Common bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) and halo blight (HB), caused by *Pseudomonas syringae* pv. *phaseolicola* (*Psp*) are among the major production constraints of common bean (*Phaseolus vulgaris* L.) in Castilla y León. They are widespread in the region and their severity and yield losses depend on the weather, cultivars grown, and levels of the pathogen populations. Both are seed-borne and their chemical and cultural control is difficult. Use of resistant cultivars alone or in combination with other methods is the best alternative to control these diseases. Thus, availability of resistant cultivars to both bacterial diseases will help reduce chemical use and production costs. It will also increase and stabilize cultivar yield for a sustainable and environment friendly cropping systems. Our objective was to study the effectiveness of F_1-selection in double crosses, first step in Gamete selection (Singh, 1994), for simultaneous improvement of resistance to CBB and HB of common bean cultivars.

Gamete selection is based on multiple-parent crosses, which offers the opportunity to evaluate and select for dominant and codominant traits in heterogametic and heterogeneous F_1 hybrids. The selected F_1 plants are harvested separately to develop F_1-derived F_2 families ($F_{1:2}$ families). This process reduces the population size in the subsequent segregating generations. The second step of Gamete selection involves early generation evaluation and selection between and within $F_{1:2}$ families.

Pod resistance reaction to CBB were inherited quantitatively (Aggour and Coyne, 1989), although it can be controlled by a single major and a few minor genes (Silva *et al.*, 1989) depending on the source of resistance used. Pod resistance reaction to HB was controlled by a single dominant gene (Hill *et al.*, 1972).

Five double crosses were made. They were coded as follow: Morada Larga / Montcalm // Harris / BRB 131 as **ZARA VI**, Harris / BRB 131 // Harris / VAX 3 as **ZARA VII**, Harris / VAX 3 // BRB 131 / Harris as **ZARA VIII**, Harris / BRB 131 // Harris / VAX 4 as **ZARA IX**, and Harris / VAX 4 // BRB 131 / Harris as **ZARA X**. Disease evaluations were conducted in field during 1999 (F_1) and 2000 (F_2). Plants were inoculated by aspersion method at the first trifoliate leave stage of development with a mixture of *Psp* isolates representing the most important races in Spain (Asensio *et al.*, 1998), and at flowering stage with an isolate of *Xap*. A combined evaluation of pods for both diseases was carried out at maturity using a 1 (resistant) to 9 (susceptible) scale (Schoonhoven and Pastor-Corrales, 1987). Only progenies of F_1-resistant (scores of 1,2,3) and intermediate (scores of 4,5,6) plants were tested in F_2. An average of 20 plants per family were evaluated. Diseases reactions were averaged for crosses in F_1 and $F_{1:2}$ families.

All crosses segregated in F_1 and F_2 (Table 2). **ZARA VI** had the highest average disease score because of the high proportion of susceptible plants. All other crosses had lower disease scores and differences among them were not significant. This disease score was because of higher proportion of resistant parents in each cross. In **ZARA VI**, two of the four parents (Morada Larga and BRB 131) are extremely susceptible to CBB and HB, but in the rest of crosses, only one parent (BRB 131) was susceptible to both bacteria (Table 1).

If we evaluate double crosses for characters controlled by dominant genes, the progeny of F_1-susceptible plants are assumed to be uniformly susceptible, and progeny of F_1-resistant plants are supposed to segregating. Thus, F_1-susceptible plants can be discarded because there will not be any descent with the character of interest. We could verify that all $F_{1:2}$ families derived from resistant F_1 plants segregated for resistant, intermediate and susceptible plants as we expected (Table 3). Some families had average disease score above 6, due to the number of susceptible plants. These families were discarded. Some families did not segregate for susceptible plants. This could be due to higher number of resistant genes present in each cross.

We conclude that F_1-selection was effective for simultaneous improvement of resistance to CBB and HB.

Table 1. Response to infection in pods with common bacterial and halo blights of genotypes used as parental of studied crosses in Valladolid (Spain) during 1999 and 2000.

Genotype	Growth habit	Seed color	Seed size (g)	HB	CBB
BRB 131	I	White	29.90	9	9
Harris	III	White	22.90	1	6
Montcalm	I	Red	52.74	1	2
Morada larga	I	Red	44.02	9	9
VAX 3	II	Red	30.48	1	2
VAX 4	II	Creme	23.20	8	3

Table 2. **Number of plants evaluated, frequency of resistant (R), intermediate (I), and susceptible (S) plants and mean bacterial blight scores for pods evaluated for five double crosses at Valladolid during 1999 and 2000.**

Cross	F_1						F_2					
	Number of evaluated plants	R (1,2,3)	I (4,5,6)	S (7,8,9)	Mean evaluated	Mean selected	Number of evaluated plants	R (1,2,3)	I (4,5,6)	S (7,8,9)	Mean evaluated	Mean selected
ZARA VI	40	11	8	21	5.7	2.7	274	162	45	67	3.7	2.2
ZARA VII	62	47	5	10	2.4	1.4	1018	598	226	194	3.4	2.4
ZARA VIII	59	39	5	15	3.2	1.5	710	472	119	118	3.1	2.1
ZARA IX	18	11	1	6	3.8	1.3	229	171	37	21	2.6	2.0
ZARA X	19	11	5	3	3.1	2.2	295	188	61	46	3.2	2.3
LDS (0.05)					1.6	0.9					0.4	0.3

Table 3. Frequency of resistant, intermediate, and susceptible plants and mean bacterial blight scores for evaluated and selected plants in $F_{1:2}$ families of ZARA VI and ZARA IX at Valladolid during 1999 and 2000.

Cross	**Family $F_{1:2}$**	**F_1**	**F_2**					
			Number of evaluated plants	**Resistant (1,2,3)***	**Intermediate (4,5,6)***	**Susceptible (7,8,9)***	**Mean of evaluated plants**	**Mean of selected plants**
ZARA VI	1D-260-1/99	1	20	8	6	6	4.9	1.9
	1D-260-2/99	1	20	14	2	4	2.9	1.4
	1D-260-3/99	1	20	14	3	3	3.0	1.4
	1D-261-2/99	1	20	15	4	1	2.5	1.5
	1D-262-1/99	5	16	3	2	11	6.5	1.7
	1D-263-1/99	5	20	15	1	4	2.7	1.1
	1D-264-4/99	5	20	6	0	14	6.7	2.0
	1D-267-3/99	5	20	17	0	3	2.2	1.1
	1D-269-3/99	5	18	7	7	4	4.3	1.1
	1D-274-4/99	1	20	13	6	1	2.7	1.3
	1D-274-5/99	5	20	15	3	2	2.7	1.4
	1D-275-1/99	1	20	16	0	4	2.6	1.3
	1D-277-2/99	1	20	5	8	7	5.3	2.2
	1D-278-2/99	1	20	14	3	3	3.0	1.3
	Total	**2.7**	**274**	**162**	**45**	**67**	**3.7**	**1.5**
	LSD (0.05)						**1.7**	**1.2**
ZARA IX	1D-347-1/99	1	20	10	9	1	3.4	1.3
	1D-349-3/99	1	20	18	1	1	1.6	1.2
	1D-350-4/99	5	20	16	3	1	2.4	1.6
	1D-350-5/99	1	20	18	1	1	1.8	1.3
	1D-352-2/99	1	20	15	3	2	2.3	1.2
	1D-352-3/99	1	9	7	0	2	3.1	1.7
	1D-352-4/99	1	20	17	1	2	2.2	1.2
	1D-353-1/99	1	20	10	5	5	4.0	1.2
	1D-355-1/99	1	20	20	0	0	1.0	1.0
	1D-355-2/99	1	20	5	10	5	5.1	2.6
	1D-356-3/99	1	20	15	4	1	2.6	1.5
	1D-357-3/99	1	20	20	0	0	1.6	1.6
	Total	**1.3**	**229**	**171**	**37**	**21**	**2.6**	1.4
	LSD (0.05)						**1.3**	**0.9**

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SEXUAL STAGE DEVELOPMENT OF *Uromyces appendiculatus*

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Abstract

Bean rust, *Uromyces appendiculatus,* sexual stages (pycnia and aecia) have been observed on infected volunteer beans in eastern Colorado and the surrounding region during 11 years from 1989 to 2001. Aecial infection was increased for pinto U. I. 114 and Olathe seedlings that germinated through field-overwintered, rusted bean debris. Greenhouse studies demonstrated that sources of rust-infested, conditioned bean debris produced different levels of sexual infection on different pinto cultivars. Research results and illustrations of bean rust sexual stages are presented.

Methods

Field Surveys

- Observations of bean rust outbreaks (July – August) in commercial pinto bean fields throughout northeast Colorado, northwest Kansas and southwest Nebraska were recorded during the growing seasons of 1988 to 2000.
- Fields where bean rust outbreaks occurred were then surveyed the following spring (May – July) during 1989 to 2001.
- Special attention was given to fields rotated into winter wheat.

Bay Farm Field Study

- Plants heavily infested with rust from inoculated research test plots at Colorado State University provided the source of infested plant debris with teliospores.
- Debris was used to simulate the effects of bean debris carryover and survival within a winter wheat canopy.
- Sprinkler irrigation was utilized to achieve maximum wheat canopy development in the Fall and Spring.
- During this time period, the bean debris and winter wheat crop were left to naturally condition and overwinter.
- Volunteer and seeded bean plants were monitored for the presence of rust development during May and June, 1992, paying attention to infection locations.

Eastern Colorado Study

- Initial studies to verify overwintering of the bean rust fungus involved collection of naturally overwintered debris plus conditioned telia from bean growing regions where rust outbreaks occurred during the 1993 growing season.
- Replicated flats with 22 grams of finely ground bean debris (< 0.36 mm) from Yuma, Burlington or Fort Collins, CO, and 30 seeds of Pinto U I 114, Olathe or Chase in all combinations were randomly placed on a greenhouse bench at 25 C average daily temperature under a plastic tent, and misted (on 15 sec. / off 45 sec., 24 hr per day) using an automatic overhead misting system.
- Hypocotyls, stems, petioles, and leaves were inspected after 30 days incubation to enumerate pycnial and aecial lesions.

Results

Field Surveys

- Evidence of the bean rust sexual stage has been confirmed 11 years during 1989 to 2001 in eastern Colorado and the surrounding region (Table 1).
- Rusted bean fields that are rotated into winter wheat are especially favorable for bean rust overwintering.
- Conditions favorable for volunteer bean plant emergence apparently are also favorable for basidiospore germination and subsequent infection of the volunteers.
- Basidiospores can infect bean plants on hypocotyls, stems, petioles and leaves.

Bay Farm Field Study

- Winter wheat provides a compatible environment for teliospore conditioning and completion of the bean rust sexual stage.
- Both pycnia and aecia were found on volunteer and seeded plants, which germinated through rusted bean debris piles and on top of barren soil surfaces.
- Bean plants that germinated through bean debris had higher incidence and severity of aecial infection.
- Leaf tissues had higher incidence and severity of aecial infection than did stems.

Eastern Colorado Study

- Naturally overwintered bean debris, which contains conditioned teliospores, can complete the sexual stage in the greenhouse.
- Pinto cultivars U I 114 and Olathe had significantly more infections by the sexual stages of bean rust than did Chase in greenhouse conditions.
- Pinto cultivar Chase which is resistant to the asexual stage is susceptible to the sexual stage of one or more of

the locally occurring rust races from Colorado and the surrounding region.

Discussion

Overwintering of *Uromyces appendiculatus* has been only briefly mentioned in the literature. Although several early dry bean researchers including C. F. Andrus (1) and W. J. Zaumeyer (3) have mentioned the sexual stage during discussions on bean rust biology and management, almost all references indicate that this is a rare occurrence. Schwartz et al. (2) reported the first evidence of the bean rust pathogen overwintering in Colorado in a winter wheat field planted minimum tillage into bean straw. Since that time, evidence of teliospore conditioning and sexual stage development have been found during eleven of the past 13 years from 1989 - 2001 in eastern Colorado and the surrounding region.

Potential causes for the increased observations and variations of the sexual stage might include: it is more common than previously suspected; weather patterns have been more favorable for development; monoculture cropping and varying crop acreage have impacted the potential for infection; minimum tillage operations which do not incorporate previously infected crop debris; varietal selection in the absence of sexual stages which may result in cultivars more susceptible to sexual spore stages; a shift in the pathogen population to a race or group of races which utilize the sexual cycle more commonly; and/or improved and more timely scouting techniques. Regardless of the reason that the sexual stage was not previously reported, the increased awareness now may provide clues to more timely and effective management of bean rust in the high plains of Colorado and surrounding states.

Table 1. Field Survey **Colorado bean (primarily pinto) acreage, regional average rainfall (inches), and observation dates of aecia and uredia during 1992 – 2001.**

Year	Acres Planted	Total monthly rainfall – in. (regional average)				Occurrence of	
		May	Jun	Jul	Aug	Aecia	Uredia
1992	164,000	0.76	3.86	6.17	4.30	7/10	7/17-8/28
1993	205,000	3.65	2.55	4.11	4.45	6/10-6/17	7/21-8/16
1994	205,000	1.11	2.81	3.72	1.20	6/7	7/21-8/16
1995	190,000	6.38	2.60	2.95	0.56	6/1-6/23	7/18-8/30
1996	145,000	5.75	2.05	4.37	5.47	6/25-7/16	7/25-8/6
1997	135,000	0.64	3.60	2.90	3.43	6/17-6/20	7/15-9/8
1998	170,000	2.88	1.52	5.28	2.42	6/23	7/27-8/25
1999	155,000	1.92	4.87	1.41	6.11	none	8/4-8/5
2000	120,000	0.37	0.97	2.72	1.90	none	8/9-9/6
2001	115,000	2.49	1.33	2.73	2.00	6/1	none

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EFFECTS OF ADJUVANTS ON COVERAGE, ABSORPTION, AND EFFICACY OF BEAN RUST FUNGICIDES

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Introduction:

Adjuvants have the potential to significantly improve pesticide coverage, absorption, and efficacy, but their use with fungicides and in disease management has been largely ignored. Laboratory and greenhouse studies evaluated coverage, absorption, and efficacy of commercially-accepted adjuvants with diverse chemistries using *Uromyces appendiculatus* and *Phaseolus vulgaris* L. as a model host-pathogen system. Coverage of uv-fluorescent dye was captured and quantified via black light photography and subsequent digital image analysis. Organosilicone-based adjuvants improved coverage 80-89% compared to latex spreader-stickers and untreated controls. Absorption of 14-C azoxystrobin was improved 73% with the addition of methylated seed oil. Translocation of azoxystrobin was negatively impacted by latex-based spreader-stickers. Field trials in Fort Collins, CO found that bean rust control was significantly improved with organosilicone or nonionic-surfactants.

Materials and Methods:

Leaf discs excised from greenhouse grown 'Olathe' pinto bean were treated in a spray chamber with the highest labeled rates of various commercially-accepted adjuvants + uv-fluorescent dye (5 reps/run x 4 runs). Digital images were captured under black light and pixels counted in Adobe Photoshop to quantify coverage.

14-C azoxystrobin absorption with each adjuvant treatment was measured by leaf dosing, rinsing in a methanol and surfactant solution, and subsequent liquid scintillation spectrometry (LSS). Azoxystrobin volatility was measured with each adjuvant treatment to account for all disintegrations not recovered from treated leaves. Preliminary translocation data were generated by biological oxidation of plant tissues followed by LSS.

Field trials with treatments that provided the most thorough coverage or highest levels of absorption were conducted at the CSU Agricultural Research, Development, and Education Center in Fort Collins, Colorado.

Results and Discussion:

All adjuvant treatments except the latex spreader-sticker Bond improved coverage. Organosilicone-based adjuvants consistently improved coverage 80-89% compared to water controls and Bond (Figure 1).

Azoxystrobin absorption was improved 73% over controls with the methylated seed oil SunIt (Figure 2). Although Figure 2 suggests that Bond had the highest level of absorption, later oxidation of treated and untreated leaves revealed that the fungicide was bound within the spray droplet and did not absorb into the cuticle or translocate.

In field trials, bean rust suppression was improved greater than 50% (nonsignificant) over Maneb alone with the addition of an organosilicone or nonionic surfactant within label-recommended spray intervals. Twenty one days after treatment, Maneb + Kinetic or Latron significantly improved rust control compared to Maneb alone. Some phytotoxicity was observed in treatments with organosilicone surfactants in this study. Careful selection of adjuvants and rates are essential as phytotoxicity can be significant with certain crops, varieties, and/or pesticides.

Future studies should examine the ability of adjuvants to allow lower application volumes, lengthen spray intervals, and/or reduced rates of pesticides, while not compromising disease control, yield, or quality.

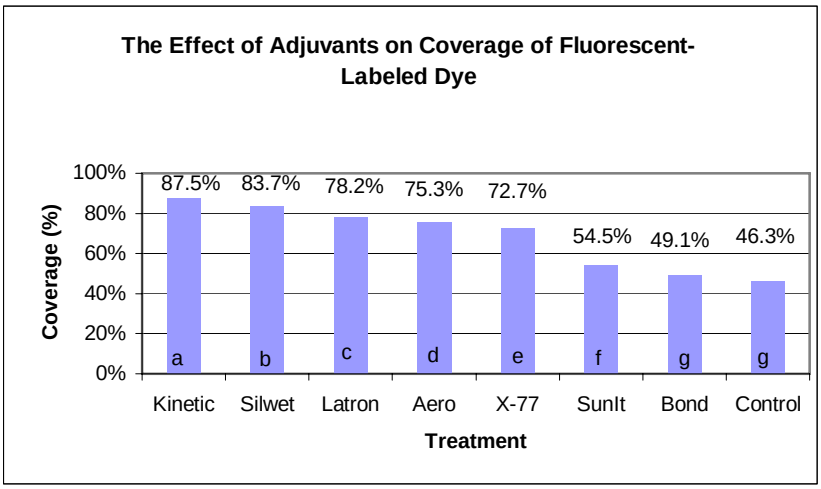

Figure 1. **The Effect of Adjuvants on Coverage of Fluorescent-labeled dye. Treatments followed by the same letter are not significantly different ($LSD_{.05}$).**

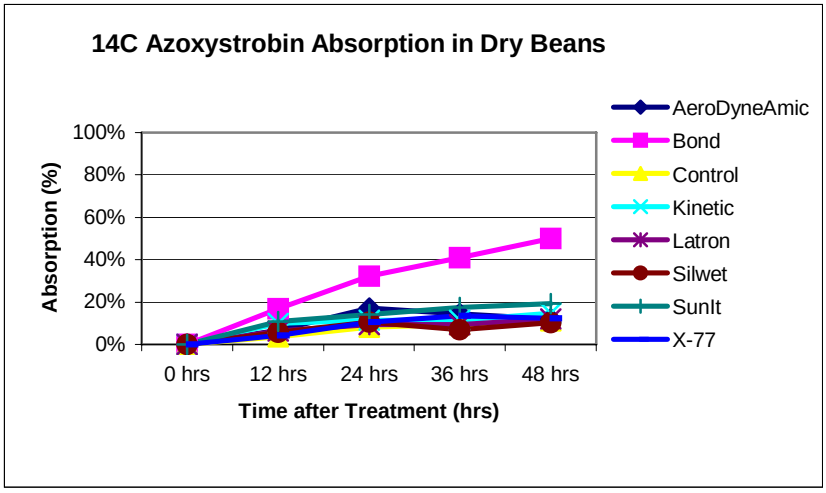

Figure 2. 14-C Azoxystrobin apparent absorption in 'Olathe' pinto bean.

GENETICS OF RUST RESISTANCE IN COMPUESTO NEGRO CHIMALTENANGO (CNC)

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Introduction

Rust, caused by *Uromyces appendiculatus* (Pers. Ex Pers.) Unger, is a major disease of dry bean (*Phaseolus vulgaris* L.) in many production areas of the world. Several dry bean varieties released from United States breeding programs in recent years possess rust resistance against North American races of the fungus. However, in many cases, this resistance is conditioned by a single locus, often *Ur-3*. The natural population of the bean rust pathogen can shift virulence patterns and can overcome genetic resistance over time. New races may develop in the future that could parasitize varieties protected by *Ur-3* only.

Compuesto Negro Chimaltenango (CNC) is one of 19 dry bean lines that make up the set of differentials used to detect and identify races of the bean rust pathogen. The resistance in CNC is effective against all races of the bean rust fungus found in the northern Great Plains (3,4). Despite this, CNC has not been widely used as a source of rust resistance for cultivar development and little is known of the genetics of resistance in the line. The objectives of this project are to develop a basic understanding of the genetics of rust resistance in CNC and to identify molecular markers linked to the resistance gene(s) that may facilitate the use of this resistance in cultivar development.

Methods and Materials

Pathogen. *U. appendiculatus* races 49 (incompatible with CNC) and 67 (compatible with CNC) were obtained from Dr. R. Stavely, USDA, Beltsville, MD in 1999. Each race was subjected to single pustule purification, increased in the greenhouse, and was tested over the set of differentials to confirm the race identity.

Plant material. Genetic crosses were made between CNC and Othello (susceptible parent) in the greenhouse. F_3 families, derived from 100 F_2 individuals, were produced in the greenhouse.

Inoculations & disease evaluations. One hundred F_2 plants were inoculated with races 49 and 67 by a modification of the procedure of Stavely (2). One unifoliate leaf from each F_2 individual was spot-inoculated with 42 µl of a suspension of race 49 rust spores at 120 µg/ml in water containing 0.01% Tween 20; the other unifoliate leaf was then inoculated with race 67. Inoculated plants were held at 100% humidity for 16 hours, then were incubated in the greenhouse for nine days. Disease evaluations for each race were on a scale of 1 (highly resistant/immune type reaction) to 6 (3). The plants were allowed to produce F_3 seed. F_3 families were evaluated for reaction to race 49 only. Sixteen plants per family were inoculated with spores using Soltrol 170 oil as a carrier. Inoculated plants were incubated and evaluated for disease as described above.

Molecular markers. AFLP markers were produced by PCR as described (5). Bulked segregant analysis (1) was used to facilitate the identification of markers linked to the resistance phenoptype. Disease reaction data to race 49 were used from both F_2 individuals and F_3 families to form the susceptible and resistant bulks. After PCR, AFLP bands were subjected to electrophoresis through a 5% acrylamide gel, then were visualized by silver staining.

Results

Parental reactions. Othello pinto bean was susceptible to race 49 (mostly infection type 5) and CNC was resistant (mostly infection types 2 and 3). As expected, both Othello and CNC were susceptible to race 67.

Segregation data. Seventy-four of the 100 F_2 progeny were resistant to race 49. These plants gave infection types 2 and 3, consistent with those observed with the parental CNC. By comparison, 26 F_2

individuals developed mostly infection type 5 and were susceptible. This segregation fits a 3:1 ratio (χ^2 = 0.018, P = 0.75 – 0.90) expected for a single dominant gene conditioning resistance to race 49. F_3 families derived from the same F_2 individuals segregated 29:45:26 (homozygous resistant:heterozygous:homozygous susceptible) for reaction to race 49. This fits a 1:2:1 ratio (χ^2 = 1.18, P = 0.50 – 0.75) expected for a single dominant gene.

Molecular markers. Preliminary experiments with approximately 50 arbitrary RAPD 10mer primers and bulked segregant analysis failed to identify polymorphic bands unique to the resistant bulks. However, the use of AFLP markers has revealed at least four molecular markers found in CNC that are unique to the resistant bulk.

Discussion

Bean rust is one of the major diseases of dry beans in the United States and in many other bean producing areas of the world. *Ur-3* has been deployed in many dry bean varieties recently released in the United States to control rust. This locus is effective against most or all rust races common to dry bean production areas in North America. The use of *Ur-3* in commercially-accepted cultivars has reduced the incidence and severity or rust epidemics in the late 1990s. However, the reliance on a single gene that is widely deployed may not be a wise strategy for long-term control of the disease. The natural population of the bean rust fungus can produce new races through mutation and presumably through sexual recombination, so single gene resistance may not be durable.

The rust resistance in CNC is effective against all known races of bean rust found in the northern Great Plains (3,4). Thus, CNC may be an excellent source of genetic resistance to combine with *Ur-3* in future cultivar releases. In the work reported here, genetic analyses indicate that CNC possesses a single dominant gene that conditions rust resistance effective against race 49 of the bean rust fungus. Rust resistance in dry bean and in other agricultural plants such as wheat and flax is often controlled by dominant genes that fit the gene-for-gene model. It appears that the reistance gene in CNC fits this model. It is also possible that the CNC gene that controls reaction to race 49 is effective against other races of the fungus, but this has not yet been demonstrated. Another possibility is that the ability of CNC to resist multiple races results from the presence of multiple resistance genes in the line.

AFLP and bulked segregant analysis techniques were used to identify molecular markers that appear to be linked to the rust resistance gene in CNC. Experiments are underway to determine the precise linkage of these markers and to convert the markers to more user-friendly SCAR markers. Molecular markers with reasonably tight linkage (< 5 cM) would greatly facilitate efforts to combine this gene with *Ur-3* in breeding germplasm. Other experiments planned or in progress for this project include: 1) allelism tests between the CNC gene and known rust resistance loci, and 2) inoculation of F_3 families with other bean rust races to determine whether CNC possesses other rust resistance loci.

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Molecular Mapping of Root Rot Resistance in Common Beans

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Introduction

Fusarium root rot, caused by *Fusarium. solani* f. sp. phaseolus , is a major disease of beans in southwestern Ontario. All currently recommended Ontario Cultivars of beans are susceptible to root rot disease complex. However, root rot resistant germplasms are available. Incorporation of genes from source germplasm to commercially grown cultivars can be accomplished through a marker assisted selection program. The present experiment was undertaken to identify molecular marker(s) linked to *Fusarium* root rot resistance in common beans. Our objectives were to determine inheritance and linkage of root resistance genes**,** map QTLs relative to molecular markers and map molecular markers relative to each other**.**

Materials and Methods

Plant Material: A cross was made between a root rot susceptible navy bean cv. AC Compass and NY2114-12, a germplasm highly resistant to *Fusarium* root rot. $F_{2:6}$ recombinant inbred lines from this cross were used as a mapping population.

Disease and Seed Trait: Three weeks after inoculation severity of root rot was visually scored using a 0-10 scale: 0 = no root rot; 1 = 1-10% of root area affected; 2 = 11 - 20% of root area affected, 3 = 21-30% of root area affected, and so on (Tu and Park 1993). Seed luster was rated using a scale of 1-5 where, 1 for very shiny and 5 for very dull. AC Compass has dull oval white seed and Ny2114-12 has elongated shiny dark brown seed. Seed shape was rated using a scale of 1-5 where 1 for round and 5 for elongated shape. Seed coat colour was rated using a scale of 1-7 where, 1 for white and 7 for dark brown.

DNA extraction and PCR: DNA extraction was done following a protocol described by Yu et al. (1999). PCR was done following a protocol described by Chowdhury et al. (2000).

Marker Analysis: RAPD markers have been used for mapping. Bulked Segregant Analysis (BSA) have been used to detect putatively linked (linked to root rot) markers. General linkage analysis was performed to detect additional linkage.

Statistical Analysis: Linkage among molecular makers was computed using MAPMAKER/EXP. The putative location of QTLs was determined using MAPMAKER/QTL. The analysis of variance for quantitative traits was performed using SAS.

Results and Discussion

Genetic control of the characteristics: The disease scores, averaged over five plants and two replications (i.e. 10 plants), were used for checking their distribution. The normal distribution of disease reaction indicated quantitative inheritance for resistance for *Fusarium* root rot (Fig 1). Analysis of variance revealed significant differences between the RILs for each trait. High broad sense heritability estimates were observed in all traits indicating less environmental influence over these traits.

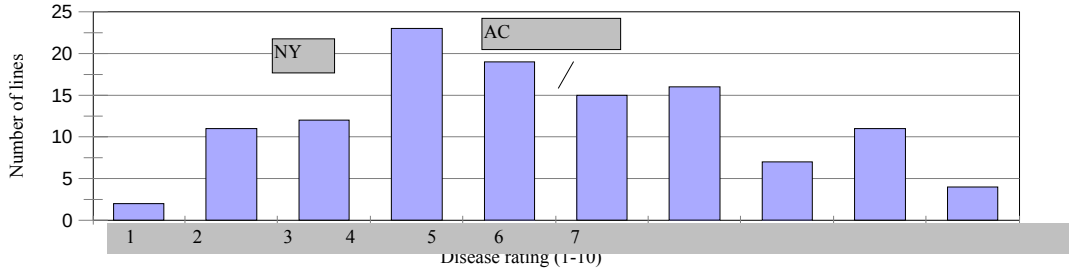

Figure 1. Frequency distribution of root rot caused by *F. solani* in a cross, AC Compass / NY2114-12, F2:6.

UBC218$_{1200}$ UBC218$_{850}$ UBC563$_{1390}$

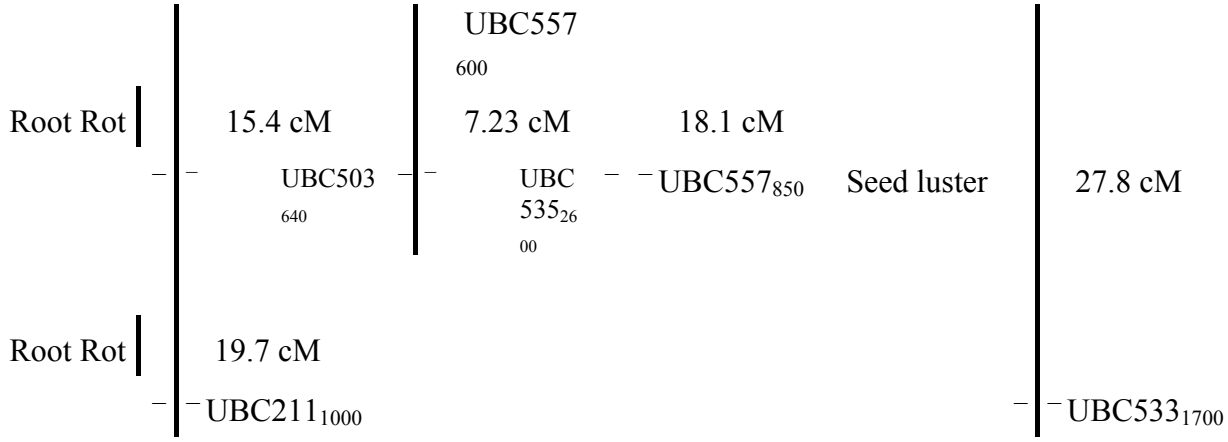

Fig 2. Linkage map in common bean

Linkage Map: After screening 400 RAPD primers two markers ($UBC218_{1200}$ and $UBC503_{640}$) were identified polymorphic between resistant and susceptible lines and parents. Two possibly linked RAPD markers along with 40 other polymorphic RAPD markers were scored for 117 individual RILs and data analysed. Out of 42 RAPD markers nine markers were linked to form four linkage groups (Fig 2) spanning a total distance of 88 cM. Linkage groups ranged from 7.2 cM to 35.1 cM with an average distance of 17.6 cM. Two markers that were polymorphic in the bulks are in the same linkage group along with a third RAPD marker $UBC211_{1000}$.

Detection of QTL: Interval mapping of QTLs revealed two QTLs for root rot resistance, one located between the markers $UBC218_{1200}$ and $UBC503_{640}$ and the other located between the markers $UBC503_{640}$ and $UBC211_{1000}$. Of two QTLs one that is located between $UBC218_{1200}$ and $UBC503_{640}$ was detected with a LOD 8.0 and explained 30% of phenotypic variation. The other QTL was detected with a LOD 5.0 and explained about 20% of phenotypic variance. Seed lustre showed one QTL located between the markers $UBC563_{1390}$ and $UBC533_{1700}$ as detected with a LOD score 2.8 and explained about 15% of phenotypic variation.

In present experiment quantitative inheritance was observed for root resistance that agrees with previous results reported by Azzam (1957), Bravo et al. (1969) and Park and Rupert (2000). For root rot resistance, total phenotypic variation explained by two QTLs is about 50% indicating major effect of these two QTLs on root rot resistance. Higher heritability was observed for seed lustre, seed shape and seed coat colour indicating negligible effect of environment on these traits. Though several reference RAPD markers have been included in this experiment no linkage could be establishes with them may be due to insufficient number of polymorphic markers in this study. More polymorphic markers needed to be analyzed in this population to obtain a closer linkage between markers (<5 cM) and to obtain a more saturated linkage map in common bean.

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Acknowledgement

This research was partially supported by grants from Ontario Colored Bean Growers Association and Ontario White Bean Growers Association. Technical help of T. Rupert is gratefully appreciated.

THE EFFECT OF *Bacillus subtilis* AND *Rhizobium* INOCULATION OF DRY BEAN SEED ON ROOT ROT SEVERITY AND YIELD IN MINNESOTA

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Dry bean root rot in Minnesota is caused primarily by *F. solani* f. sp. *phaseoli* in a complex with *R. solani* and *F. oxysporum*. Seed treatment with *Bacillus subtilis* reduces disease severity (DS) but *Rhizobium* inoculation can result in significantly reduced root rot and increased yield. The objectives of this study were 1) to determine the effect of *Bacillus subtilis* on the incidence and severity of bean root rot, 2) to compare a conventional and granular formulation of *Bacillus subtilis*, and 3) to determine effectiveness of root-rot control with co-inoculations of *Bacillus subtilis* (MBI600 or GBO3) and *Rhizobium tropici* UMR 1899 and *Rhizobium leguminosarum* RCR3622 (HiStick). Two experiments were conducted in Verndale, MN in a sandy loam (USDA classification). In the first experiment the seed treatments had no effect on plant emergence. However, seed treated with either *B. subtilis* MBI600 or GBO3 and *Rhizobium* UMR 1899 reduced bean root rot and increased yield, when compared to untreated plants (Table 1). All treatments that included *B. subtilis* or *Rhizobium* outyielded the standard seed treatment (Captan+Lorsban+ Streptomycin) (SST) by approximately 120 to 400 kg ha^{-1}. Co-inoculation with *B. subtilis* MBI600 and *Rhizobium* UMR1899+RCR3622 reduced DS and enhanced yield (1,904 kg ha^{-1}) relative to the untreated control (1,498 kg ha^{-1}) and SST (1,415 kg ha^{-1}) treatments (Table 1).

In the second experiment no differences were detected in plant emergence. Disease severity decreased with all treatments and the lowest DS was obtained with *Bacillus subtilis* MBI600 applied to the seed plus a granular application of *Rhizobium* UMN 1899 (DS 3.1) (Table 2). The use of a granular formulation with *Rhizobium* UMR 1899 applied to the soil as well as the peat formulation was efficient in significantly increased yield (2,302 kg ha^{-1}) relative to the untreated control (1,812 kg ha^{-1}). In contrast the granular formulation of *Bacillus subtilis* MBI600 was not as efficient as the seed application (Table 2). In most of the treatments the response to inoculation with *Rhizobium* UMR 1899 improved yield probably due to a combination of factors, improved nitrogen fixation and decrease of disease severity. When *Bacillus subtilis* MBI600 alone was applied to seed (2,167 Kg/ha) or combined with a granular treatment of *Rhizobium* UMR 1899 (2,019 Kg/ha) yields were increased, compared to the untreated (1,812 Kg/ha). Seed inoculation of *Rhizobium* had an effect on dry bean, reducing DS, increasing root dry weight and improving yield. Seed inoculation with *Rhizobium* alone increased dry bean yield (2,040 kg ha^{-1}). Inoculation of dry beans with a co-formulation of *Bacillus subtilis* and *Rhizobium* in a peat carrier can alleviate the effects of bean root rot.

Table 1. Effect of dry bean seed inoculation with *Rhizobium* and *Bacillus subtilis* on disease severity, root and plant dry weight and yield in Verndale, MN in 2001

Treatment	Disease severity 1-9	Root dry weight (g)	Plant dry weight (g)	Yield Kg/ha
Rhizobium tropici 1899 + *Rhizobium* RCR3622 + *Bacillus subtilis* MBI600 (seed)	4.2 b	1.98 a	7.32 ab	1,904 a
Rhizobium tropici 1899 + *Rhizobium* RCR3622	4.1 b	1.70 ab	9.00 a	1,806 ab
Bacillus subtilis GBO3 (seed)	3.7 b	1.76 ab	8.70 a	1,782 ab
Rhizobium 3622 (seed)+*B. subtilis* GBO3 (seed)	4.1 b	1.83 a	7.21 ab	1,779 ab
Bacillus GBO3 (seed)	4.3 b	1.81 a	8.94 a	1,763 a
Rhizobium 3622 (seed)	3.6 b	2.10 a	8.78 a	1,630 abc
Bacillus MBI600 (seed)	4.2 b	1.36 ab	7.09 ab	1,626 abc
Untreated Seed	6.3 a	0.89 b	6.00 b	1,498 bc
Captan+Streptomycin+Lorsban	5.8 a	1.78 a	7.00 ab	1,415 c

[1]Different letters within a column are significant different by LSD 5%

Table 2. Effect of dry bean inoculation with *Rhizobium* and *Bacillus subtilis* on disease severity, plant and root dry weight and yield in Verndale, MN in 2001

Treatment	Disease severity 1-9	Root dry weight (g)	Plant dry weight (g)	Yield Kg/ha
Rhizobium tropici 1899 (granular-soil)	4.6 ab[1]	0.94 ab	7.4 ab	2,302 a
Bacillus subtilis MBI600 (seed)	4.8 ab	0.92 ab	6.8 ab	2,167 ab
Captan+Streptomycin + *Rhizobium* 1899 (granular)	4.3 b	0.8 b	7.4 ab	2,066 ab
Rhizobium 1899 (seed)	4.3 b	0.8 b	6.8 ab	2,040 ab
Rhizobium 1899 (granular) + *Bacillus* MBI600 (seed)	3.1 c	0.8 b	6.9 ab	2,019 ab
Rhizobium 1899 (seed) + *B. subtilis* MBI600 (seed)	4.8 ab	0.9 b	7.0 b	1,985 ab
Captan+Streptomycin + MBI600 (granular) + *Rhizobium* 1899 (granular)	5.0 ab	0.75 b	5.9 ab	1,956 ab
Captan + Streptomycin	4.6 ab	0.96 ab	8.0 ab	1,907 ab
Captan+Step +*B. subtilis* MBI600 (granular)	4.5 b	1.32 a	8.6 a	1,904 ab
Rhizobium UMR1899 (granular) + *B. subtilis* MBI600 (granular)	4.2 b	1.09 ab	7.8 ab	1,883 ab
Untreated Seed	5.8 a	0.89 b	5.6 b	1,812 b
B. subtilis MBI600 (granular)	4.8 ab	0.82 b	6.8 b	1,746 b

[1]Different letters within a column are significant different by LSD 5%

TILLAGE AND SEED INOCULATION EFFECTS ON BEAN AND SOYBEAN ROOT ROT IN TWO SOILS IN MINNESOTA

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Root rot of dry bean and soybean has become an important yield limiting disease problem in Central Minnesota. The increase in root rots, caused by *Fusarium solani* f. sp. *phaseoli* in complex with *Rhizoctonia solani* and *Fusarium oxysporum,* has been attributed to shortening of rotation intervals, the use of susceptible cultivars, and soil compaction. The effects of chisel (CP) and moldboard tillage (MB) and a combined *Bacillus* and *Rhizobium* seed treatment on root rot of dry bean and soybean were evaluated in field experiments planted in a split-split plot design with six replications at Staples and Verndale, MN. Main plots were a comparison of tillage: MB or CP. Subplot treatments were the two crops: dry bean (Montcalm) or soybean (McCall). Sub-subplot was the seed treatment: inoculation of dry bean with a combination of *Bacillus subtilis* MBI600 *and Rhizobium leguminosarum* strain RCR3622 or soybean with a combination *B. subtilis* MBI600 and *Bradyrhizobium japonicum* RCR3407 and an untreated control. The inoculant (MicroBio RhizoGen Corp.) was applied in a peat carrier. At Staples, nitrogen was applied in a split treatment at a rate of 50 Kg/ha at sowing and at 45 days after sowing. No nitrogen was applied at Verndale. Soil penetration resistance was measured from 6 to 24 cm and *Fusarium* colony counts determined at 0-5 and 5-10 cm. Plant growth parameters measured were: stand counts two weeks after sowing; disease severity, and plant height at flowering; and grain yield at maturity. Early spring temperatures were below average and rainfall was excessive, delaying emergence and plant growth at both locations. Emergence of dry beans and soybeans at both Staples and Verndale was unaffected by tillage or treatment. Populations of *F. solani* were not affected by tillage or seed treatment at either location. MB reduced penetration resistance to less than 2000 kPa at both locations. At Staples seed treatment reduced disease severity in dry bean (Fig. 1b) while tillage had no effect. Disease severity in dry beans was affected by the interaction of tillage with seed treatment at Verndale ($P = 0.5$) (Fig 1a). Plant height of dry bean was greater in MB than in CP. Dry bean yield was increased by seed treatment and also by MB (Fig. 2b and 2c). At Verndale plant height of dry bean was affected by the interaction of tillage x treatment with taller plants in MB than CP. Plant height of soybean was greater in MB than CP. Seed treatment reduced disease severity in dry beans at both locations (Fig. 1a and1b) while tillage had no effect. Seed treatment reduced disease severity in soybean at both locations but the reduction was not significant. Dry bean yields (Fig. 2b,c) were low at Staples because of poor early season growing conditions, however yields were increased by MB tillage and seed treatment (Fig. 2b,c). At Verndale, seed treatment increased soybean yields from 2204 Kg/ha to 3022 Kg/ha. Soybean yields were greater in MB than CP at Staples (2,988 vs. 2,835 Kg.ha) although the difference was not significant.

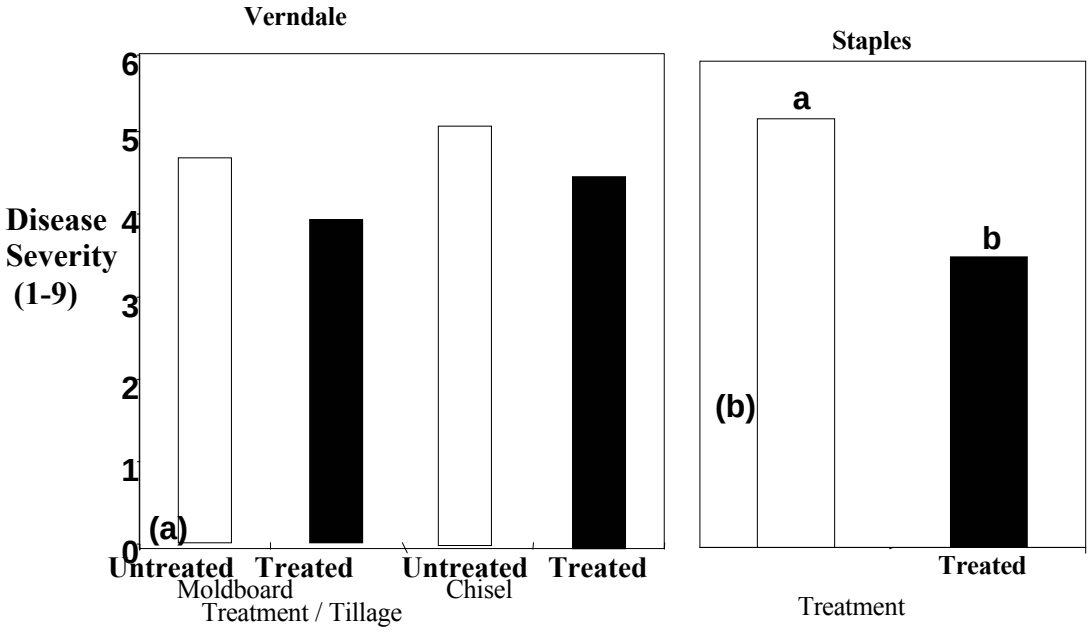

Figure 1a and 1b. Significant factors affecting disease severity in dry bean at Verndale (a) and Staples (b) in 2001.

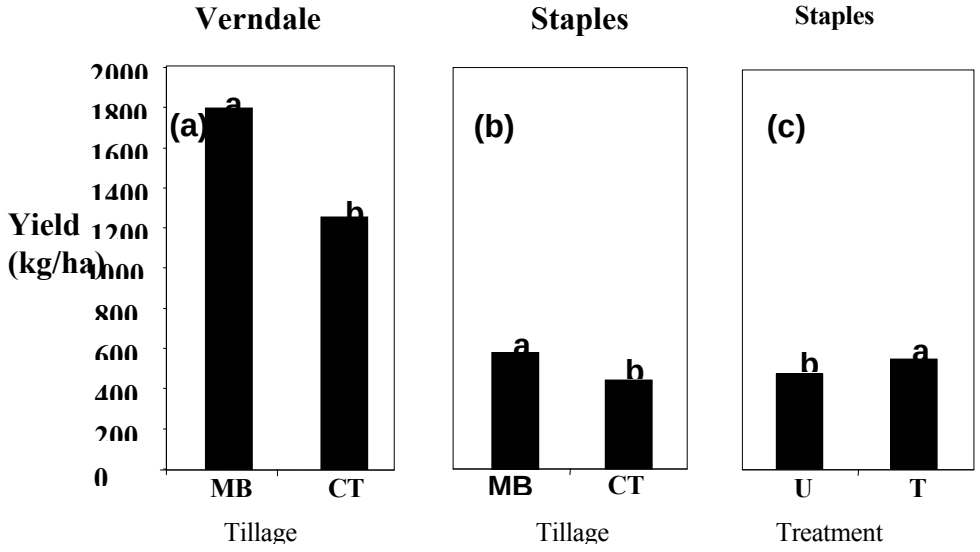

Figure 2a and 2b,c. Significant factors affecting yield of dry bean at Verndale (a) and at Staples (b,c) in 2001.

CODOMINANT INTERPRETATION OF A DOMINANT SCAR MARKER LINKED WITH POTYVIRUS RESISTANCE IN COMMON BEAN

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A major disadvantage of many PCR based RAPD and SCAR markers is that they exhibit dominant inheritance, and thus cannot be used to discriminate between homozygous (AA) and heterozygous (Aa) genotypes. For marker-assisted selection purposes codominant markers provide greater efficiency than dominant markers (reviewed by Kelly and Miklas, 1998).

For conventional PCR twice as much template DNA of the target sequence (dominant DNA marker) is present in the PCR reaction for a homozygous (AA) versus heterozygous (Aa) individual; therefore, in principal twice as much product (dominant DNA marker) should be amplified for the homozygous genotype (AA). Unfortunately, this difference in the amount of dominant DNA marker amplified between AA versus Aa individuals by conventional PCR is undetectable by gel electrophoresis. An F_2 population segregating for the *bc-1*^{2} gene and linked dominant SCAR marker $SBD5_{1300}$ (Miklas et al., 2000) was used to investigate whether codominant interpretation of a dominant SCAR marker was plausible using quantitative PCR techniques.

The segregating population consisted of 59 F_2 plants derived from a cross between pinto bean breeding lines P94207-43 (*bc-1*^{2}*//bc-1*^{2}) and P94207-189 (*bc-1//bc-1*) that were near-isogenic except for resistance to BCMV (Miklas et al., 2000). Both parents possessed the *I* gene. P94207-43 was released as the pinto bean cultivar Kodiak (Kelly et al., 1999). DNA was extracted from the first trifoliolate leaf of all plant samples using the Fast-DNA kit (BIO 101, Inc., Carlsbad, CA) according to manufacturer's recommendations. DNA was quantified with a fluorometer (TD-700; Turner Designs, Inc., Sunnyvale, CA), and diluted to 20 ng/ul for use in quantitative PCR reactions. The 1329 bp DNA sequence corresponding to the $SBD5_{1300}$ SCAR marker was analyzed using Primer Express software (Applied Biosystems, Foster City, CA) to identify candidate sequences for PCR primers and probes.

Foward primer p43335F: 5′- d-TGTACTGTGCTACCACTGCTACATCTT-3′
Reverse primer p43424R: 5′-d-CAGAGCTCAGAATTGCAGCAA-3′.
Taqman probe p43T369C: 5′-ATGCTCCCTCACATTC ATTTAAGTTTGCTGCATAT-3′

PCR for each plant sample was performed in 50 ul reactions containing100 ng of purified genomic DNA, 900 nM forward primer p43335F, 900 nM reverse primer p43424R, 100 nM TaqMan™ probe p43T369C, 5 ul ddH_2O, and 25 ul of 2X TaqMan™ Universal PCR Master Mix (Applied Biosystems). Amplifications and detection of fluorescence were done using a GeneAmp 5700 Sequence Detection System (Applied Biosystems). All PCR reactions were performed using the manufacturer's suggested default cycling profile, which consists of an initial cycle of 2 min at 50^o C, then a single cycle of 10 min at 95^o C, followed by 40 cycles of 15 s at 95^o C and 1 min at 60^o C.

The relative amount of *bc-1*^{2} present in 100 ng of total genomic DNA for each plant sample was determined by plotting the C_T value for the PCR reaction on a standard curve plot generated using total genomic DNA of the homozygous dominant *(bc-1*^{2}*//bc-1*^{2}*)* parent P94207-43. Discrimination between plants that were homozygous dominant (*bc-1*^{2}*//bc-1*^{2}) or heterozygous (*bc-1*^{2}*//bc-1*) was based on comparisons between the results for segregating F_2 plants with results for the reference sample of four comparative heterozygous F_1 plants.

A group mean (y) and standard deviation (s_y) was calculated for the four comparative heterozygous F_1 control plants based on the combined analysis of three PCR reactions for each plant. This group of comparative heterozygous F_1

controls fit a normal distribution, thus a 99% confidence interval for all heterozygotes was determined using the formula y ± 2.58s_y. F_2 plants that fell within the confidence interval were classified as heterozygotes (*bc-1^2//bc-1*). F_2 plants which fell outside the tail area to the right of the confidence interval were considered to be homozygous dominant (*bc-1^2//bc-1^2*). F_2 plants with no fluorescence were classified as homozygous susceptible (bc-1//bc-1).

Twenty F_3 progeny from each F_2 plant were inoculated with the NL-3 strain of BCMNV. Segregation or lack thereof for resistance and susceptibility to NL-3 strain within an F_3 family enabled genotypic classification of the 59 F_2 plants as either homozygous resistant (*bc-1^2//bc-1^2*), heterozygous (*bc-1^2 //bc-1*), or homozygous susceptible (*bc-1//bc-1*).

Quantitative PCR of the Taqman probe, specifically developed for the dominant SBD5 SCAR marker, correctly (100%) discriminated heterozygous *bc-1//bc-1^2* plants from homozygous *bc-1^2//bc-1^2* plants in the F_2 generation as confirmed by F_3 progeny tests for reaction to NL-3 strain of BCMNV. The effective application of real time fluorescent PCR for assigning genotype to plants was demonstrated previously for the *Rhg 4* locus in soybean (Meksem et al., 2001), by a process known as allelic discrimination. However, allelic discrimination requires the availability of a codominant PCR marker.

Our results indicate that the method employed in this study for assigning plant genotype based on quantitative PCR may be broadly applicable to the genotyping of diploid plants for other loci of interest for which only dominant PCR linked markers are available. The application of the quantitative PCR assay described herein will result in more timely population improvement and reduce greenhouse and field space requirements dedicated to progeny testing for disease resistance, as plants that are homozygous for dominant marker-linked resistance genes can be identified as seedlings.

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RACES OF *Pseudomonas syringae pv. phaseolicola* IN NORTH DAKOTA

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Bacterial blights are considered the third most important disease affecting dry bean production in North Dakota (2). Halo blight, caused by *Pseudomonas syringae* pv. *phaseolicola (Psp)*, is one of the three bacterial blights present in the region. The existence of different races of this pathogen in other parts of the world (9) indicate the necessity to identify those present in North Dakota in order to help the breeding program select the right parental materials.

Material and Methods

A total of 161 *Psp* isolates, including 148 collected in 2000, six in 1995, three in 1997 and ten in 1999, were retrieved from infected bean leaves and pods. The identity of the isolates was confirmed by culturing in King's B medium, and standard biochemical tests (3). Pathogenicity of isolates was confirmed on 'Charveloix' a dark red kidney bean. Race identification was ascertained

by inoculation of these isolates on a set of eight standard differentials obtained from Deidre Fourie (ARC-Grain Crops Institute, Private Bag X1251, Potchefstroom 2520, South Africa). Reference cultures of *Pseudomonas syringae* pv. *phaseolicola*, obtained from the same source were used as controls. Seeds of differentials were planted in the greenhouse in 7 cm plastic pots containing Sunshine Soil Mix. Each *Psp* isolate, obtained from 24-48 hours old colonies, was standardized at a concentration of ~10^8 cfu/ml and inoculated using a Paasche airbrush set at 20 psi when differentials were at the primary leaf stage. The inoculum was forced into a small area (1 cm diameter) on both sides of the midrib of primary leaves and also sprayed on the adaxial side until runoff. Inoculated plants were incubated at >90% relative humidity for 24 hrs and then returned to the greenhouse.

Disease reaction was recorded 10 days later using a 1 to 5 scale (4), where 1 = highly resistant (red-brown necrotic spots in the area of maximum inoculation); 2 = resistant (red-brown necrosis with a trace of water-soaking); 3 = slightly susceptible (some necrosis but more water-soaking that is confined to the area of maximum inoculation); 4 = susceptible (water-soaked lesions <1 mm diameter distributed randomly over abaxial side of leaf); and 5 = fully susceptible (water-soaked lesions 1-3 mm in diameter distributed at random over the leaf underside). Differentials were considered resistant if a rating of 1 or 2 was observed.

Results and discussion

Table 1 presents the reaction of selected isolates to a set of standard differentials to illustrate race identification. Out of 161 *Pseudomonas syringae* pv. *phaseolicola* isolates examined during the study, 148 were identified as race 6, three as race 2 and two as race 8. Race 6 was found in samples collected from ten counties (Dickey, Foster, Grand Forks, Griggs, Nelson, Richland, Sargent, Steele, Traill and Walsh) in 2000, as well as in all samples collected in 1995, 1997, and 1999. Race 6, considered the most prevalent race worldwide (4), has been previously reported in Nebraska (1). Race 2, considered the most predominant race in Latin America (4), was identified for the first time in the US on samples collected in 2000 from North Dakota counties Sargent, Foster and Stutsman. Race 8 was identified from a sample from a North Dakota field of unknown location in XXXX. Race 8 has been previously identified in Africa (4), and to our knowledge, this is the first report on its presence in North America. This is the first report on the identification of these three races of *Psp* in North Dakota. The reaction of 8 *Psp* isolates, grouped in two different patterns, could not be matched to that of any of the established races. One group of seven isolates, illustrated by isolate 2000-46, was pathogenic on all differentials but '1072'. Isolate 1995-29 was pathogenic on all differentials but 'A52' and 'A53'. Further tests are in progress to confirm these patterns as that of new races. Finding resistant materials against race 6 will require extensive search within and outside of the genus *Phaseolus*, since race 6 has the broadest spectrum of virulence of all races known to date.

Table 1. Reactions of North Dakota isolates of *Pseudomonas syringae* pv. *phaseolicola* on eight differential cultivars.

		Differentials							
Isolate	Race	CW	A52	TG	U13	1072	A53	A43	196-B
2000-1	6	+	+	+	+	+	+	+	+
2000-6	2	+	+	+	+	-	+	-	+
2000-46	Unknown	+	+	+	+	-	+	+	+
1999-11	8	+	+	+	+	+	+	-	+
1999-15	6	+	+	+	+	+	+	+	+
1997-1	6	+	+	+	+	+	+	+	+
1995-29	Unknown	+	-	+	+	+	-	+	+

1995-50	2	+	+	+	+	-	+	-	+
1995-83	6	+	+	+	+	+	+	+	+

CW = Canadian Wonder, A52 = ZAA54, TG = Tender Green, U13 = Red Mexican, 1072 = 1072, A53 = ZAA55, A43 = ZAA12, 196-B = Guatemala G-196

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BEAN RUST RACES IN NORTH DAKOTA

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Introduction

Bean rust (Uromyces appendiculatus (Pers.:Pers.) Unger) has periodically caused economically important losses to dry edible bean (Phaseolus vulgaris L.) in North Dakota. As market classes, pinto beans have been most susceptible. In the early 1980s seven races of bean rust were identified from leaves collected in North Dakota (3). Development of new races is probable since the bean rust is macrocyclic, and its sexual stages (pycnia and aecia) have been found on beans in North Dakota (5). Because the rust epidemics were particularly severe from 1994 to 1996 and appeared to be increasing in fields planted to cultivars with known resistance to rust, a study to detect the presence of new races of this pathogen was conducted.

Materials and Methods

Rusted bean leaves were collected from forty fields in North Dakota and four in Minnesota during the 1996-2000 growing seasons. Samples were taken from pinto (23), navy (12), snap bean (2), kidney (1), and pink beans (2). Four samples from unidentified varieties were also collected. Samples were taken to the laboratory and immediately stored at -15 ^{o}C. Urediniospores were collected by scraping each frozen leaf over a sheet of aluminum foil, and funneling them into No. 00 gelatin capsules. Spores of each collection were augmented by inoculating them on 'Othello' pinto beans following the procedures described elsewhere (2). The oil was allowed to evaporate, and the inoculated plants were gently misted with an aqueous solution of Tween 20 at 0.1% (v/v). The seedlings were incubated in humid chambers at, >90% RH, and 18 ^{o} C for 16 hr in the dark.

Inoculated seedlings were then transferred to individual chambers in the greenhouse for the following 12 days. Rust spores collected from these seedlings were inoculated separately onto a set of 19 bean rust differentials to obtain single pustules isolates. Differentials were incubated as described above. Two weeks after differentials were inoculated, spores from 2-5 single pustules from each collection were transferred by camel hairbrush (#1) from the differentials onto Othello’

pinto bean seedling to increase the number of spores. Rust spores collected from single pustules (1.2 mg), were suspended in 10 ml of 0.1% Tween 20, then rapidly vortex stirred twice for 5 sec each time, and inoculated onto each of the 19 standard differential varieties for race identification. Spores were delivered using a new HAC-4061 nasal pump sprayer (McKernan Packaging Clearing House, Chicago, IL 60650), which delivered 0.042 ml per spray. Each sprayer was fitted with a 3 cm long plastic extension tube to restrict the spray pattern. The underside of each leaf on each differential was inoculated at a spot formed by a single pump of the sprayer.

Differentials were incubated as described above, and after two weeks, were evaluated for rust reactions using the methods adopted at the 1983 International Bean Rust Workshop (1, 4).

Results and Discussion

Forty-three of the forty-four field collections, were inoculated onto the 19 standard differentials. Bean rust races 52, 54, 69, 70 and 71 were identified and their pathogenic reaction type described (Table 1). Race 54 was the most prevalent race, being retrieved from 90% of the collections evaluated. Races 52 and 70 were retrieved from 33% of the field collections while races 69 and 71 were present in only 7 and 9% of the collections, respectively. This is the first report on the presence of races 69, 70 and 71 in North Dakota. Races collected from Minnesota fields were identified as 52 and 54.

Table 1. Reaction profile of the five bean rust races found in North Dakota 1996-2000.

Differential cultivar[a]	races[b] 52	54	69	70	71
U.S. 3	6	6,5	5,6	5	5,6
C.S.W. 643	2,3	3,2,2^{+}	2,4,3	5,6	3,2
Pinto 650	5,6	5,6	5	6	5,6
K.W. 765	5	6	5	5	4,5
K.W. 780	5,6,4	2^{+},2^{++}	2^{+},2^{++},3	$2,2^{+}$	2^{+},2
K.W. 814	5,6	6,5	5	5	2,3
Golden Gate Wax	5,6	5,6	5	5	4,5
Early Gallatin	4,5	2,2^{+}	2^{+},2	2^{++},2	2^{+},2
Redland Pioneer	3,2	3,2	5,4	5	3,2,4
Ecuador 299	2	2^{+},2	2	2	2
Mexico 235	2	2	2	2	2
Mexico 309	2^{+},2	2^{++},2	3,2	2,3	3,2
Brown Beauty	5,4	2	2^{+},2,3	2^{++},2^{+},2	2^{+},2
Olathe	6,5	6	5	5	5,6
AXS 37	2^{+},2	2	2,3	$3,2^{+}$	2,2^{+},3
Nep-2	2,2^{+}	2^{+},2	2,2^{+}	2	2,2^{+}
Aurora	2^{+}	2	2,2^{+}	2,2^{+}	2,2^{+}
51051	2,3	2	2,2^{+}	2,2^{+}	2,2^{+}
CNC	2	2,3	2	2	2

[a] U.S. = United states; CSW = California Small White; K.W.= Kentucky Wonder; AXS 37=Actopan X Sanilic Selection 37; CNC= Compuesto Negro Chimaltenango (Stavely, 1984).

[b] Reaction grades: 2= necrotic sports without sporulation and < 0.3 mm in diameter; 2^{+} = necrotic spots 0.3-1 mm in diameter; 2^{++} = necrotic spots 1-3 diameter; 3 = uredinia <0.3 mm; 4 = uredinia 0.3-0.5 mm in diameter; 5 = uredinia 0.5-0.8 mm in diameter; 6 = uredinia larger than 0.8 mm

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MAJOR DRY BEANS MARKET CLASS IN BRAZIL AND PERFORMANCE OF SELECTED LARGE-SEEDED TYPES

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Brazil is the largest producer and consumer of dry bean (*Phaseolus vulgaris* L.) in the world and grows 4.4 million hectares yearly, producing about 3.4 million tons (Table 1). Demand for dry bean is increasing because of the population growth rate at 1.8% year^{-1}. To keep the annual consumption at 16 kg capita^{-1} or about 50 000 tons or equivalent to 250,000 ha of new land would have been added yearly. Small seeded bean of Meso-american race dominates 90% of the domestic market e.g. Carioca (striped bean with cream background), black and too lesser extend mulatinho (cream) and Jalo (medium size yellow bean). Brazilian grain types (except black seeded) do not belong to international market class, hence no supply is available elsewhere when import is needed for satisfying internal demand. Only carioca and to a lesser extend black color are consumed all over Brazil. To meet the domestic demand, several hundred thousand tons have to be imported from Argentine (black seeded 100 000t, and white large seeded 15 000t year^{-1}).

Small amount of medium to large seeded beans of Andean race are sold in small quantities in the local market, and consumer preference varied from one region to other. These beans command at least 50% higher price than the small seeded, and can be classified according to international market class: Cranberry/Sugarbean, Calima and Pompadour type, large yellow and white bean.

CIAT (Centro Internacional de Agricultura Tropical - Cali, Colombia) has generated different market classes bean advanced breeding lines adapted for the tropics and these lines are now available in Brazil.

The Brazilian Savannah during winter offers a suitable environment for large-scale production of beans with high input and irrigation, less insect and pest incidence. Small farmers can take the advantage of growing small quantity of these beans to supply the local market. This is a good alternative to escape the already crowded area in producing the traditional grain type among thousands of small and large farmers.

Sixty one advanced breeding lines were grouped into 5 market classes and each group was evaluated separately in a randomized block design with four repetitions in a net plot size of 10.0 m^2. The common checks are Irai (Sugar bean market class) and Jalo Precoce (Yellow market class). Group 1, White seeded (15 lines), Group 2, DRK and LRK (15 lines), Group 3, Calima and Pompadour (15 lines), Group 4, Cranberry and Sugarbean (9 lines) and Group 5, Large Yellow bean (7 lines).

The experiment was conducted with irrigation at Santa Helena, GO and at Anapolis, GO, during winter 2000 on an Oxisol and was fertilized by 400 kg ha^{-1} of fertilizer 4:30:16. Santa Helena farm is under minimum tillage system for more than 10 years. Additional N was side dressed at the rate of 18 kg ha^{-1} , 21 days after germination. Crop was irrigated with 40 mm/week and was protected against insects and diseases.
Results show that the large seeded beans are well adapted to the Savannah in winter season (May - August) agroecosystem. The incidence of diseases, angular leaf spot, rust and powdery mildew incidence was low. White mould did not proliferated because no tilled management used the *Brachiaria* as mulch that protects the soil. The main stem of almost all tested lines remained green up to physiological maturity. This may be a limiting factor for direct mechanized harvesting.

Average yields of the two sites are shown in Table 3. About 10% discount should be made considering high commercial standard. On average bean yield of these large seeded is less than those Meso-american grain type (>4t ha^{-1}) under this farm condition. The outstanding white seeded lines are: WAF 83, WAF 74 WAF 90, WAF 124 and WAF 75, which can compete with the Alubia bean from Argentine; dark and light red kidney: DRK 18, DOR 831, AFR 331, DOR 837 and AFR 329; Calima and Pompadour market class: PVD 92, AND 670, PVAD 1184, AFR 197, and PVA 992; Cranberry/Sugar bean: SUG 31, AFR 245, DOR 868, SUG 33 and CD 8117; and large yellow: BAN 30, A 195, G 9603 SIN 15 and A 463.These results showed that it is possible to produce good quality large seeded beans under tropical growing conditions during the dry season with irrigation, when nutrient and water are not limiting factors. Further studies are needed to evaluate the performance of these beans in other growing seasons (September and March planting date). Cost benefit studies should be conducted before these large-seeded beans can be recommended to farmers.

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Table 1. Bean production at each planting season in 1999*

Planting Season	Planted Area (1000 ha)	Production (1000 kg)	Yield (kg ha^{-1})	Seed color Relation**
Wet season : October - January				
	1,542.3	1,311.5	Roxo	32/60/6
Dry season : March - June				
	2,716.0	1,491.8	549	9/50/51
Winter season : June - September				
	184.0	292.0	1,592	4/96/0
Total	**4,4423**	**3,387.3**	**997**	

* Adapted from IBGE-Survey'. Pesq. Orçamento Familiar, Consumo alimentar per capita 1999. ** Seed color relation of non-black-black and non *Phaseolus.*

Table 2. Bean consumption in Brazil (kg cp-1) by region and estimated seed color and estimated seed color relation**.

Seed color	Market class	North east	Centralwest	South	Average	%
Small seeded						
Black	Black	0.52	3.39	7.90	3.94	39.00
	Carioca*	2.66	4.35	0.11	2.37	23.50
	Cream	6.09	0.34	0.25	2.22	22.04
	Purple	0.02	2.01	0.01	0.68	6.75
	Yellow	0.08	0.30	0.00	0.13	1.24
	Rosinha	0.01	0.06	0.04	0.04	0.38
Subtotal		8.86	7.06	0.41	5.44	53.91
Large seeded						
Medium yellow	Mayocoba	0.79	0.54	0.02	0.45	4.46
Large yellow		0.08	0.11	0.61	0.27	2.63
Subtotal large seeded		0.87	0.65	0.63	0.27	7.09
Total		10.25	11.10	8.94	10.11	100.00

*Carioca = Brown stripes with cream background.

Table 3. Yield of the outstanding large-seeded bean-breeding lines, grouped into five market classes.

	Market class													
	Large seeded white		Light and Dark Red Kidney			Calima and Pompadour			Cranberry and Sugar bean			Large yellow		
Lines	**Yield Kg ha^{-1}**	**100 seed weight**	**Line**	**Yield Kg ha^{-1}**	**100 seed weight**	**Line**	**Yield Kg ha^{-1}**	**100 seed weight**	**Line**	**Yield Kg ha^{-1}**	**100 seed weight**	**Line**	**Yield Kg ha^{-1}**	**100 seed weight**
WAF 83	1923	51.7	DRK 18	1954	67.6	PAD 92	2030	55.3	SUG 31	1772	44.8	BAN 30	1986	57.6
WAF 74	1852	56.1	DOR 831	1907	58.3	AND 670	1914	54.2	AFR 245	1658	43.5	A 195	1838	64.2
WAF 90	1825	51.9	AFR 331	1844	57.9	PVAD1184	1866	40.5	DOR 868	1672	43.0	G 9603	1665	49.8
WAF124	1761	61.0	DOR 837	1692	51.2	AFR 197	1637	56.8	SUG 33	1617	56.1	SIN 15	1459	25.3
WAF 75	1701	61.5	AFR 329	1705	52.6	PVA 992	1616	51.9	CD 8117	1498	36.8	A 463	1408	40.4
Exp. mean	1691			1676			1719			1637			1653	
LSD (5%)	340			331			355			266			348	
CV (%)	18			17			18			14			18	

ALLELISM STUDIES FOR ANTHRACNOSE RESISTANCE GENES OF COMMON BEAN CULTIVAR WIDUSA

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Cultivar Widusa is a differential cultivar for common bean anthracnose. In Brazil it has been shown that this cultivar is resistant to 30 pathotypes of *Colletotrichum lindemuthianum* and susceptible to pathotypes 31, 86, 121, 217, 249 and 320 (Rava et al., 1994; Andrade et al., 1999; Thomazella et al., 2000). However, the anthracnose resistance genes present in Widusa have not been characterized. Previous work shows that no segregation was observed in F_2 populations derived from the cross PI 207.262 vs. Widusa indicating that this cultivar carries at least one of the resistance genes present in PI 207.262. In the same work no segregation was observed in crosses involving TO and cultivar PI 207.262 indicating that PI 207.262 harbors an allele of the resistance gene *Co-4* (*Co-4*^{3}) present in TO (Alzate-Marin et al., 2001). This observation raised the question if Widusa also harbors and allele of the *Co-4* gene. The present work aimed at: 1) determining the number of anthracnose resistance genes present in Widusa using populations derived from a cross between this cultivar and susceptible cultivar Rudá; 2) determining the allelic relationships between these gene(s) and the *Co-4* allele present in TO and the allele *Co-4*^{2} present in SEL 1308.

The F_2 seeds (Rudá vs Widusa and Rudá vs SEL 1308) and those of their respective progenitors and one susceptible control were sown in the greenhouse. Spores (1.2 x 10^6 conidia/ml) from *C. lindemuthianum* pathotype 65 were applied with a aid of a De Vilbiss n$^{o.}$ 15 atomizer powered by an electric compressor to one primary leaf of 10-day-old plants. The plants were incubated in a mist chamber (20-22^o C, 100% relative humidity) for seven days and then the disease symptoms were scored visually using a 1 to 9 scale. Resistant (R) phenotype was assigned to plants with no or limited symptoms (grades 1 to 3) whereas plants graded 5 or greater were considered to be susceptible (S) (Pastor-Corrales et al., 1992).

The inheritance studies showed a 3:1 ratio in the F_2 generation (Rudá vs Widusa), indicating that anthracnose resistance in Widusa is determined by one dominant gene (Table 1).

The allelism studies showed that a segregation ratio of 15:1 for crosses involving Widusa and cultivars TO and SEL 1308, indicating that two independent dominant genes govern anthracnose resistance in these segregating populations.

According to our results, the *Co-4* gene is not present in cultivar Widusa. Consequently, the gene shared by Widusa and PI 207.262 is distinct from all anthracnose resistance genes characterized in allelism studies so far (Table 1).

Table 1. Crosses used for the genetic characterization of anthracnose resistance in cultivar Widusa using pathotype 65 of *Colletotrichum lindemuthianum*

Population	Reaction*	Gene or allele	Observed ratio		Expected ratio	χ^2	P value
			R	S			
Rudá x Widusa	*SxR*	?	72	30	3:1	1.0588	30.35
Widusa x TO	RxR	*Co-4*	119	13	15:1	2.9171	8.76
Widusa x SEL 1308	RxR	*Co-4*^{2}	124	4	15:1	2.1333	14.41

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INHERITANCE OF ANTHRACNOSE RESISTANCE IN COMMON BEAN DIFFERENTIAL CULTIVAR PI 207.262

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Inheritance of anthracnose resistance of the common bean (*Phaseolus vulgaris*) differential cultivar PI 207.262 to races 65, 73 and 79 (binary system designation) was studied in crosses with the cultivar Rudá and Ouro Negro (Table 1). Rudá is a "carioca" type cultivar, with a good yield potential but susceptible to most races of anthracnose present in Brazil, including races 65, 73 and 79. This cultivar is the recurrent progenitor in our backcross breeding program assisted by molecular markers for the creation of cultivars resistant to anthracnose. The black seeded Mesoamerican cultivar Ouro Negro presents excellent agronomic characteristics and good cooking qualities, is resistant to several races of *Colletotrichum lindemuthianum*, but is susceptible to race 65.

Seeds from each progenitor and F_2 populations (Rudá vs. PI 207.262 and Ouro Negro vs. PI 207.262) and twelve plants from each of 100 $F_{2:3}$ families from the cross Rudá vs. PI 207.262 were sown. Fourteen days later, the first expanded leaf from each plant was inoculated on the lower and upper surfaces with *C. lindemuthianum* spore suspensions (1.2×10^6 spores/ml) with the aid of a De Vilbiss no. 15 atomizer powered by an electric compressor in separate experiments for each race (Table 1). The plants were then incubated for seven days in a mist chamber, which was maintained at 20 – 22°C and 100% relative humidity. After this period, each plant was scored visually for the disease symptoms using a 1-9 scale based on Rava et al. (1993).

The segregation ratios in the F_2 population and $F_{2:3}$ derived families involving cultivar PI 207.262 were 57:7 or 15:1, indicating that resistance in this cultivar is determined by two independent dominant genes (Table 1). The 57:7 segregation ratio suggests complementarity between the resistance gene(s) present in PI 207.262 and a gene present in the susceptible progenitor when the populations were inoculated with races 65 or 79. Similar results were reported by Vidigal et al. (1997) using C. lindemuthianum races alpha and delta.

Table 1. Inheritance studies of anthracnose resistance in cultivar PI 207.262

Cross	Race	Generation	Observed ratio		Expected ratio	χ^2	P
			R	S			
Rudá x PI 207.262	65	$F_{2:3}$	87	13	57:7	0.43	50.87
Ouro Negro x PI 207.262	65	F_2	81	13	57:7	0.81	36.89
Rudá x PI 207.262	73	F_2	92	09	15:1	1.22	26.92
Rudá x PI 207.262	79	F_2	74	12	57:7	0.80	37.01

Acknowledgement

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VALIDATION OF RAPD MARKERS LINKED TO *Co-4* ANTHRACNOSE RESISTANCE ALLELES IN COMMON BEAN CULTIVAR PI 207.262

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The common bean breeding program which is being conducted at BIOAGRO/UFV, in Vicosa, MG, Brazil, uses the cultivar PI 207.262 as a source for anthracnose resistance and the "carioca-type" cultivar Rudá as the recurrent progenitor. Previous works have reported the presence of two independent dominant anthracnose resistance genes in PI 207.262, one of them being an allele of the Co-4 gene designated Co-4^3 (Alzate-Marin et al., 2000). Other alleles of this gene are present in cultivars TO (Co-4) and G 2333 (Co-4^2). RAPD markers OPY20 and OPJO1 are linked to the Co-4 gene in cultivar TO (Arruda et al., 2000) and markers OPH18 and OPAS13 are linked to the Co-4^2 allele in cultivars SEL 1308 and G2333 (Alzate-Marin et al., 2001; Young & Kelly, 1998). These markers are potential candidates to aid the selection of plants harboring the Co-4^3 allele derived from the cross between cultivars Rudá and PI 207.262. The main goals of the present work were: 1) to test markers OPY20, OPH18, OPJO1 and OPAS13 in two contrasting bulks of plants selected from an F_2 population from the cross Rudá vs PI 207.262 to identify the one showing the lowest number of recombinants in the susceptible bulk; 2) to use the selected marker to identify $F_{2:3}$ families with the Co-4^3 allele and 3) to determine the genetic distance between the selected marker and the Co-4^3 allele in $F_{2:3}$ families segregating for only one anthracnose resistance gene.

Population F_2 derived from crosses between Rudá (susceptible to most races races of Colletotrichum lindemuthianum) and the resistant cultivar PI 207.262 were used. Leaf DNA was extracted from the parents and from two bulks of F_2 plants contrasting for resistance to C. lindemuthianum pathotype 65, and amplified with primers flanking the markers OPY20, OPH18, OPJO1 and $OPAS13_{950C}$. Marker $OPAS13_{950C}$ was present in all resistant plants and absent in all susceptible plants (Figure 1). Ten $F_{2:3}$ families were obtained from individual F_2 plants harboring the $OPAS13_{950C}$ marker. These plants were inoculated with spores (1.2×10^6 spores/ml) from C. lindemuthianum pathotype 65 to select those segregating for only one anthracnose resistance gene.

The inoculation results showed that three out of the 10 $F_{2:3}$ families segregated for one gene only. DNA from 67 plants of these three families was tested positive for marker $OPAS13_{950C}$, confirming that this marker was indeed linked to the Co-4^3 allele. The genetic distance between the gene and the marker was 3.5 cM (Table 1). Our data demonstrated that the $OPAS13_{950C}$ marker can be used to follow alleles Co-4^2 and Co-4^3 in a breeding program. In our breeding program at BIOAGRO/UFV, this marker can be used to identify lines harboring the Co-4^3 in the cross Rudá vs PI 207.262 or to indirectly select for lines carrying the second gene present in cultivar PI 207.262.

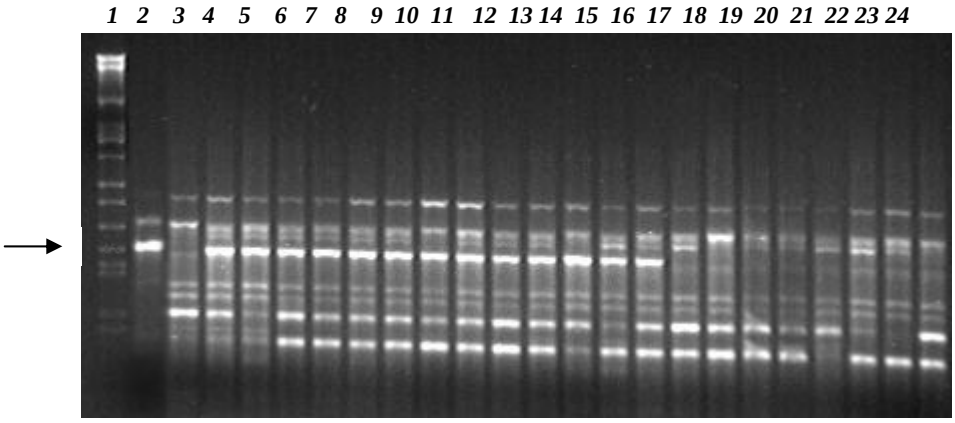

Figure 1. Electrophoretic analysis of amplification products obtained with primer OPAS13. Lanes are as follows: 1, lambda DNA digested with *Eco*RI, *Bam*HI and *Hin*dIII (size markers); 2, PI 207.262; 3, Rudá; 4- 16, F_2 plants resistant to *C. lindemuthianum* pathotype 65; 17-24 F_2 plants susceptible to to *C. lindemuthianum* pathotype 65. The arrow indicates marker $OPAS13_{950C}$ linked in coupling phase to the *Co-4*^{3} gene.

Table 1. Linkage analysis between molecular marker $OPAS13_{950C}$ and resistance allele *Co-4*^{3} in crosses involving cultivars Rudá and PI 207.262

Cross	***Locus tested***	***Expected ratio****	**Observed ratio***	χ^2	***P***	***cM******
Rudá x PI 207.262	**Co-4^3**	*3:1*	*55:12*	*1.796*	*70.54*	-
Rudá x PI 207.262	**Co-4^3/*OPAS13$_{950C}$***	*9:3:3:1*	*53:0:2:12*	*42.24*	*0.00*	*3.5*

* Three $F_{2:3}$ families were tested

** Distance, in centimorgans, between marker and resistance gene.

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DEVELOPMENT OF A SCAR MARKER LINKED TO *Co-9* IN COMMON BEAN

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In order to introduce the genetic resistance to anthracnose in the cultivar 'Andecha', a backcross breeding program was developed in SERIDA (Villaviciosa, Asturias, Spain), using line 'A493' as donor, and 'Andecha' as the recurrent parent. 'Andecha' is a very large white seeded cultivar, highly susceptible to anthracnose, proceeding from a selection of Asturian landraces. Line 'A493' (Alubia / BAT93) proceeded from CIAT (Cali, Colombia). The analyses of reaction to race 38 of *C. lindemuthianum* in the F2 plants from the cross 'Andecha' x 'A493' indicated that 'A493' has only one dominant resistant gene. After six backcross generations, the line 'A1220' was obtained, carrying a single dominant resistant gene, obviously proceeding from 'A493' and, in turn, from 'BAT93', (Alubia is highly susceptible to anthracnose). Line 'BAT93', which carries the anthracnose resistance gene *Co-9*, located in linkage group B4 (Geffroy et al., 1999), was derived from a 4-way cross of which the differential cultivar 'PI207262' is a parent (S. Singh, personal communication).

In this article, we report on the linkage between three RAPD markers and the anthracnose resistant gene present in the new breeding line 'A1220', and on the development of a SCAR from one of such RAPD. The results obtained strongly support that *Co-9* is the gene introduced in breeding line 'A1220' and that this gene is also present in 'PI207262'.

In order to find molecular markers linked to the resistant gene present in line 'A1220', the amplification products of 373 10mer primers (Operon Technologies) were compared in lines 'Andecha', 'A493' and 'A1220'. Line 'A1220' conserved five RAPD markers from the donor parent 'A493': $OB12_{350}$, $OAH18_{1100}$, $OH19_{425}$, $OI19_{460}$ and $OZ13_{325}$. A F2 population of 73 individuals derived from the cross 'Andecha' x 'A493' was used to test the linkage of these five markers to the resistance (Table 1). Three of these markers were significantly linked to the resistant gene.

Table 1. Chi-square (χ^2) and linkage analysis of RAPD markers $OB12_{350}$, $OAH18_{1100}$, $OH19_{425}$, $OI19_{460}$ and $OZ13_{325}$, and resistance to pathotype 38 of *C. lindemuthianum* in 73 F2 individuals derived from a 'Andecha' x 'A493' cross.

Marker	Expected ratio	Observed ratio	χ^2	P	cM *
$OB12_{350}$	3:6:3:1:2:1	19:27:0:0:2:17	66.2	0.00	2.9
$OAH18_{1100}$	3:6:3:1:2:1	20:29:1:0:2:16	57.1	0.00	4.6
$OH19_{425}$	3:6:3:1:2:1	13:23:15:7:8:2	3.6	0.16	unlinked
$OI19_{460}$	3:6:3:1:2:1	4:29:17:16:3:1	45.0	0.00	12.5
$OZ13_{325}$	3:6:3:1:2:1	14:23:17:5:8:1	4.4	0.11	unlinked

* Distances (cM) between each RAPD marker and the resistant gene were calculated using MAPMAKER software (Kosambi function).

Since RAPD marker $OI19_{460}$ had been previously converted to SCAR SI19 by Melotto et al (1998) and mapped in linkage group B4 (Miklas et al., 2000), the F2 'Andecha' x 'A493' population was tested for this SCAR. A co-segregation between RAPD $OI19_{460}$ and SCAR SI19 was observed, indicating that the resistant gene present in 'A493' and 'A1220' is located in linkage group B4, and therefore, it is *Co-9*.

The DNA fragment corresponding to RAPD $OB12_{350}$ was purified, cloned, sequenced and converted to the SCAR marker SB12. The primer sequences for this SCAR are: 5'-CCT TGA CGC ACC TCC ATG-3' (forward) and 5'-TTG ACG CAT GGG TTG GCC-3' (reverse).

The amplification of SB12 was carried out in a 25 µl solution containing 25 ng of DNA, 100 mM Tris-HCl, 100 mM KCl pH 8. 3, 5 mM $MgCl_2$, 0.2 mM of each dNTP (Roche), 0.2 µM of each primer and 1.25 U of Stoffel fragment DNA polymerase (Perkin Elmer). Amplification was performed in a 9600 Perkin Elmer DNA termocycler programmed as follows: 94ºC for 2 min, 35 cycles of 94ºC for 1 min, 68ºC for 1 min and 72ºC for 1 min, followed by 7 min extension at 72ºC.

A co-segregation between RAPD $OB12_{350}$ and SCAR SB12 was observed in the F2 'Andecha' x 'A493' population. Figure 1 shows a genetic map including the resistant gene *Co-9* and markers $OAH18_{1100}$, SB12 and SI19.

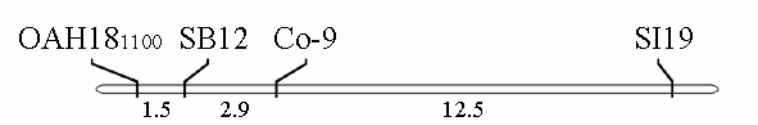

Figure 1. Relative positions of markers OAH181100, SB12, and SI19, and the anthracnose resistant gene *Co-9*. Distances are expressed in cM.

On the other hand, the amplification pattern of SB12 was analyzed on the twelve differential cultivars as well as in other frequently used bean lines (Figure 2). The SB12 amplification band was only observed in genotypes 'A493', 'BAT93', 'A1220' and 'PI207262', suggesting that *Co-9* is also present in this differential cultivar.

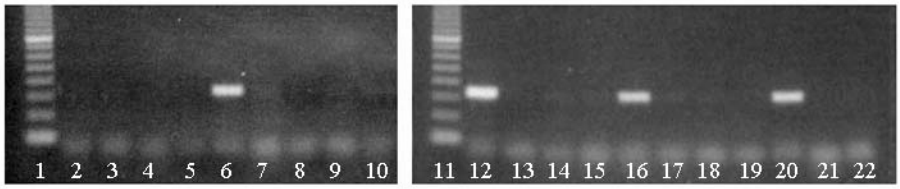

Figure 2. Electrophoretic analyses of the amplification product obtained with SCAR SB12. Lanes 1 and 11: 100bp ladder; lane 2: Perry Marrow; lane 3: MDRK; lane 4: Michelite; lane 5: Kaboon; lane 6: PI207262; lane 7: Cornell 49242; lane 8: Mexico222; lane 9: To; lane 10: Tu; lane 12: A493; lane 13: AB136; lane 14: G2333; lane 15: Widusa; lane 16: BAT 93; lane 17: JaloEEP558; lane 18: Catrachita; lane 19: Andecha; lane 20: A1220; lane 21: SEL1308; lane 22: SEL1360

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EFFECT OF POPULATION SIZE AND SPATIAL ARRANGEMENT IN A NEW ERECT COMMON BEAN GENOTYPE (*Phaseolus vulgaris* L.), COMPARED WITH COMMERCIAL CULTIVARS IN LOW INPUT SYSTEM

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Before releasing new genotypes, it is essential to test them in field conditions utilized by farmers. In Brazil, common bean average farmers utilize medium-low input system, with suboptimal disease control.

The aim of this research is to test a new erect genotype at medium-low input system, in contrasting population size and spatial arrangement.

A factorial field experiment was carried out in split randomized blocks with five replicates. The treatments were: distance between rows: 45,0 or 22,5 cm; seed density: 10 or 20 seeds per linear meter; fertilization: 500 or 1000 kg/ha (8-28-16) and 3 common bean genotypes: Erect, Carioca and Perola.

The Erect genotype was developed by the Brazilian company "FT-Pesquisas e Sementes Ltda" (FT –Research and Seeds Ltd).

According to Table 1, Erect genotype produced more than traditional cultivars Carioca and Perola at all treatments tested.

Table 1. Seed density, distance between rows, fertilizer level, yield and weight of 100 seeds of an three genotypes of common bean.

Seed density (Seed/meter)	Distance between Rows (meter)	Fertilizer level (Kg/ha)	Yield (Kg/ha)			Weight of 100 seeds (g)		
			Erect	Carioca	Perola	Erect	Carioca	Perola
10	45.0	500	1416	904	590	18.1	21.5	20.8
10	45.0	1000	1928	1341	974	17.8	19.2	20.1
20	45.0	500	1621	911	705	17.7	17.7	19.5
20	45.0	1000	2060	986	1015	18.4	18.2	20.5
10	22.5	500	753	345	280	14.4	17.0	17.0
10	22.5	1000	1662	481	229	18.4	16.0	17.0
20	22.5	500	704	435	274	14.9	17.0	17.0
20	22.5	1000	1832	321	450	18.0	17.0	17.5

Low distance between rows affected Carioca and Perola productivity more than erect genotype due to plant architecture, which was less, affected by distance between rows, mainly when 1000 Kg/ha of fertilizer was utilized.

In spite of 2 fungicides application, according to Figure 01 antracnosis also limited strongly yield mainly for Carioca (r^2 = -0.99) and Perola (r^2 = -0.98). The new erect genotype had very low antracnosis symptoms (Table 2 and Figure 01). Considering 500 Kg fertilizer/ha, at higher population size, the competition effect for nutrients was more higher then anthracnose symptom, decreasing the antracnosis correlation with yield (r^2 = -0.60).

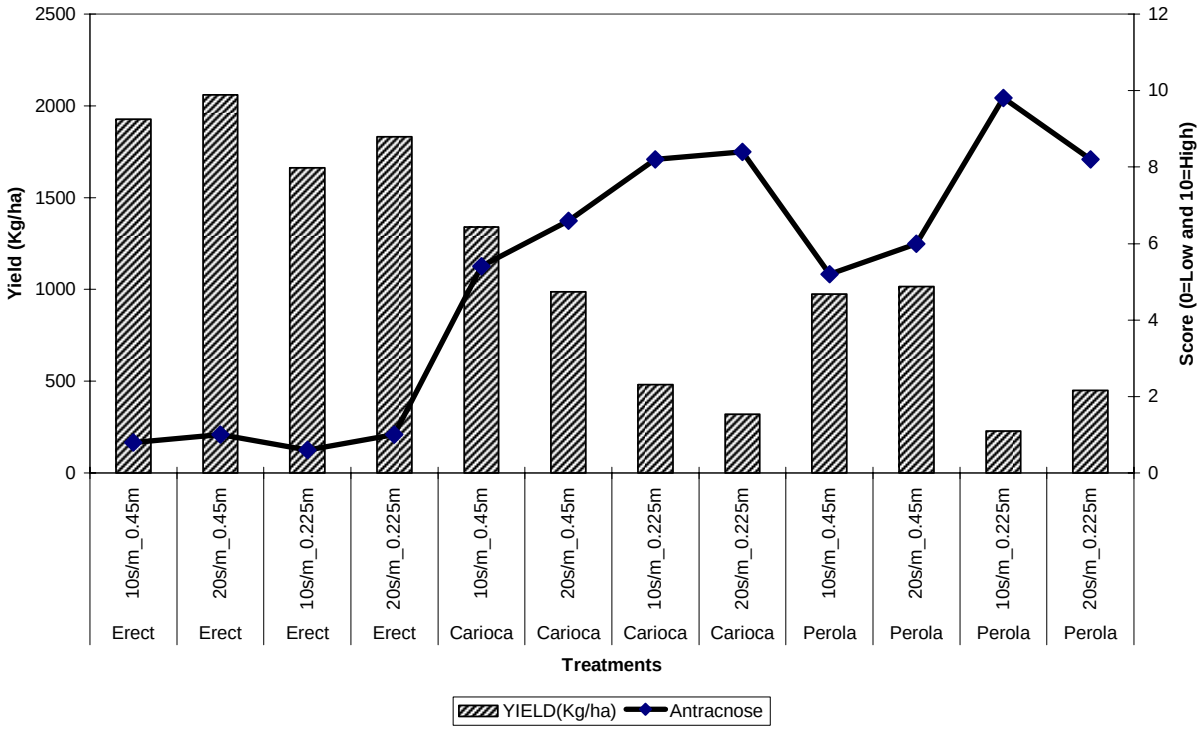
At usual 45 cm between rows and 10 seeds per meter, Erect genotype had lower weight of 100 seeds than Carioca and Perola, but similar to Carioca with 20 seeds per linear meter.

On the other hand, at 22.5 cm between rows, Carioca and Perola had similar weight of 100 seeds, lower than erect genotype at high fertilization level and higher than Erect genotype at low fertilization level. This means that at 22.5 cm between rows, there was higher competition for fertilizers

Table 2. Score of Anthracnose symptoms (*Colletotrichum lindemuthianum*), seed density, distance between rows, fertilizer level, yield and weight of 100 seeds of three genotypes of common bean.

Treatment	Seed density (Seed/meter)	Distance between Rows (meter)	Fertilizer level (Kg/ha)	Anthracnose score (0 = Low and 10 = High)		
				Erect	Carioca	Perola
1	10	0.45	500	2	7	6
2	10	0.45	1000	1	5	5
3	20	0.45	500	1	7	7
4	20	0.45	1000	1	7	6
5	10	0.225	500	1	7	6
6	10	0.225	1000	1	8	10
7	20	0.225	500	1	6	5
8	20	0.225	1000	1	8	8

Figure 01 - Score of Antracnosis attach (Colletotrichum lindemuthianum), seed density, distance between rows, fertilizer level of 1000 Kg/ha, yield of an three genotype of common.



According to the results, in the environmental conditions tested, with suboptimal disease control, the new Erect genotype produced more than traditional cultivars at usual and high size population, due to antracnosis tolerance and plant architecture.

An Overview of the 3rd Bean Rust and 2nd Bean Common Bacterial Blight International Workshops, March 4-8, 2002 Pietermaritzburg, South Africa

J.R. Steadman, M.A. Pastor-Corrales and J.S. Beaver

There were 31 participants from 10 countries and 30 institutions at these two workshops. Countries in East and Southern Africa were well represented as was CIAT (International Center for Tropical Agriculture), Spain and the USA. The objectives of the workshop were to discuss past and current information on management of bean rust and bacterial diseases, plan future research, especially on pathogen variation and breeding for disease resistance and identify opportunities for research collaboration.

Rust is the most important disease of dry beans in South Africa where 100% losses have been reported for rust susceptible varieties. Rust is a recurrent problem in small white beans in Ethiopia. Rust is also an economically important disease in Lesotho, where they grow mostly very susceptible pinto beans, in the southern highlands of Tanzania, as well as in Kenya, Zimbabwe, Madagascar, Mozambique and other bean producing countries of Eastern and Southern Africa.

In describing the life cycle of the rust pathogen, *Uromyces appendiculatus*, the potential of the sexual stage to produce variation was emphasized. However, with the exception of telial germination studies on the High Plains of the USA, nothing is known about the role that this stage plays in most bean production areas with rust problems. Pathogenic variation studies indicate that this fungus is one of the most variable known plant pathogens. Using 19 standard differentials and a standard grading scale adopted in 1983 at the First International Bean Rust Workshop, more than 90 races have been identified, and selected races have been used to identify rust resistance genes. With improved information on putative rust resistance genes, redundancies in these genes and ineffective sources of resistance have been documented in the 19 standard differentials. A new set of differentials needs to be identified. The existence of Andean and Middle American bean gene pools, and information indicating the existence of Andean-specific pathotypes of *U. appendiculatus* highlight the need to have more balance in gene pool representation in the differential set. Co-evolution of the rust pathogen and other bean pathogens has been suggested in recent studies. Further evidence of co-evolution may come from studies involving rust pathotypes found on wild and weedy beans in Andean and Middle American areas of domestication.

The new set of differential lines and cultivars that were chosen after considerable discussion are presented in Table 1. A modified binary system was also chosen for identifying races. The modification is that the Middle American and Andean values will be separated by a hyphen.

Table 1. New standard set of bean rust differentials

Gene pool	Entry	Binary system value	Resistance gene(s)
Andean	A. Early Gallatin	1	Ur-4
	B. Redland Pioneer	2	Unknown
	C. Montcalm	4	Unknown
	D. PC-50	8	Ur-9, Ur-12
	E. Golden Gate Wax	16	Ur-6
	F. PI 260418	32	Unknown
Middle American	A. GN1140	1	Ur-7
	B. Aurora	2	Ur-3
	C. Mexico 309	4	Ur-5
	D. Mexico 235	8	Ur-3+
	E. CNC	16	Unknown
	F. PI 181996	32	Ur-11

Grades 1, 2 and 3 = Resistant; Grades 4, 5 6 = Susceptible.

Until a set of isolines with single rust resistance genes is developed, the new differentials or a modification of these with the addition of some germplasm lines exhibiting multiple rust resistance genes can be used to identify pathogen variability (12 new differentials) or guide the deployment of rust resistance genes. A mobile nursery technique for determination of useful resistance genes in bean production areas may be helpful in guiding rust resistance breeding programs. Because the technique is inexpensive, needs small seed numbers, provides data within 3 weeks of planting and is flexible for resistance gene and gene combination sources in different regions and countries, it will be helpful for developed as well as in resource limited areas of the world. The disadvantage for use in some areas is the need to have transportation of the seedlings in vehicles with protection from environmental stresses such as temperatures above 30°C and high wind. This nursery does not detect adult plant resistance or slow rusting due to abaxial leaf pubescence, both of which are only found in trifoliolates produced above the fourth node. These resistance mechanisms can be selected in the field.

Rust disease management strategies include early planting date, destruction of infested debris and volunteer bean, fungicide applications and disease resistance. Fungicides are expensive, not compatible with environmental sustainability and are limited to protectants in many countries. Thus, disease resistance offers the most effective and least expensive grower control option. Rust resistance genes exhibit dominant heritability and thus can be selected for by specific pathogen races. A set of races has been used to select for Ur-3, -3+, -4, -5, -6, -7 and -11. However, when using races to follow the resistance gene in a backcross method of pyramiding genes, a cluster of tightly linked genes may not be entirely transferred from the source. This has happened with Ur-11, which has resistance to a wider number of races in the PI 181996 source than in the derived line BelMiNeb-RR-1. The Ur-5 and - 11 rust resistance genes, as well as Ur-3, are useful for breeding beans with rust resistance for South Africa. Similarly, CAL 143, a bean line with a red mottled seed from CIAT, that apparently derives its rust resistance from Redlands Pioneer, suffers minimum defoliation and limited yield loss in the presence of rust in South Africa. Isolates of the bean rust pathogen that overcome the Ur-11 have been found in Tanzania; however, Ur-3 and -5 are resistant to these isolates while Ur-4 is not. In Puerto Rico Ur-4 and -5 are reliable sources of rust resistance.

The use of molecular markers can facilitate pyramiding rust resistance genes. Molecular markers are available for Ur-3, -4, -5, -6 and -11 but not all are useful in different gene pools. A linkage map of published rust resistance genes has been constructed and some markers were co-integrated on the BAT 93 X Jalo genetic map. Some of the rust resistance genes are clustered on the same linkage group, e.g. Ur-3, -3+ and -11. This knowledge can help reduce the number of allelism tests needed to determine if resistance genes are independent and assist in estimating map distances. Mapping and tagging genes may be easier than conducting allelism tests to confirm gene independence. Research groups are using RAPD, AFLP or SCAR markers to move rust resistance genes into improved, adapted germplasm. More robust molecular markers need to be developed. As an example, the marker for Ur-4 cannot be identified in an Andean gene pool background.

The following priorities were established for naming rust resistance genes:

1. Establish a core group of named genes (Ur-3, -4, -5, -6, -11).
2. Accept Ur-9 (present in PC-50) into the core map.
3. If genetic map locations of the core group loci are known mapping alone can name the new resistance gene.
4. If the proposed gene is located on a linkage group, allelism tests are only needed for the resistance genes in that linkage group
5. Officially eliminate Ur-8 (present in broadly rust susceptible U.S. #3).
6. Officially name Ur-10 (present in Cape and Resisto).
7. Name previously unnamed rust resistance genes such as those in Ouro Negro, BAC-6, Dorado, and CNC. Inheritance studies and allelism tests will be necessary.

The use of individual rust resistance genes may be temporarily useful in bean production areas where pathogenic variability is low, but gene pyramids will be needed to avoid the 'boom and bust cycle' or where pathogen variation is high, e.g. Honduras.

Although isolines of single and multiple genes would be very useful, no one program has sufficient resources to develop these lines. Before any isolines can be developed, a backcross parent needs to be identified. Either Aztec or Othello were suggested as a potential recurrent parent, but they will have to be tested first for resistance genes in their background. Minimally, the rust resistance in PI 181996 and PI 260418 needs to be transferred to a photoperiod insensitive background.

A collaborative program with Bean/Cowpea CRSP, CIAT and East and Southern African Bean Network scientists was established. Members, advisors and a coordinator were established each for rust and bacterial blights. Very little is known about the diversity of the bean rust fungus in most countries of Africa. For the rust, a mobile nursery comprised of the new 12 differentials will be distributed to four countries to initiate pathogen variation studies. The usefulness of specific resistance genes in specific locations can also be determined. Pathogen race determinations would be conducted in South Africa and the USA. Similar to the rust work, pathogen variation and useful resistance gene studies are planned for the bacterial blights among the Bean/Cowpea CRSP, CIAT, National and University programs in East and Southern Africa.

Common bacterial blight [caused by two different bacteria, *Xanthomonas campestris* pv. *phaseoli* = *Xanthomonas axonopodis* pv. *phaseoli* (Xcp), and *X.c. phaseoli* var. *fuscans* (Xcpf) known as the fuscans strains], halo blight [*Pseudomonas syringae* pv *phaseolicola* (Psp)] and brown spot [*Pseudomonas syringae* pv. *syringae* (Pss)] occur separately or in combination almost anywhere beans are grown. Bacterial brown spot (BS) is the most widely distributed bacterial disease of beans in South Africa. Common bacterial blight (CBB) is also widely distributed and halo blight (HB) occurs only in cool-climate production regions. Several races of the HB pathogen have been identified in South Africa. CBB is the most important disease of beans in Northern Uganda.

The primary causes of these disease problems are contaminated seed and infested crop debris. Crop rotation to avoid inoculum found in infested debris is practiced in the USA and on larger farms in other countries; however, it is difficult for small landholders in Africa and Latin America to rotate on small (1-2 ha) farms. Bean debris can remain a source of inoculum on the soil surface for 18 months in the temperate regions while in the tropics 6-8 months is maximal for survival of the pathogen. Also, the cost and availability of improved seed often force many small landholders to reuse some of their own seed or obtain seed from a neighbor. In other instances, the absence or poor management of seed certification programs leads to contaminated seed.

Detection of the bacterial pathogens in seed can be accomplished by seed washing and plating on MXP medium for Xcp and Xcpf, PCR and bioPCR among a number of methods. Nonpathogenic Xc can be separated from Xcp and Xcpf by a DNA probe for the plasmid present in Xcp and Xcpf but absent in Xc. Nonpathogenic Xc can be found associated with seed and especially in infested debris. The fuscans strains of Xcp produce a dark pigment due to an intermediate compound in tyrosine utilization and thus can be distinguished from Xcp on MXP medium, and using rep-PCR fingerprinting has shown clonal distribution in East Africa. The fuscans strain does not show preference for Andean bean genotypes as originally thought. The fuscans strain is more common in East Africa and shows unique types compared to the old world. The use of the 30 bean landraces representing six races of *P. vulgaris* as hosts can determine if there is specificity for bean gene pools as there was with the bean rust pathogen, and was suggested for anthracnose and angular leaf spot pathogens. Fuscans strains also were reported to be more pathogenic than the regular Xcp strains, and RFLP polymorphisms can separate Xcp from Xcpf. Evidence is accumulating from pathogen storage, antibiotic sensitivity as well as DNA analysis that the fuscans strain is different enough to possibly warrant a new species designation.

Pathogenic variation in Xcp has been reported for many regions. However, the existence of races of Xcp has been debated. Using tepary (*P. acutifolius*) lines as hosts, evidence of a gene for gene relationship was found. Virulence differences could be seen on *P. vulgaris* with some isolates, but the issue may be resolved when a large isolate by resistance source study is completed later this year. A standard set of tepary differentials and a standard rating scale would allow Mendelian separation of resistance genes. However, there is greater need to have differentials in *P. vulgaris*. The investment in time and resources does not seem to justify working in a tepary system, and *P. vulgaris* does not appear to have that degree of specificity.

The use of three screening methods, large nurseries (6 ha) and a large technical staff combined with excellent breeding leadership facilitated the development of the VAX 1-6 series of pyramided sources of CBB resistance. These lines show superior resistance compared to PI 207262, Great Northern #1 sel 27 (Montana #5), XAN 159, 160, 161 and OAC 88-1. Table 2 shows sources of common blight resistance in derived lines. The level of resistance needed in a region depends on the risk that favorable disease conditions will develop and whether other CBB disease management strategies can be successful. Resistance in tepary was found to be major gene dominant or recessive while in *P. vulgaris* major and minor Quantitative Trait Loci (QTLs) were responsible for the resistance reaction. The resistance expressed in earlier sources has always been affected by temperature, plant maturity, organ specificity, photoperiod and screening method. VAX lines do not have these problems but do have resistance instability that needs to be selected out by making single plant selections.

Table 2. Sources of common bacterial blight resistance in derived cultivars and germplasm.

Montana #5 x Tepary #4

↓

GN NE#1 → Sel 27 → Tara, Jules

↓ ↓

Montcalm, Red Kote Harris, Valley, Beryl

↓ Star, Chase, Montrose

SUG lines XAN lines, BAC lines

WBB lines, VAX lines

↑ ↑

PI 207262 XAN 159

A number of mapping populations in tepary and *P. vulgaris* have been co-integrated and used to group QTLs of CBB as well as HB and BS. Linkage groups B 6, 7, 8 and 10 are important for major resistance clusters, and there are SCARs for these areas. The map also provided evidence for the resistance in GN#1 sel 27 coming from Montana #5 rather than tepary as had been thought for many years. The use of gene tags such as SCARS can facilitate CBB resistance improvement programs as well as trace the QTLs in improved resistance lines. More robust markers are needed to pyramid CBB resistance in Andean beans. Marker assisted selection often does not recover parental levels of resistance. Flanking markers will guarantee that the locus of interest is transferred in a cross. Breaking the linkage of QTLs for CBB resistance and the v gene allowed CBB resistant pintos with good seed type to be developed. Allelism tests and a search for unique QTLs are needed to improve selection of genes that can be pyramided. Some HB, BS and CBB loci appear to be linked, e.g. Montcalm was selected for HB resistance but also has CBB resistance.

Disease resistance must be part of an integrated disease management program. Since resistance often is partial, clean seed, crop rotation, volunteer bean and debris elimination and occasionally copper sprays early in the season to control epiphytic populations of Xcp, Psp and Pss are needed. Breeders seed contaminated with CBB, HB or BS can be cleaned up by increasing the seed in a greenhouse.

Intercropping beans with corn or sorghum is still a major crop management strategy in Africa, but it is declining in Central and South America. Studies have shown that a reduction in CBB and rust diseases and increased yield can be obtained when compared to a sole cropping system. Breeding programs should test new lines in intercropped as well as monocrops, although there is evidence that performance is similar in these two cropping systems.

The proceedings of the Workshop will be published separately but a few selected reports presented at the workshop are published in the current issue of the BIC.

Comprehensive linkage map of bean rust resistance genes

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Bean rust disease [caused by the hypervariable fungal pathogen *Uromyces appendiculatus* (Pers.:Pers) Unger] limits common bean (*Phaseolus vulgaris* L.) production worldwide. As with most host/hypervariable pathogen interactions, co-evolution has led to numerous resistance gene and pathotype interactions for bean rust (Sandlin et al., 1999). Kelly et al. (1996) recently characterized the source and provided symbols for most of the resistance genes. Sources for the three resistance genes *Ur-1*, *Ur-2*, and *Ur-2*^{2} previously described by Ballantyne (1972) are no longer available. Eight other named genes: *Ur-3* (Haley et al., 1994; Nemchinova and Stavely, 1998), *Ur-4* (Miklas et al., 1993), *Ur-5* (Haley et al., 1993; Melotto and Kelly, 1998), *Ur-6*, *Ur-7* – tentatively named (Park et al., 1999b), *Ur-9* (Jung et al., 1998; Park et al., 1999a), *Ur-11* (Johnson et al., 1995; Boone et al., 1999), and *Ur-12* (Jung et al., 1998), have been tagged with RAPD or SCAR markers. Note that the *Ur-12* gene conditions adult plant resistance (APR) that is initially expressed at the fourth trifoliolate leaf stage or later (Jung et al., 1998). Two tentatively named specific rust resistance genes, *Ur-8* = *Ur*-US#3 (Christ and Groth, 1982) and *Ur-10* = *Ur*-Resisto (Webster and Ainsworth, 1988), have neither been tagged nor mapped.

Four additional unnamed rust resistance genes: one from BAC 6 assigned the temporary symbol *Ur*-BAC 6 (Jung et al., 1996); one from Ouro Negro = *Ur*-Ouro Negro (Corrêa et al., 2000); and two from Dorado = *Ur*-Dorado-108 with resistance to Race 108 and *Ur*-Dorado-53 with resistance to Race 53 (Miklas et al., 2000), have also been tagged. Two other genes affecting bean rust, *Pu*-a conditioning abaxial leaf pubescence (Jung et al., 1998) and *Crg* (complements resistance gene) necessary for expression of the *Ur-3* gene (Kalavacharla et al., 2000), have also been tagged and previously mapped to linkage groups B3 and B8 (not shown), respectively.

The differential reaction of the specific resistance genes across a subset of nine races is given in Table 1. Note the different responses for *Ur-3* gene from the different sources Aurora and NEP-2 to Race 44, and likewise for *Ur-6* from the sources Golden Gate Wax and Olathe to Races 49, 67, and 108. The *Ur-3* and *Ur-6* genes that were previously tagged, and mapped herein, derive from NEP-2 and Olathe, respectively. Of the genes mentioned above, *Ur-4*, *Ur-6*, *Ur*-US#3, *Ur-9*, *Ur*-Resisto, *Ur-12* and *Pu*-a genes are thought to be of Andean origin (A), and the other genes of Middle American origin (MA). The nine races in Table 1 do not generate a recognizable disease reaction pattern based on origin of the resistance genes, however.

Our objective was to co-integrate all the tagged specific resistance (SR) genes onto the core map (Gepts, 1999) in order to provide a guide or framework from which to determine appropriate allelism tests for characterizing and naming new SR genes. Based on published information and marker work conducted in our laboratory, we were able to map all the genes except the untagged *Ur*-US#3 and *Ur*-Resisto genes (Fig. 1). Because the *Ur*-US#3 and *Ur*-Resisto genes have little value to bean breeding programs, it is unlikely that these genes will be tagged, mapped or allelism tests will be conducted to fully characterize and officially name them. Thus, the temporary *Ur-8* and *Ur-10* gene symbols assigned to the resistance loci in US#3 and Resisto should be retired from further use by the bean research community.

There are two primary groups of Middle American genes clustered on linkage groups B4 and B11 (Fig. 1). The *Ur-5*, *Ur*-Ouro Negro, and *Ur*-Dorado-108 genes cluster toward the end of B4. The *Ur-3*, *Ur-11*, and *Ur*-Dorado-53 genes cluster toward the end of B11. Ballantyne (1972) and Stavely (1984; 1998) previously observed linkage of bean rust resistance genes. Stavely (1984) showed that the *Ur-5* locus was comprised of a block of linked dominant genes, each conditioning resistance to a different race. The *Ur-3* and *Ur-11* genes were shown to be linked (Stavely, 1998). The clusters on B4 and B11 indicate that even more complex rust resistance gene blocks, which span wider genomic regions, exist in common bean.

The *Ur*-BAC 6 gene is located on linkage group B11, but independent of the gene cluster. The *Ur-6* gene is 60 cM from the *Ur-3* locus, but on the same B11 1inkage group, as determined in a Sierra/Olathe RIL

mapping population. This association with *Ur-3* places *Ur-6* in the vicinity of *Ur*-BAC 6 (Fig.1). Because of genomic independence from the other named genes, a single allelism test between *Ur-6* and *Ur*-BAC 6, should suffice for officially assigning the latter with an appropriate gene symbol. Mapping and allelism tests for officially naming the *Ur-7* gene is in progress (Park et al., 1999b).

The *Ur-4* and *Ur-9* genes of Andean origin map independently from the other named genes, on linkage groups B6 and B1, respectively. Note that the location of *Ur-4* on B6 differs from B4 in previous reports (Gepts, 1999), but this information should still be considered tentative because its position is based upon a single linked A14.1100 RAPD marker (Miklas et al., 1993), not the gene itself nor any corroborating linked markers. It will be interesting to see if Andean genes like *Ur-4*, *Ur-6*, and *Ur-9* contribute to gene clusters like their MA counterparts on B4 and B11, or if clusters can be identified which contain both Andean and Middle American genes.

The partial map (Fig. 1.) clearly indicates allelism tests necessary for characterizing the four undefined genes. For instance, crosses among Dorado, Ouro Negro, and *Ur-5* source will provide allelism tests for naming *Ur*-Dorado-108 and *Ur*-Ouro Negro as either new loci or new alleles of *Ur-5*. Similarly, crosses between *Ur*-Dorado-53 with *Ur-3* and *Ur-11* will suffice for officially naming the SR gene conditioning resistance to Race 53 from Dorado.

To determine independence of a new SR gene in the absence of mapping data, at this time, would require initial allelism tests with a gene member of the B4 (*Ur-5*) and B11 (*Ur-3*) clusters, and *Ur-4*, *Ur-6*, and *Ur-9* genes. A protocol for naming new bean rust resistance genes based upon the combination of genetic mapping information and allelism testing is forthcoming. Note that phenotypic segregation data (Kelly et al., 1996) also fully supports independence of *Ur-3* (cluster), *Ur-4*, *Ur-5* (cluster), and *Ur-6*.

The map also indicates linked genes to recombine in coupling for more durable resistance, as conducted recently by Stavely (1998) for the *Ur-3* and *Ur-11* genes formerly linked in repulsion on B11. Based on current data (Table 1), recombining *Ur*-Dorado-108 and *Ur*-Ouro Negro in coupling (if possible) would provide the broadest level of resistance available at the B4 gene cluster.

The partial map (Fig. 1) provides other interesting information. For example *Ur-9* (B1), and the *Ur-3* (B11) and *Ur-5* (B4) clusters co-locate with anthracnose [caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams.-Scrib] resistance genes (clusters) of similar Andean (*Co-1* cluster, B1) and MA origin (*Co-2*, B11; *Co-9* cluster, B4), respectively (Kelly et al., 2003; Geffroy et al., 1999, 2000). Thus, it appears likely that resistance genes to the hypervariable rust and anthracnose fungal pathogens derive from an ancestral resistance gene or gene cluster (Geffroy et al., 1999; Witsenboer et al., 1995) that has undergone cycles of duplication and divergence of function. Duplication and divergence combined with chromosomal translocation events and additional cycles of duplication and divergence could explain the clustering of different rust and anthracnose resistance genes across linkage groups B1, B4, and B11 of the bean genome.

QTL conditioning resistance to bean golden mosaic virus (BGMV) and ashy stem blight disease [caused by *Macrophomina phaseolina* (Tassi) Goid.] also co-locate with the B4 rust and anthracnose resistance gene cluster (Miklas et al., 2000), suggesting even wider divergence of function from an ancestral gene may have occurred in this genomic region. Recombination of rust and anthracnose resistance genes into coupling phase linkages on B1, B4, and B11 may assist breeders in developing cultivars with combined rust and anthracnose resistance.

A survey of the rust-linked markers across a subset of host-gene differentials clearly indicates the potential utility of certain markers for marker-assisted selection (MAS) in different genetic backgrounds (Table 2). For instance, markers linked with the Andean genes *Ur-4* and *Ur-9* will be most useful for MAS in a MA background; whereas, markers linked with MA genes *Ur-3* and *Ur-11* (AC20) appear most useful for MAS in an Andean background. The *Ur-5* marker has broad application and conversely the GT02 marker for *Ur-11* has virtually no application. Studies verifying the utility of the markers for MAS of the linked *Ur*-genes outside the original mapping populations are generally lacking. Therefore, use of these markers should proceed with caution until their cosegregation with resistance is validated in other populations.

Table 1. Differential reaction of specific resistance (SR) genes across a subset of nine rust races.

		Races								
SR gene (source)	**Map**	**41**	**44**	**47**	**49**	**53**	**54**	**67**	**73**	**108**
Middle American origin										

Ur-5 (Mexico 309)	B4	3,2	3,2	3,2	5,6	3,2	3,2	4,5	5,6	5,6
Ur-Dorado-108	B4	5,4	5,4	4,5	5,4	5,4	5,4	5,4	1	1
Ur-Ouro Negro	B4	3,2	3,2	3,2	3,2	3,2	ND	3,2	3,2	5,4
Ur-3 (Aurora)		$2,2^{+}$	4,5	4,5	4,5	$2,2^{+}$	$2,2^{+}$	4,5	5,6	$2,2^{+}$
Ur-3 (NEP-2)	B11	$2,2^{+}$	3,2	4,5	5,4	$2,2^{+}$	$2,2^{+}$	4,5	5,6	$2,2^{+}$
Ur-Dorado-53	B11	5,4	5,4	4,5	5,4	$2^{+},3$	$2^{+},3$	5,4	5,4	5,4
Ur-11 (PI 181996)	B11	2,3	f2,3	2	f2	f2	f2	f2	f2,3	5,6
Ur-BAC 6	B11	3,4	3,2	3,2	3,2	5,4	4,5	3,2	3,2	5,4
Ur-7 (GN 1140)		3,4	3,4	3,2	3,2	5,6	ND	3,2	5,6	3,2
Andean origin										
Ur-4 (Early Gallatin)	B6	4,5	$2^{+},2$	4,3	$2^{+},2$	4	$2^{+},2$	4	$2^{+},2$	$2^{+},2$
Ur-6 (G.G.W)		$2^{+},2^{++}$	$2^{+},2$	$2,2^{+}$	4,5	4,5	5,4	4,5	2	5,4
Ur-6 (Olathe)	B11	$2^{+},2^{++}$	$2^{+},2$	$2,2^{+}$	3,2	6,5	5,4	3,2	2	3,2
Ur-US#3 (tentative *Ur-8*)		5,4	5,4	4,5	5,6	5,6	6,5	5,4	4,5	5,6
Ur-9 (Pomp.Checa)	B1	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ur-Resisto (tentative *Ur-10*)		ND	ND	ND	ND	ND	ND	ND	ND	ND

Scale: 1 to 3 = resistant reactions (light shading) and the 4 to 6 susceptible reactions (no shading). ND= No data.

Table 2. Rust resistance gene-linked markers assayed across a differential set of resistant bean genotypes.

	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	$Ur\text{-}3^{+}$,*Ur-4*	-	+	-	-	-	+	-
BARC-RR-4,13	*Ur-4,Ur-5*	-	+	+	+	+	-	-
BARC-RR-25	*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*	+	+	-	-	-	+	-

A shaded box (+) indicates a false positive. Shaded lines are of Andean origin and non-shaded of Middle American origin.

$Ur\text{-}3^{+}$ derives from MEX 235 while *Ur-3* derives from NEP-2.

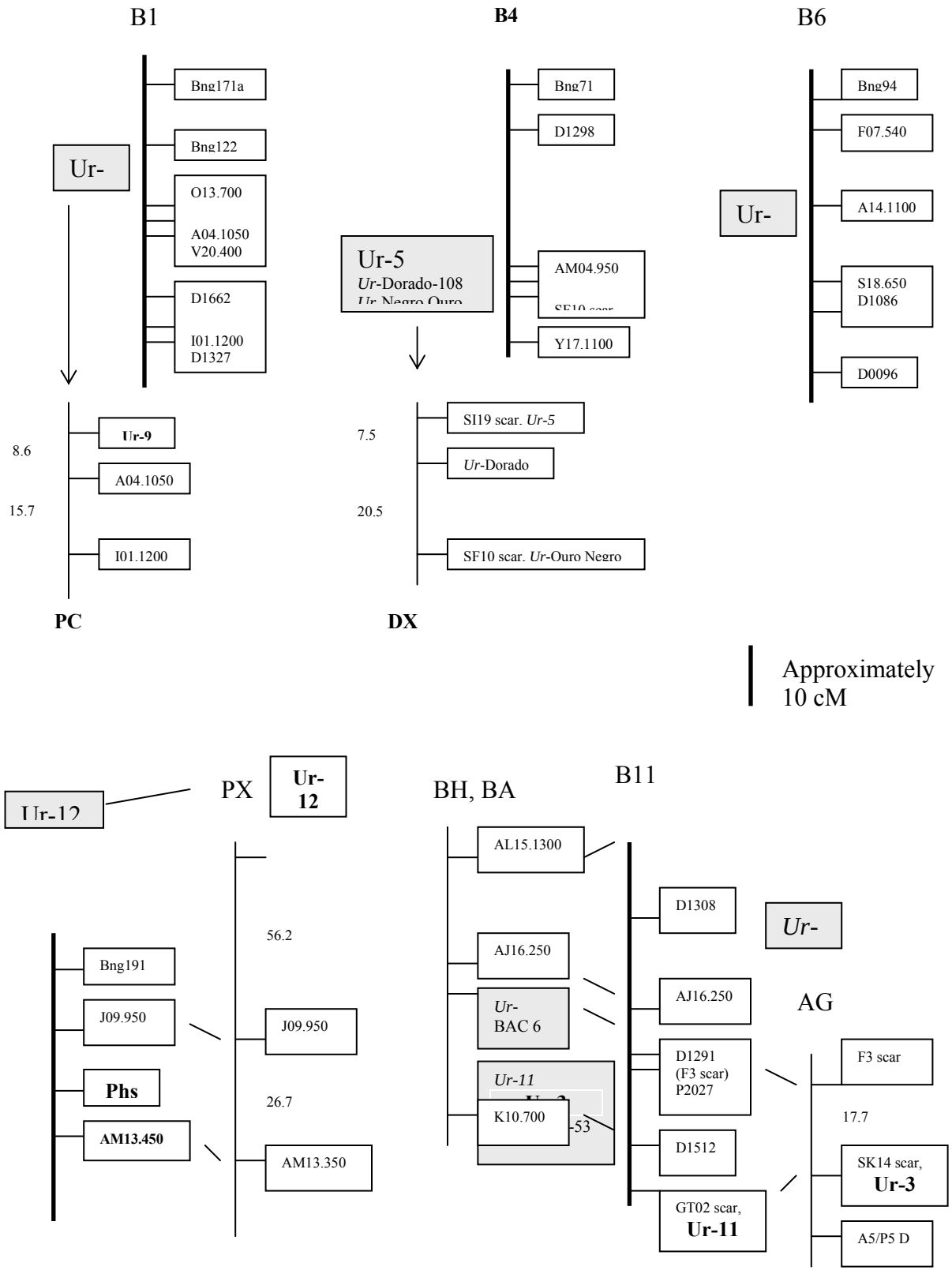

Fig.1. Partial core map indicating general location of bean rust resistance genes mapped indirectly in BAT93/Jalo-EEP-558 via linked markers or co-integrated from PC=PC-50/Chichara-83-109, DX=Dorado/XAN176, PX=PC-50/XAN159, BA=Belneb-RR-1/A55, and AG=A55/G122 mapping populations.

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RUST RESISTANCE GENE BLOCK IN COMMON BEAN CV. OURO NEGRO IS NOT *Ur-5* OR *Ur-11*

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Identification of different rust resistance genes with large resistance spectra and knowledge about the allelic relationship among them are basic conditions for works aiming at pyramiding rust resistance genes in common bean. Cultivars Ouro Negro, Mexico 309 and Belmidak RR-3 are resistant to various *Uromyces appendiculatus* pathotypes identified in Central Brazil (Faleiro et al., 1999; 2001). In order to determine the allelic relationships between the resistance locus present in Ouro Negro, a widely used rust resistance source in Central Brazil, and genes *Ur-5* (Mexico 309) and *Ur-11* (Belmidak RR-3), we analyzed the inheritance of rust resistance in bean segregating populations derived from crosses between Ouro Negro and these two cultivars. The inheritance of the rust resistance present in Mexico 309 and Belmidak RR-3 was also analyzed in crosses involving susceptible cultivar Rudá.

$F_{2:3}$ populations derived from crosses between Rudá and Belmidak RR-3 and F_2 populations derived from crosses between Rudá and Mexico 309 were obtained. Twelve seeds each of 57 $F_{2:3}$ families derived from the cross Rudá vs. Belmidak RR-3, and 222 F_2 plants derived from crosses between Rudá and Mexico 309 were sown in the greenhouse. Fourteen days after sowing the first leaf from each plant was inoculated on the lower and upper surfaces with spore suspensions (2×10^4 spores/ml) of *U. appendiculatus* race 6 (Rudá vs. Belmidak RR-3) and race 10 (Rudá vs. Mexico 309), with a aid of a De Vilbiss no. 15 atomizer powered by an electric compressor. The plants were then incubated for two days in a mist chamber, which was maintained at 20-22 ^{o}C and 100% relative humidity. The plants were returned to the greenhouse where they were evaluated for disease symptoms 15 days after inoculation, using a scale reported by Stavely et al. (1983). Resistant (R) phenotype was assigned to plants with no or limited symptoms (grades 1 to 3), whereas plants graded 4 or greater were considered to be susceptible (S). The phenotypic class frequencies obtained were tested for goodness-of-fit to theorical ratios with chi-square tests. To avoid cross-contamination, each experiment was done in a separate chamber.

For allelism tests seeds from cultivars Ouro Negro, Mexico 309, Belmidak RR-3, and F_2 seeds derived from crosses Ouro Negro vs. Mexico 309 and Ouro Negro vs. Belmidak RR-3 were sown in the greenhouse. The inoculation conditions and symptom evaluations were as described before, but in this case *U. appendiculatus* race 10 was used.

The good fit to the segregation ratio expected (homozygote resistant:heterozygote: homozygote susceptible) ratio in the $F_{2:3}$ families and F_2 plants derived from crosses between Rudá vs. Belmidak RR-3 and Mexico 309, respectively, confirm that resistance of these cultivars is controlled by single dominant genes (Table 1). In the allelism tests the segregation ratios of rust resistance genes (Table 2) were of 15 resistant (R) to 1 susceptible (S) plant in the F_2 populations derived from crosses between Ouro Negro and Mexico 309 (*Ur-5*) and Belmidak RR-3 (*Ur-11*) indicating that two independent genes govern resistance in these populations. These results showed that the gene (or complex gene locus) present in Ouro Negro is different from genes *Ur-5* and *Ur-11*. Thus, cultivars Ouro Negro, Mexico 309 and Belmidak RR3 can be used simultaneously as rust resistance sources in breeding programs for Central Brazil.

Table 1- Segregation for resistance to rust in the crosses between Rudá vs. México 309 (*Ur-5*) and Belmidak RR-3 (*Ur-11*)

Locus tested	Race	Generation	Expected ratio	Observed ratio	χ^2	P
Ur-11	6	$F_{2:3}$	1:2:1	12RR:28Rr:13S	0.578	74.87
Ur-5	10	F_2	3:1	183:29	1.3	24.9

Table 2. Allelism studies of the rust resistance gene present in cultivar Ouro Negro using race 10 of *Uromyces appendiculatus*

Cross	Gene	Observed ratio		Expected ratio	χ^2	P value
		R	S			
Ouro Negro x Mexico 309	***Ur-5***	193	15	15:1	**0.328**	**56.67**
Ouro Negro x Belmidak RR-3	*Ur-11*	60	4	15:1	0.000	100.00

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BEAN RUST RESEARCH IN THE CARIBBEAN

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The first International Bean Rust Workshop was held in Puerto Rico (PR) in 1983 (Stavely et al., 1983). The 1-6 rust evaluation scale based on pustule (uredinium) absence or presence and size and a set of differentials was established at that workshop. The rust differentials have been planted in PR since 1983. Early Gallatin with the Ur-4 gene has been the most durable source of resistance to rust. Pustule size of Early Gallatin has never been > 3 (considered resistant) and the leaf area infected with rust has not exceeded 1%. Mexico 309 (Ur-5 gene) has generally had rust severity < 2% although in 1985 and 1997 large pustule sizes (> 5) were observed. Yield loss caused by rust on susceptible bean lines Pinto 650 and Pinto 114 were 75% and 64%, respectively (Velez-Martinez et al., 1989). A positive correlation between pustule size and % of leaf area infected was also reported.

Many Andean bean lines have durable levels of rust resistance in the Caribbean. Bean rust infection has generally been low in the Caribbean Adaptation Nurseries conducted in PR, the Dominican Republic (DR), and Haiti. The light red kidney cultivar 'Indeterminate Jamaica Red' and the dark red cultivar 'Montcalm' have maintained an immune reaction to rust for more than 15 years in PR. These Andean cultivars have been crossed with 'Pinto 650' to develop populations that will be used study the inheritance of their rust resistance and identify linkage map position. The red mottled bean cultivar 'PC-50' had moderate resistance to rust races prevalent in the DR in the 1980s (Saladin et al., 2000). PC-50 has specific rust resistance determined by the *Ur-9* gene and adult plant resistance determined by the *Ur-12* gene (Bokosi, 1996). In the 1990s, rust races including Andean-specific races appeared in the DR that rendered PC-50 susceptible. In addition, rust resistance genes Ur-3, -4, and -5 have not provided consistent resistance in the DR. Haiti is located in close proximity to the DR and these aggressive pathotypes would be expected to challenge bean production under favorable environments.

Bean rust research has been conducted in PR for several decades. In 1973, the black-seeded bean cultivar 'La Vega' (a bulk of individual plant selections from PI 287536) was released. It was identified as "slow rusting" with small pustule size in the field. The snap bean cultivar 'Palmarejo', a selection from PI 207139 which was collected in Colombia as 'Algarrobo L-101' and released in 1976, was highly resistant to rust races endemic in PR. In 1979, rust resistant black-seeded bean germplasm lines B-190, derived from the cross Mexico 309 / 50600, and 2B-5-1, derived from the cross La Vega / 15R-55 // Mexico 309 / La Vega, were released. In the 1970's, Mexico 309 was immune to rust in PR. The white bean MITA 6383 (an individual plant selection from PI 151395) was also released in 1979. The white bean breeding lines L226-10 and L227-1, released in 1983, derive their resistance from 2B-5-1 (Freytag et al., 1985). Stavely found that these lines were resistant to 13 rust races and the pattern of rust resistance was similar to Mexico 309. The white bean cultivar 'Arroyo Loro', derived from the cross Bonita / La Vega, combines rust resistance and the dominant *I* gene for resistance to bean common mosaic (BCM), (Beaver et al., 1990). The small red line PR9357-107 combines rust resistance and the recessive genes *bgm-1* for bean golden yellow mosaic (BGYM) and *bc-3* for BCM and BCMN resistance (Beaver et al., 1998). Researchers in PR participated in the release of a pole snap bean cultivar 'Genuine' that combines BGYM and rust resistance (Stavely et al., 1996). The white bean cultivar 'Morales", derived from the cross

Arroyo Loro/Don Silvio, is resistant to rust, BCM and BGYM (Beaver et al., 1999a). The pink bean cultivar 'Rosada Nativa', derived from the cross DOR483 / BelNeb RR1, is resistant to endemic races of rust in PR and the DR (Beaver et al., 1999b). The light red bean kidney line PR9443-4 combines BGYM, common bacterial blight and rust resistance (Beaver et al., 1999c). Bean breeding lines with the *Ur-11* gene are resistant to rust in PR and the DR. Pinto bean breeding lines that combine *bgm-1* gene for BGYM resistance with the *Ur-6* and *Ur-11* genes for rust resistance have been developed in PR in collaboration with USDA-ARS researchers. Black- and white-seeded breeding lines that pyramid specific genes for rust resistance are being developed for the Caribbean. TARS VCI-4B was released as a multiple disease (including rust) resistant small-seeded dry bean germplasm derived from an interspecific cross between *Phaseolus vulgaris* and *P. coccineus* (Miklas et al., 1994a). Seven rust resistant tepary bean (*P. acutifolius* L.) lines were also identified in PR (Miklas et al., 1994b). Collaborators at the University of Florida have developed interspecific populations, which will be used to broaden the genetic base of rust resistance for beans in Central America and the Caribbean.

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RESEARCH ON BEAN RUST IN EAST AND CENTRAL AFRICA: STATUS AND FUTURE DIRECTIONS

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Introduction

Bean rust, caused by *Uromyces appendiculatus* is an important constraint to bean productivity in many countries of East, Central and Southern Africa. Among the major diseases, rust is ranked as the fifth most important constraint. Annual yield losses due to this disease are estimated at 191,400 t /year in Africa; 119, 000 t (Wortmann et al, 1998). Rust is of high importance (losses of 200 kg/ha) in Madagascar, Ethiopia, Mozambique, Malawi, Zimbabwe and South Africa; and of moderate importance (losses of 100 kg /ha) in Kenya, Uganda, Tanzania, Zambia, Lesotho, Swaziland, Rwanda, Burundi and DR Congo and Sudan. Rust management methods used by farmers include cultural practices especially sanitation and intercropping, fungicides and tolerant varieties. Use of resistant varieties is regarded as the most effective and economically viable strategy for rust management. The objective of national and regional breeding programs is to develop rust resistant cultivars. However, the achievement of this objective is constrained by lack of good sources of resistance, limited information on pathogen diversity, human and operational resources. Our objective is to describe the progress made in realizing these objectives and suggest directions for future research.

Results and Discussion

Research on rust in Africa has focused on identification, distribution, prevalence and economic importance, pathogenicity analysis, epidemiology in pure stands and mixtures, survival and spread, non-genetic control strategies, identification of sources of resistance and evaluation/screening for resistance by CIAT and national program scientists. Work on distribution, prevalence and economic importance culminated in publication of rust distribution map for Africa (Wortmann et al ,1998). Epidemiology work indicated that the disease is spread principally through windborne urediospores, contact with man and animals and implements, crop residues and volunteer crops (Allen, 1987). Seed transmission plays a minor role. Conditions favouring disease spread include cloudy, humid weather with heavy dew and temperatures of 21-27^{0}C. Telia are rarely seen eastern Africa and epidemics probably depend on transport of urediospores. There is no evidence that other legume species or alternative hosts serve as significant reservoirs of infection. Fininsa and Yuen (2001) showed that intercropping with maize and sorghum reduced mean rust incidence and severity was reduced by 25 and 16% in eastern Ethiopia. Although fungicides effective on rust have been identified, dry bean growers for economic considerations rarely use them

Available evidence indicates that considerable pathogenic variation occurs in Africa. Howland and Macartney identified six races in eastern Africa; six new races were reported in Tanzania (Macarteny,1966); Allen (1975) reported six races in Malawi. Habtu (1990) reported variability in Ethiopia; Rakotomalala and Rabakoarihanta (1995) in Madagascar and Liebenberg and Liebenberg (2000) in southern Africa. Comparison of these races is difficult either because standard differentials were not used or assessment methods were different A regional rust nursery coordinated by Ethiopia with 103 entries was constituted in 1989 and distributed for evaluation in Kenya, Uganda, Madagascar, Zambia, Mauritius, DR Congo. Twenty-four lines were rated resistant in Uganda, 40 in Ethiopia and 12 in Madagascar. Only PAN 134 was rated resistant in the three countries. This suggested pathogenic diversity. ExRico (Awash 1) rated resistant in Ethiopia was susceptible in Madagascar. Further pathogenic diversity was indicated by variable reaction of the 20 standard differentials (Table 1).

Table 1. Reaction of 20 rust differentials to six isolates from Ethiopia and Madagascar.

Line	Ur gene	Ethiopian isolates			Madagascar isolates		
		Ambo	AN*	AW*	AKZ*	ATS*	FNR*
US No 3	Ur-3	R	I	R	S	S	S
CSW 643	Ur-3	R	R	R	-	-	-
Pinto 650	Ur-3	R	I	I	S	S	S
Kentucky Wonder 765	Ur-3	I	I	I	-	-	-
Kentucky Wonder 780	Ur-3	I	I	I	I	I	R
Kentucky Wonder 814	Ur-4	R	R	R	S	S	I
Golden Gate Wax	Ur-4	R	I	I	S	I	R
Early Gallatin	Ur-4	R	R	R	R	?	?
Mountaineer	unknown	I	I	I	I	I	R
Redlands Pioneer	Ur-5	I	R	R	I	I	R
Ecuador 299	Ur-6	R	R	R	R	R	I
Mexico 309	Ur-8	R	R	R	-	-	-
Brown Beauty	Unknown	R	I	R	R	I	I
Olathe	Unknown	I	I	I	I	R	R
AxS37	Unknown	R	R	R	R	S	I
NEP 2	Unknown	R	R	R	R	R	S
Aurora	Unknown	R	R	R	R	I	R
51501	Unknown	R	R	R	R	I	I
CNC	unknown	R	R	R	R	R	R

Sources of isolates: AN= Arsi Negelle, AW= Awassa, AKZ=Ankazobe, ATS=Antisirabe and FNR=Fianarantsoa; seeds of CSW 643, Kentucky Wonder 765 and Mexico 309 failed to germinate in Madagascar.

In the field studies, the differentials showed reactions that varied with locations and seasons. However, Kentucky Wonder 814, Ecuador 299, Mexico 309, NEP 2, Aurora, 51051 and CNC showed consistent resistant reactions at Ambo, Awassa, Debre Zeit and Melkassa in Ethiopia for two years. This indicated that deployment of ur-4, ur-6 ,ur-8 and other genes may be effective against races prevalent in this region.

Efforts to incorporate rust genes in popular local cultivars started in 2001 in the recently developed market led programs linking 20 national programs in Eastern, Central and southern Africa. Rust is a major problem in small reds, pinto, navy and snap beans. To develop cultivars with potential for production in many countries, it will be necessary to determine the pathogen diversity in these countries and identify rust genes that could be deployed. The current differentials do not represent all the known rust genes and needs to changed to better reflect the current status of knowledge. An African Bean Rust working group was formed in March 2002 during the Third International Rust Workshop in South Africa to coordinate research on bean rust in Africa.

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INHERITANCE OF RESISTANCE TO RUST IN TWO BEAN CULTIVARS IN KENYA

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Introduction

Bean (*Phaseolus vulgaris*) is a major source of dietary proteins to the majority of the human population in Eastern Africa, which cannot afford the more expensive animal proteins. Although the total area under bean production has increased over time, the yields have remained low due to various agronomic constraints but mostly as a result of diseases and pests. Bean rust caused by *Uromyces appendiculatus* (Pers.) Unger var *appendiculatus* is a major constraint to production in this region. The cheapest and most effective control measure is to use resistant varieties. However, development of resistant cultivars has been constrained by lack of information on the nature of inheritance of resistance to rust especially among recently introduced germplasm. Many physiological races of the bean rust fungus have been reported since the first systematic identification of races in 1941 (Stavely, 1984). Numerous reports have been published on the genetics of resistance to certain races of *Uromyces appendiculatus* in certain cultivars of beans. The bean improvement program of the University of Nairobi, Kenya identified two bean cultivars (L226-10 and NB 123) which had proved resistant to bean rust in several evaluations (Mukunya and Keya, 1978) and are potential sources of resistance in future breeding work. The purpose of this study was to determine the inheritance of resistance to rust in these two cultivars.

Materials and Methods

Crosses were made between two *cultivars resistant* to rust (L226-10 and NB 123) and two susceptible cultivars (GLP X92 and NB 86) in a complete dialer scheme to produce F_1, F_2 and F_3 generations. GLP X92 is a pinto type of bean, which has gained popularity among Kenyan consumers since its release by the grain legume project of the Ministry of Agriculture in the 1984. However, it is very susceptible to bean rust. NB 123 is indigenous in Kenya (Mukunya and Keya, 1978). It has small black seeds and is resistant to anthracnose, halo blight and rust. NB 123 reaction to bean rust is indicated by small necrotic spots without sporulation. NB 86 is a small red seeded type which is susceptible to bean rust (Mukunya and Keya, 1978). L226-10 is a small white seeded type of navy bean, which was developed and released co-operatively by the ARS-USDA the Agricultural Experimental Station, Michigan State University and the University of Puerto Rico (Freytag et al, 1985). It is has shown high resistance reaction ranging from no visible symptoms to mild hypersensitivity. Test plants were inoculated with a suspension with approximately 3×10^4 urediospores/ml from a single spore isolate in a greenhouse at Kabete. CIAT rust scale was used for disease rating.

Results and Discussion

Inheritance of resistance. The F_1 plants of all resistant x susceptible crosses and their reciprocals showed a uniform resistant reaction. The F_2 generation of these crosses segregated for resistance, the segregation ratio fitting a 3:1 (resistant: susceptible) ratio (Table 1). The testcrosses gave the expected 1:1 ratio, confirming the F_2 test. The F_3 progeny obtained from the resistant F_2 plants from all resistant x susceptible crosses and their reciprocals gave segregating to non-segregating lines in proportions that fitted a 2:1 ratio. Further, segregation for resistance among the segregating lines of the F_3 generation fitted the expected 3:1 ratio.

Allelic relationship. In the cross NB 123 X L226-10, both the parents, the F_1 and the F_2 progenies were resistant to the bean rust isolate. However, their resistance levels were different. All the F_1 plants gave the L226-10 type resistance reaction, which ranged from no visible symptoms to mild hypersensitivity while their F_2 progenies segregated for resistance between the L226-10 and NB 123 reaction of small necrotic spots without sporulation in the ratio 3:1. The reciprocal cross also showed a 3:1 segregation ratio. The cross NB 86 X GLP-X92 gave a uniform susceptibility reaction among the parents, the F_1 and the F_2. In all the

tests for goodness of fit, the Chi- square values were not significant indicating a good fit to the expected ratios.

We concluded that resistance was monogenically inherited, with L226-10 type of resistance being dominant to the NB 123 type. Crosses between these two resistant cultivars and two susceptible cultivars (GLPx92 and NB 86) showed that, in both the resistant cultivars, resistance was dominant to susceptibility and was monogenically inherited. The lack of reciprocal differences showed that there were no maternal effects. U_r allele was proposed for the resistant cultivar NB 123 with the L226-10 type of resistance determined by U_{ra} allele while the susceptibility in both the GLPx92 and NB 86 was conferred by the same u_r allele in the homozygous state. Dominance hierarchy series $U_{ra} > U_r > u_r$ in that order of predominance was indicated. The gene identified has been assigned these tentative symbols due to lack of sufficient information about its relationship with already named rust resistance genes in beans. The U_r symbol approved by the germplasm committee of the bean improvement cooperative and used by Stavely 1984 has been adapted here.

Table 1. Segregation for resistance to a bean rust (*Uromyces appendiculatus)* isolate in F_2 and testcross progenies.

Cross*	Total plants	Observed		Expected		Expected ratio	Chi-square #	Probability
		Resistant	Susceptible	Resistant	Susceptible			
NB 123 x GLPX92	369	288	81	276.8	92.3	3: 1	1.671 ns	0.1 - 0.25
Reciprocal	530	389	141	397.5	132.5	3: 1	0.644 ns	0.25 - 0.5
Testcross	59	28	31	29.5	29.5	1: 1	0.068 ns	0.75 - 0.9
NB123 x NB86	282	214	68	211.5	70.5	3: 1	0.075 ns	0.75 - 0.9
Reciprocal	197	146	51	147.75	49.3	3: 1	0.042 ns	0.75 - 0.9
Testcross	51	27	24	25.5	25.5	1: 1	0.078 ns	0.75 - 0.9
L226-10 x GLPX92	319	242	77	239.3	79.8	3: 1	0.086 ns	0.90 - 0.99
Reciprocal	582	436	146	436.5	145.5	3: 1	0.00 ns	> 0.99
Testcross	57	28	29	28.5	28.5	1: 1	0.00 ns	> 0.99
L226-10 x NB 86	126	90	36	94.5	31.5	3: 1	0.677 ns	0.25 - 0.5
Reciprocal	243	186	57	182.3	60.75	3: 1	0.289 ns	0.5 - 0.75
Testcross	62	32	30	31	31	1: 1	0.016 ns	0.5 - 0.75

Resistant parental lines, L226-10 and NB123 had a rust reaction rating of 1 and 3, respectively. The rust susceptible lines rating were 7 to9 for GLP X92 and 7 for NB 86. # ns= not significant at P=0.05, 1 df.

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PHYSIOLOGICAL VARIATION OF BEAN RUST IN ECUADOR

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Introduction

Common bean (*Phaseolus vulgaris* L) is one of the most important crop in Ecuador. Approximately 37000 ha are cultivated as bush beans in the main valleys. The area of climbing beans in the highlands that is associated with maize is approximately 32000 ha (Instituto Nacional de Estadísticas y Censos, 1995). Bean rust caused by *Uromyces appendiculatus* is a major threat to the bean production in the country (Lepiz et al., 1995). Breeding for disease resistance is the most desirable approach to control bean rust. In order to identify sources of resistance an adequate knowledge of the pathogen populations are required. This paper describes the variation of bean rust populations in beans in Ecuador.

Materials and Methods

Twenty two isolates of bean rust that were collected from various bean growing areas in Ecuador were tested against 20 bean differential varieties as described Stavely, et. al 1989. The differentials included commercial varieties and advanced lines. The study was conducted in the green house at 19 ºC in the Santa Catalina Experimental Station of INIAP-Ecuador. Inoculation was made at the primary leaf stage by using suspension of 30,000 urediospores per ml of water. Infection types of bean rust were evaluated 16 days after inoculation by using the 0-6 scale described by Stavely et. al., 1989. Infection types 1, 2 and 3 were considered incompatible (resistant) and infection types 4, 5 and 6 were considered compatible (susceptible).

Results and Discussion

A total of 14 differentials out of 20 proved to be susceptible to the isolates examined. (Table 1) According to compatible/incompatible reactions on the differential set, 17 rust populations were detected. This indicates that the virulence of bean rust population is of a complex nature. The virulence of the examined isolates ranged from very simple as A13 to highly complex as A26. The results indicates that the Ecuadorian commercial varieties could also be used as a suitable differentials to distinguish between the virulence of bean rust populations in Ecuador. Bush and climbing bean isolates did not differ in the virulence composition pattern. Inoculum movement between valleys and highland seems to be common in bean rust in Ecuador. All local and released varieties were susceptible to at least one isolate. Line G2233 was resistant to all isolates. It is worth noting that the varieties INIAP 418, Cocacho, Canario Imbabura and CAP 9 were found to be resistant in the field but in the green house they have shown compatible reaction against some isolates (Table 1). This might be due to adult plant resistance or temperature sensitivity resistance. Lack of adaptation of virulent isolates in location where these varieties are cultivated should also not be excluded.

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Table 1. Bean rust infection types of differential varieties, Ecuadorian varieties and promising advanced lines to 22 isolates of bean rust collected in the mean bean growing areas of Ecuador.

Varieties	A1	A2	A5	A6	A10	A11	A12	A13	A14	A15	A16	A17	A18	A19	A20	A23	A24	A26	A27	A28	A30	A32
Differential varieties																						
US-3	3[1]	2	**4**	2	2	3	1	2	**4**	**5**	1	2	1	1	**4**	**4**	**4**	**5**	2	3	**4**	2
CSW 643	2+	2	2	1	1	2	1	1	2	1	2	2	1	1	1	1	2	**4**	1	1	1	1
Pinto 350	1	**6**	**5**	**4**	**4**	**6**	**5**	2	1	1	1	**4**	**3**	**4**	**4**	**6**	**4**	**5**	**6**	**6**	**5**	**6**
KW 765	2	2	2	2	1	1	1	1	**4**	1	1	2	1	1	1	2	2	2	2	2	1	2
KW780	2+++	2++	2	2	1	2+	2	2	**2+**	1	2	2++	1	2+	2++	2	2	**5**	2++	2	1	2++
KW 814	1	3	**4**	**4**	1	1	1	1	2	1	1	2	1	1	1	1	3	**5**	2	**5**	1	1
Golden Gate Wax	1	5	3	2	1	2	**6**	1	2	1	1	1	1	1	3	**6**	2	**5**	2	2	**5**	2+
Eary Gallantin	**4**	**6**	3	**4**	1	1	**4**	1	**5**	**5**	1	**4**	2	**4**	**4**	**4**	**4**	**4**	**5**	**4**	**4**	**4**
Redlans Pioneer	2+	3	3	3	2	1	**4**	3	**4**	2	1	2	1	1	**4**	**4**	3	**4**	3	**6**	**4**	3
Ecuador 299	1	2	1	2	2	1	1	2	2	2	1	2	1	1	1	1	1	1	1	**4**	2	2
México 235	1	1	2	2	2	1	1	1	1	1	1	2	1	1	1	1	2	**4**	1	1	1	2
Brown Beauty	**5**	**6**	**5**	**6**	**4**	**6**	**5**	1	2	2	**5**	**5**	**5**	**4**	**4**	**5**	**5**	**4**	**5**	**5**	1	**6**
NEP 2	2	2	2	2	2	1	2	1	2	1	2	2	1	1	2	1	2	**4**	1	1	1	2
Aurora	2++	2	2	2	2	2+	1	2	2	1	2	2	1	1	2	2	2	**4**	2	1	**4**	2
51051	1	1	1	1	1	1	3	1	**4**	1	1	1	1	1	1	1	1	1	2	1	1	1
Mountain White	2+++	2	2	2+	**4**	2	1	1	**5**	1	2	2	2	1	3	2	2	2	2	1	1	2+
Ecuadorian varieties																						
INIAP- 417	3	**6**	**5**	**4**	1	**6**	**4**	**4**	**5**	4	4	4	3	4	**4**	**5**	**5**	**4**	**4**	1	**5**	**5**
INIAP-Yunguilla	**5**	**6**	**6**	**4**	**4**	**6**	**4**	1	**4**	**5**	**5**	**5**	**5**	1	**5**	**6**	**5**	**5**	**4**	**6**	1	**6**
INIAP-Vilcabamba	**5**	**6**	**6**	**4**	**4**	**5**	**5**	2	3	1	3	3	3	1	**4**	**5**	**4**	**5**	**4**	1	1	**6**
NIAP-418 (Jema)	1	2	**4**	2	2	1	1	1	**4**	1	3	3	1	1	3	3	**4**	**4**	1	1	1	3
San Anatonio	**5**	**4**	**4**	**4**	2	**5**	**4**	1	**4**	3	4	4	1	1	**4**	**4**	**4**	1	**4**	**4**	**4**	**6**
Paragachi	**4**	**5**	**4**	**5**	**4**	**6**	**4**	1	**4**	**4**	**4**	**4**	3	**4**	**4**	**6**	**4**	1	**6**	**6**	**5**	**5**
INIAP 412	1	3	**4**	**4**	1	3	**4**	1	**4**	1	3	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	3	1	**4**
INIAP 403	**5**	**4**	**5**	3	**4**	**4**	**5**	2	**5**	**6**	**5**	**5**	**6**	**4**	3	**4**	**5**	**5**	1	1	1	3
INIAP 400	**5**	**4**	**4**	**4**	1	**6**	**5**	1	**4**	1	**4**	**6**	**4**	**4**	**4**	**5**	**4**	**5**	**4**	**6**	**4**	**5**
INIAP 416	**5**	**5**	3	3	1	1	**4**	2	**4**	3	3	**4**	1	1	3	**4**	1	**6**	1	1	1	3
Advanced lines																						
CAP 9	3	3	**4**	3	1	3	**4**	3	**4**	1	3	3	1	**4**	**4**	**4**	**4**	**5**	3	**4**	1	3
Mil uno	1	**4**	**4**	**4**	1	**5**	**4**	1	**5**	1	3	3	1	**4**	**4**	**4**	**4**	1	**5**	**4**	1	**4**
CanarrioImbabura	3	2	2	3	1	1	3	1	**4**	2	3	3	3	**4**	1	3	1	1	1	1	1	2
Cocacho	3	2	3	3	2	1	**4**	1	3	1	2	2	1	3	3	3	3	3	1	1	1	3
LAS 298	1	**5**	**4**	3	1	**6**	**4**	1	**4**	1	1	**4**	**5**	1	**4**	**4**	**4**	**5**	**4**	**5**	**5**	**4**
SCC 2	**5**	3	**4**	**4**	**4**	2	**4**	1	3	**4**	**4**	**4**	3	1	**4**	**4**	**4**	**4**	**4**	1	1	3
TIB 3042	**5**	3	**4**	3	1	**4**	**4**	1	3	**4**	3	**4**	**4**	**4**	**4**	**4**	**4**	**5**	**4**	3	1	3
OBN 104	**5**	3	**5**	**4**	3	1	**4**	1	**4**	**4**	**4**	**4**	**6**	**4**	**4**	**5**	**5**	1	**5**	**5**	**5**	**4**
G 2333	3	2	2	2	1	2	1	2	3	1	2	2	1	1	1	1	2	1	2	1	1	2
Red small garden	1	**6**	**6**	**5**	**4**	**6**	**6**	**5**	**6**	**6**	**6**	**6**	**5**	**6**	**4**	**5**	**6**	**6**	**5**	**6**	**5**	**6**

[1] Infection types on the 0-6 scale proposed by Stavely et al 1989. + = necrosis between 300-500 *um*, ++ = necrosis between 500-800 *um* , +++ necrosis more than 800 *um*.

USE OF BELMIDAK RR-3 AS A SOURCE FOR RUST RESISTANCE IN CENTRAL BRAZIL

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Conventional breeding methods have been widely used to develop new common bean cultivars resistant to rust. However, resistance is easily overcome by the extensive pathogenic variability of the rust fungus *Uromyces appendiculatus*. Pyramiding of resistance genes assisted by molecular markers has been proposed as an alternative solution to overcome this type of problem. 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. Faleiro *et al*. (2001) demonstrated that cultivar Belmidak RR-3 (*Ur-11*) is resistant to *U. appendiculatus* races 2, 6, 9 and 10 detected in the state of Minas Gerais. In the United States, Belmidak RR-3 was tested with races 1, 3, 4 and 7 also from Minas Gerais, confirming the resistance of *Ur-11* (Stavely, personal communication). *Ur-11* also confers resistance to 89 out of 90 *U. appendiculatus* races of the USDA Bean Rust Fungus (Stavely, 2000). Johnsons et al. (1995) reported the identification of molecular marker $OPAE19_{890}$ as linked to the *Ur-11* gene of cultivar PI 181996, the resistant progenitor of Belmidak RR-3 (Stavely et al., 1994). Data obtained in our group (not published), indicate that resistance of Belmidak RR-3 to *U. appendiculatus* race 10 is controlled by a single dominant gene. The main goal of this work was to evaluate if the RAPD marker $OPAE19_{890}$ would be useful in our breeding program which involves crosses between cultivar Rudá (susceptible to rust) and cultivar Belmidak RR-3.

Primary leaves from 53 F_2 plants derivated from crosses between Belmidak RR-3 and Rudá were collected and kept at -80° C for DNA extraction. Homozygous F_2 resistant and suscetible individuals were identified by rust resistance evaluation in the 53 $F_{2:3}$ derived families. These individuals were used to construct two contrasting bulks according to Michelmore et al. (1991) which were tested with molecular marker $OPAE19_{890}$. The genetic distance between the RAPD marker and the resistance gene was determined in the F_2 and $F_{2:3}$ plants with the aid of MAP-MAKER III (Lander et al., 1987) using a LOD score minimum of 3.0.

The bulk analyses (Figure 1) indicated that marker $OPAE19_{890}$ was indeed linked to the resistance gene. The analysis of the F_2 population showed that marker $OPAE19_{890}$ is linked in repulsion phase at 1.0 cM of the *Ur-11* gene (Table 1). This marker will be used in the bean breeding program at the Federal University of Viçosa to help developing common bean cultivars resistant to rust and adapted to Central Brazil.

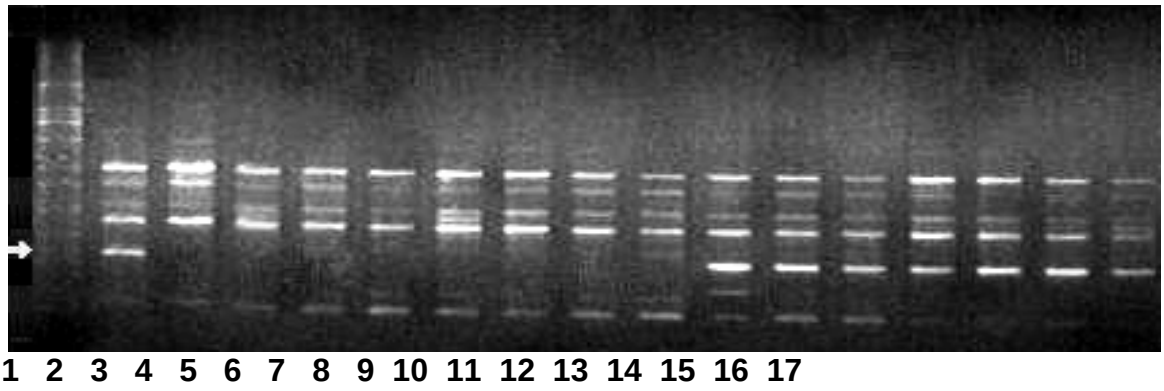

Figure 1. Electrophoretic analysis of amplification products obtained with primer $OPAE19_{890}$. Lanes are as follows: 1, lambda DNA cut with *Eco*RI, *Bam*HI and *Hin*dIII (size markers); 2, Rudá; 3, Belmidak RR-3; 4-10, F_2 plants homozigous resistant to race 10; 11-17, F_2 plants susceptible to race 10 of *Uromyces appendiculatus*. The arrow indicates a DNA band of 890 bp linked in repulsion phase to the resistance gene *Ur-11*.

Table 1. Segregation for resistance and linkage analysis between molecular marker and resistance gene to race 10 of *Uromyces appendiculatus* in the crosses between Rudá vs. Belmidak RR-3 (*Ur-11*).

Locus tested	Generation	Expected ratio	Observed ratio	χ^2	P	CM[1]
Ur-11	$F_{2:3}$	1:2:1	12RR:28Rr:13S	0.578	74.87	-
$OPAE19_{890T}$[b]	F_2	3:6:1:2:3:1[c]	0:27:12:1:13:0	44.30	0.00	1.0

[1]Distance in centimorgans in relation to *Ur-11* (resistance gene); [b] Molecular marker linked in repulsion phase to the resistance gene *Ur-11* .

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BACKCROSS ASSISTED BY RAPD MARKERS FOR THE INTROGRESSION OF ANGULAR LEAF SPOT RESISTANCE GENES IN COMMON BEAN CULTIVARS

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Angular leaf spot of common bean is caused by the fungal pathogen *Phaeoisariopsis griseola* (Sacc.) Ferraris and occurs in bean production areas in Brazil. Cultivars Mexico 54 and Mar 2 are used as donor parents in the backcross breeding program of BIOAGRO/UFV (Minas Gerais, Brazil) to create bean cultivars resistant to this disease. Mexico 54 is resistant to 20 and Mar 2 to 16 races of *P. griseola* identified in Brazil (Nietshe, 2000). The recurrent parent, Rudá, is a "carioca" type cultivar, with good yield potential but susceptible to most races of angular leaf spot.

In this work we identified homozygous resistant bean lines genetically closer to the recurrent parent using the RAPD technique. Molecular markers linked to the resistance genes were also used to ensure that these genes were maintained during the breeding process.

A BC_2F_3 population was obtained from the cross Ruda vs Mexico 54 and a BC_1F_3 population from Ruda vs Mar 2. All crosses were done in the greenhouse under controlled environmental conditions. After each backcross and selfing the plants were inoculated with spore suspensions of *P. griseola* (2 x 10^4 spores/ml) and incubated for three days in a mist chamber (20-22 ºC and 100% relative humidity). Eighteen and 25 days after inoculation the plants were scored visually for the disease symptoms using a 1-9 scale (van Schoonhoven & Pastor-Corrales, 1987). Leaf DNA was extracted from the progenitors and from the resistant BC_nF_3 plants by a mini-prep procedure based on Doyle and Doyle (1990). Amplification reactions were according to Williams et al. (1990) using different primer sets.

Two $F_{2:3}$ homozygous families were identified in each backcross all of them harboring the corresponding resistance gene (Figure 1).

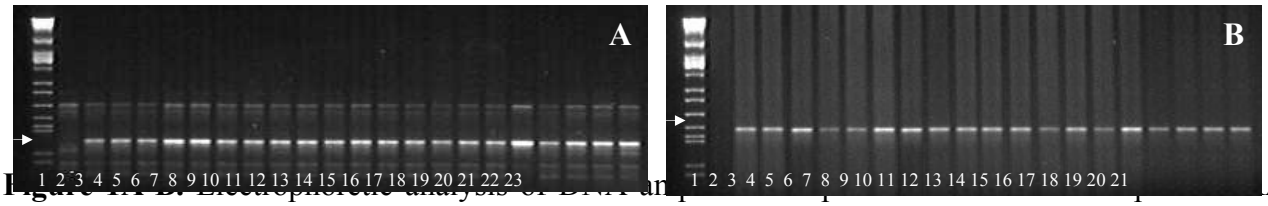

Figure 1. Electrophoretic analysis of DNA amplified by RAPD OPE04 (A) (Ferreira et al. 2000) and SCARN2 (B) (Sartorato et al. 2000). Lines are as follows: 1, lambda DNA cut with *Eco*RI, *Bam*HI and *Hin*dIII; 2, Rudá; 3, Mar 2 (A) or Mexico 54 (B); 4-23, homozygous BC_1F_3 families (A) or 4-21, BC_2F_3 homozygous families (B). The arrows indicate RAPD marker OPE04 (A) and SCAR marker N2 (B).

Pairwise genetic distances between the recurrent parent and BC_nF_3 plants were determined (Table 1). A minimum of 20 RAPD primers were used for the analyses. The data show that the

homozygous lines are still genetically distant from the recurrent parent, which would be expected after only one or two backcrosses. However, the selection of the homozygous lines was based on the presence of the resistance gene and the phenotypic aspect of their grains are indistinguishable from that of Ruda. The lines which were genetically closer to Ruda will be characterized with those *P. griseola* races previously tested in the donor parents.

Table 1. Relative genetic distances between BCnF3 homozygous resistant lines and the recurrent (Ruda) and donor parents (Mexico 54 or Mar 2). The genetic distance between the recurrent and the donor parents was considered to be 1.00.

Family A1	**Ruda**	**Mexico 54**
9-2-117-92	0.500	0.733
9-2-117-93	0.483	0.755
9-2-117-94	0.727	0.657
9-2-117-95	0.548	0.744
9-2-117-96	0.600	0.651
9-2-117-97	0.575	0.697
9-2-117-98	0.548	0.744
9-2-117-99	0.515	0.711
9-2-117-100	0.531	0.727
9-2-117-101	0.516	0.750
Family A2		
37-6-155-126	0.641	0.627
37-6-155-127	0.710	0.625
37-6-155-128	0.648	0.666
37-6-155-129	0.675	0.658
37-6-155-130	0.685	0.700
37-6-155-131	0.685	0.700
37-6-155-132	0.617	0.738
37-6-155-133	0.666	0.682

Family B1	**Ruda**	**Mar 2**
139A-142-131	0.657	0.629
139A-142-132	0.685	0.615
139A-142-133	0.794	0.590
139A-142-134	0.757	0.652
139A-142-135	0.666	0.592
139A-142-136	0.636	0.703
139A-142-137	0.657	0.629
139A-142-138	0.638	0.607
139A-142-139	0.617	0.678
139A-142-141	0.558	0.700
Family B2		
139A-120-156	0.695	0.666
139A-120-157	0.583	0.666
139A-120-158	0.541	0.684
139A-120-159	0.565	0.722
139A-120-160	0.545	0.777
139A-120-161	0.650	0.866
139A-120-162	0.521	0.736
139A-120-163	0.571	0.823
139A-120-164	0.478	0.750
139A-120-165	0.681	0.733

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COMMON BEAN GENOTYPES RESISTANT TO ANGULAR LEAF SPOT

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Bean angular leaf spot, caused by the fungus *Phaeoisariopsis griseola* (Sacc.) Ferraris, is world wide in distribution. It is found in more than sixty countries throughout the world. In recent years, in Brazil, this disease turned out to be one of the most important bean production constraints. The disease is favored by intermittent dry-wet and warm-cool weather. Losses due to the disease can be as high as 70% under disease favorable environmental. Angular leaf spot can be efficiently controlled through fungicide sprays and resistant cultivars. Although chemical control should be considered as an important control method, it can be dangerous to nature, farmer and consumer. The development of resistant cultivars, however, has been complicated by the pathogenic variability of the fungus. The objective of this study was to characterize some bean cultivars for their angular leaf spot resistance.

A set of 78 genotypes released as bean cultivars in Brazil, 35 F_6 breeding lines and two accessions were tested against six different *P. griseola* pathotypes including pathotypes 63-15, 63-23, 63-31, 63-39, 63-47 and 63-63 (Table 1). Pathotype 63-39 was collected in State of Paraíba, and were included in this study due to its high level of pathogenicity. Fourteen to sixteen days old plants, grown in the greenhouse, were inoculated with 2×10^4 conidia.ml^{-1}. Inoculations were performed on the first trifoliate leaves by spraying the inoculum to run off. Inoculated plants were incubated for 48 hours in a humid chamber (RH > 95%) at 25 ± 2°C, with a 12 h photoperiod. After this period of time, plants were transferred to greenhouse benches (28 ± 2°C). Disease severity was scored 14 to 18 days after inoculation, according to the percentage of leaf area affected and the presence or not of sporulating lesions. If in the greenhouse plants with up to 2.0 % of the leaf surface area showing few small no-sporulating lesions were observed, they were transferred to a moist chamber for 20-24 hours. After this period of time plants exhibiting non-sporulating lesions were considered resistant.

Most genotypes were susceptible to all pathotypes. In Table 1, it can be observed that only three breeding lines, three cultivars and two accessions showed some resistance to some of the pathotypes tested. Accessions AND 277 (Andean) and Cornell 49-242 (Meso American) were the two most resistant genotypes. They presented resistance reaction to four (63-15, 63-23, 63-31 and 63-63) out of six used pathotypes. However, these two cultivars are not released for commercial cultivation. Since genotype Cornell 49-242 is one of the twelve differential cultivars, it was not supposed to present a resistant reaction to the pathotype 63-63. So, the resistant reaction presented by this genotype (Table 1) during this test, maybe due to different seed source: although it remains to be checked seeds from the differential set were susceptible while those from Embrapa Rice and Beans Germplasm Bank (used in this study) were resistant. This is a very common situation in a bean germplasm bank where there are so many entries under the same name. The resistance presented by breeding lines 97200203, 97200213 and 97200311 is derived from the genotype Cornell 49242. The two most resistant cultivars released for cultivation by the farmers were IAPAR 0031 and Ouro Negro which were resistant to pathotypes 63-31, 63-63 and 63-31, 63-47, respectively. Guateiam 6662 was resistant to one pathotype only. There were no resistant cultivars for the pathotype 63-39. This fact means that the search for new resistance source to this pathotype must continue. Although cultivar Cornell 49-242 was resistant to four pathotypes, when cultivated in the field, it may react as susceptible due to the *P.griseola* pathogenic variability found in nature.

This and other results suggest that due to the great pathogenic variability showed by this pathogen it seems unlikely that, in the field, one cultivar could be resistant to all pathotypes. As a result, a breeding program, to be successful, should consider pyramiding several genes (or block of genes) in just one genotype.

Table 1. Bean genotypes and released cultivars, tested in the greenhouse that presented resistant reaction to *Phaeoisariopsis griseola*. Embrapa Rice and Beans, 2000/2001.

CULTIVARS	ISOL. [1] 525.4 PAT. [2] 63-23			ISOL. 629.2 PAT. 63-63			ISOL. 648.3 PAT. 63-47			ISOL. 384.5 PAT. 63-31			ISOL. 60.4 PAT. 63-39			ISOL. 584.3 PAT. 63-15		
	DS[3]	LS[4]	SP[5]	DS	LS	SP	DS	LS	SP	DS	LS	SP	DS	LS	SP	DS	LS	SP
97200203	2	1.5	N	5	1.5	S	80	4.0	S	2	1.5	N	10	3.0	S	1	1,0	N
97200213	20	3.0	S	0	0	N	40	3.0	S	10	2.5	S	30	4.0	S	30	3.0	S
97200311	0	0	N	0	0	N	30	3.0	S	0	0	N	60	2.5	S	0	0	N
AND 0277	1	1.0	N	0	0	N	40	2,0	S	1	2.0	N	7	2.5	S	1	1	N
CORNELL 49-242	0	0	N	0	0	N	40	4,0	S	0	0	N	50	4.0	S	0	0	N
GUATEIAN 662	2	3.0	S	1	3.0	S	60	4,0	S	20	3.0	S	80	4,0	S	0	0	N
IAPAR 0031	1	3.0	S	0	0	N	50	4,0	S	0	0	N	10	2.5	S	1	2.5	S
OURO NEGRO	99	4.0	S	20	4.0	S	0	0	N	5	1,5	N	40	4,0	S	1	2,0	S

[1] ISOL = Isolate
[2] PAT. = Pathotype
[3] DS = Disease Severity
[4] LS = Lesion Size
[5] SP = Sporulation

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PATHOGENIC VARIABILITY OF Phaeoisariopsis griseola IN COMMON BEANS

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In recent years, angular leaf spot of common bean, caused by the fungus *Phaeoisariopsis griseola* (Sacc.) Ferraris, turned out to be one of the most important bean production constraints in Brazil. Losses can be as high as 70% under disease favorable environmental conditions (intermittent dry-wet and warm-cool weather). This disease can be efficiently controlled by means of fungicide sprays and resistant cultivars. Although chemical control should be considered as an important control method, it can be dangerous to nature, farmer and consumer. Consequently, breeding for disease resistance is the most practical, economical and safe approach for angular leaf spot disease management. However, the strategy for developing new resistant cultivars requires an understanding of the genetic variation of the pathogen. Although several previous studies have reported pathogenic variation in *P. griseola* more study has to be done in order to understand the population dynamic of the pathogen and, as a result, identify new source of resistance to the disease. The objective of this study was to identify pathotypes of *P. griseola* in Brazil for later use in a breeding program to develop new angular leaf spot resistant bean cultivars.

Forty to fifty diseased bean leaves from cultivars Jalo Precoce, Rudá and Pérola was collected, at random, in Inhumas county, in the State of Goiás, Brazil. From each leaf collected in the field, it was isolated about two to three fungal colonies, resulting in 113 isolates. Up to now, only 50 of these isolates were tested. After a single spore isolation, cultures were stored either in individual glass flasks with sterile distilled water in the refrigerator (± 4 ^{o}C) or by the filter paper method. A set of 12 differential cultivars established at CIAT - Centro Internacional de Agricultura Tropical - besides the line AND 277 and the susceptible cultivar Rosinha G-2 were used for inoculation with *P. griseola* single spore isolates. Bean seeds were sown in aluminum pots with 2,0 kg of soil at the rate of 5 seeds/pot. Spores for inoculation were obtained by culturing the fungus in Petri dishes containing bean leaf-dextrose-agar medium kept in a BOD chamber at 24 ± 2 ^{o}C in darkness. After 14 days, 5-10 ml of sterile distilled water was added to each plate and the spore suspension was filtered in a double layer of cheesecloth for the removal of the pathogen mycelium. The spore suspension was adjusted to a concentration of 2 x 10^4 conidia.ml^{-1}. Inoculation was done when plants were in the V3 stage (14-16 days old). After inoculation, plants were incubated in a moist chamber (RH > 95%, at 25 ± 2^{o}C) for 48 hours, with a 12 h photoperiod. Plants were maintained in greenhouse benches for another 14-18 days and evaluated for symptoms according to a 1 to 9 descriptive scale. Plant ratings 1 to 3 were considered resistant and 4 to 9 susceptible. When inoculated plants in the greenhouse showed symptoms with no sporulation, they were transferred to a moist chamber for 20-24 hours. Plants exhibiting non-sporulating lesions were considered resistant.

Isolates exhibited a different virulence pattern when inoculated on the 12 bean differential genotypes. From the 56 single spore isolates tested, it was identified nine different pathotypes of *P. griseola* (Table 1). This data shows that this pathogen is very variable even within a given area. Not including the pathotypes 31-15, 31-31, 47-31 and 55-31, all others were capable of inducing compatible reactions in all Andean cultivars. The pathotype 63-31 was the most widespread followed by the pathotypes 63-15, 31-31 and 63-63. The identification of the pathotype 63-63,

which "brokes" the resistance genes present in all differential cultivars, seems to be important for a breeding program aiming to develop new cultivars with resistance to this pathogen. This fact reinforces the necessity of a continuous search for new resistance gene sources to angular leaf spot in Brazil. Although most of the time accessions Cornell 49-242 and AND 277 have shown the same disease reaction (resistant or susceptible) when considering the accession AND 277 as a differential cultivar, the pathotype 63-63 could, some time, be divided into two different groups of isolates representing two different pathogenic entities: one that overcame the AND 277 resistance gene (s) and the other that did not. This shows that the accession AND 277 presents some genes that are different from those in the genotype Cornell 49-242.

Table 1. Reaction of differential cultivars inoculated with 56 isolates of *Phaeoisariopsis griseola* collected in Inhumas county, in the State of Goiás, Brazil. Embrapa Rice and Beans, 2000/2001.

Pathotype	Andean beans						Middle American beans						Number
	1[a]	2	3	4	5	6	7	8	9	10	11	12	of Isolates
31-15	+	+	+	+	+	-[c]	+	+	+	+	-	-	1
31-31	+[b]	+	+	+	+	-	+	+	+	+	+	-	3
47-31	+	+	+	+	-	+	+	+	+	+	+	-	1
55-31	+	+	+	-	+	+	+	+	+	+	+	-	2
63-07	+	+	+	+	+	+	+	+	+	-	-	-	1
63-15	+	+	+	+	+	+	+	+	+	+	-	-	6
63-23	+	+	+	+	+	+	+	+	+	-	+	-	2
63-31	+	+	+	+	+	+	+	+	+	+	+	-	37
63-63	+	+	+	+	+	+	+	+	+	+	+	+	3

[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) Mexico 54; (11) BAT 332; (12) Cornell 49-242.
[b] Compatible reaction (+)
[c]Incompatible reaction (-)

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FIELD RESISTANCE TO *Macrophomina phaseolina* IN BLACK BEAN POPULATIONS

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Introduction

Charcoal rot or Ashy Stem Blight (ASB) is caused by the fungal pathogen, *Macrophomina phaseolina* (Tassi) Goid. and is particularly severe in drought stressed plants. Symptoms include wilting, chlorosis, premature defoliation, and early maturity or death (Pastor-Corrales and Abawi, 1988). Advanced breeding line, TLP 19 has ASB resistance similar to BAT 477, where resistance was controlled by two pairs of dominant genes exhibiting dominant recessive epistasis (Mayek-Pérez et al., 2001). One of the two populations in this study was segregating for ASB resistance derived from TLP 19.

In Honduras, a field experiment conducted under moisture stress conditions experienced a severe infestation of ASB. Although control measures were implemented, limited effect was observed for ASB symptoms. The genetic material under investigation was segregating for drought resistance. Drought tolerant lines have also shown ASB resistance (CIAT 1983). The objective of this study was to compare two populations segregating for drought resistance to known checks for their reactions to ASB.

Materials and Methods

On January 23, 2001, two populations, three parents and seven checks were planted in a completely randomized design in Zamorano, Honduras. The two recombinant inbred line (RIL) populations, L88 and L91, were derived from crosses B98311/TLP 19 and B98311/VAX 5, respectively. The seven checks included locally adapted material, Mexican cultivars and drought resistant genotypes (Table 1).

Moisture stress and non-stress treatments were maintained through furrow irrigation. Plots were five meters long and 0.7 meters wide. Rows were thinned to 50 plants per row at 15 days after planting (dap) to ensure adequate stands. Disease incidence (DI) was recorded at 75 dap in the stressed plots as the number of dead plants per row. During harvest, 30 plants per row were recorded for plant stand and yield. Analysis of variance was calculated to adjust yield to plant stand as the covariate.

Results

The severe infestation of ASB was characterized by 99% of the plots having at least one dead plant due to Macrophomina. DI ranged from 0.05 to 0.54 in the 160 genotypes (Figure 1). Plant stand was significantly affected by DI (R^2 =16.8****), while yield was less affected by both, DI (R^2 =6.9****) and plant stand (R^2 =6.6****).

Population L88 had a 6% higher DI than L91 yet averaged 113 kg/ha more in yield (Table 1). Both populations show the combination of yield potential and drought resistance from B98311 and disease resistance traits from TLP 19 and VAX 5. In the moisture stress and non-stress treatments, B98311 yielded more, yet had a two-fold higher DI in comparison to the two other parents. TLP 19 and VAX 5 had moderately low DI values at 0.15 and 0.20. Two RILs, L91-45 and L88-76, had the lowest DI in each population. Two other RILs, L91-30 and L88-69, had moderately low DI values and were selected as drought resistant based on yield.

The ASB resistant genotypes, BAT 477 and TLP 19 (Mayek-Pérez et al., 2001), showed more susceptibility to ASB than other genotypes. Possibly BAT 477 and TLP 19 are susceptible to the isolates of Macrophomina in Honduras. V8025, SEA 5 and Tío Canela 75 had lower DI values than

BAT 477 and TLP 19. Even though TLP 19 is resistant to ASB, its progeny had a higher DI value than the progeny from VAX 5. VAX 5 was derived from tepary bean, which has resistance to common bacterial blight and ASB (Thomas et al., 1983). VAX 5 may carry ASB resistance and should be tested as a possible new source of resistance to Macrophomina.

Figure 1. Field incidence of ASB compared to yield under stress in 160 genotypes.

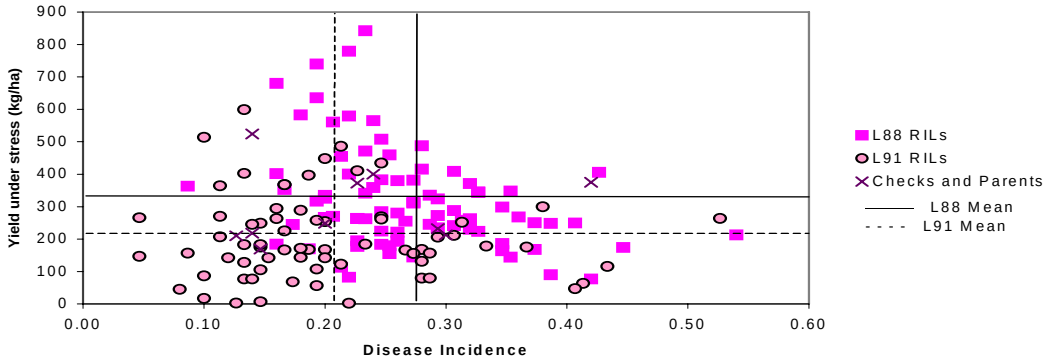

Table 1. Selected genotypes and means compared for their disease incidence (DI), plant stand, yield under stress (Yd), yield under non-stress (Yp), and geometric mean (GM).

Genotype	DI (Rank)	Stand	Yd	Yp	GM
				kg·ha^{-1}	
L91-45	0.05 (1)	29	266	2468	810
L88-76	0.09 (4)	27	363	1862	822
V8025	0.13 (13)	28	210	1783	612
L91-30	0.13 (15)	27	599	1922	1073
SEA 5	0.14 (20)	23	524	1521	893
Tío Canela 75	0.14 (22)	27	218	1657	602
TLP 19	0.15 (24)	30	169	2399	637
L88-69	0.16 (31)	27	680	2432	1286
VAX 5	0.20 (61)	27	249	1765	663
Rio Tibagi	0.23 (75)	27	372	2108	886
BAT 477	0.24 (88)	27	400	1536	784
EAP 9510-77	0.29 (120)	27	232	2112	699
Tacana	0.30 (125)	28	213	2097	667
B98311	0.42 (154)	27	375	2411	951
Mean, L88	0.27		320	2057	791
Mean, L91	0.21		207	1858	591

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AN IMPROVED ARTIFICIAL INOCULATION TECHNIQUE TO SCREEN COMMON BEAN FOR WEB BLIGHT REACTION IN THE FIELD

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Although different methods for screening common bean (*Phaseolus vulgaris*) for web blight (WB) reaction caused by *Rhizoctonia solani* (teleomorph: *Thanatephorus cucumeris*) have been reported **(3, 4, 5)**, these methods are impractical to evaluate a large number of common bean lines in the field. In the Dominican Republic, researchers screen for WB resistance in the field when climatic conditions are favorable for disease development **(1**). Unfortunately, lack of sufficient rainfall often prevents WB infection. Moreover, field screening with natural infection is limited in the Caribbean to one growing season per year. A field inoculation technique was developed that involved spraying mycelial suspensions of *R. solani* on the foliage of bean plants followed by frequent sprinkler irrigation **(2)**. The objective of this research was to test this technique for evaluating WB reaction in replicated field trials. Three experiments were planted by August, 2001 at the Isabela Substation in northwestern Puerto Rico. Thirty eight red kidney and 77 red mottled lines from the VICARIBE trials and 53 genotypes from the University of Puerto Rico (UPR) bean breeding program were evaluated for % leaf area infected and WB reaction using the CIAT scale **(6)** at 15 and 20 days after inoculation. Randomized complete block design with three replications were used. Percent leaf area infection data were subjected to an analysis of variance, and means were separated using LSD (0.05). A suspension of mycelial mats (1 g of mycelia per 300 ml of distilled water plus a surfactant agent) of *R. solani* [Rs 012 isolate, anastomosis group 1 (AG-1)] was sprayed on the foliage of the bean plants at the V4 growth stage **(6)**. A short period of sprinkler irrigation was applied every morning after inoculation. Total rainfall at the Isabela Substation was 335 mm, the temperature ranged from 21º C to 32º C and the relative humidity varied from 76% to 84%. Seven red kidney and three red mottled beans from the VICARIBE trial and 12 genotypes from the UPR showed intermediate levels of resistance to WB. The mean percent leaf area infected with WB was high (44.5%, 51.3%, and 45%) for each experiment (Tables 1, 2, and 3). The artificial inoculation technique followed by frequent irrigation used in this study were effective to provide conditions favorable for disease development, and may provide a tool to permit WB screening throughout the year in a tropical location.

Table 1. Mean percent leaf area infected and web blight score of seven red kidney common bean lines from the VICARIBE trial and the susceptible check (ck) planted by August, 2001 at the UPR Isabela Substation and artificially inoculated with *Rhizoctonia solani* (Rs 012 isolate, AG-1).

Genotype	Origin	% leaf area infected	Grade [1]	Category [1]
X003-435	UPR CIAT	26.67	5	Intermediate
DRK 152	UPR	30.00	5	Intermediate
X003-394	CIAT	33.33	6	Intermediate
AFRICA 285	UPR	33.33	6	Intermediate
X003-380	CIAT	36.67	6	Intermediate
DRK-156	UPR	36.67	6	Intermediate
PR 9840-28	UPR	36.67	6	Intermediate
Morales (ck)		40.00	7	Susceptible
Mean		44.47		
L.S.D. (0.05)		14.96		
C.V. (%)		20.67		

CIAT= Centro Internacional de Agricultura Tropical; UPR= University of Puerto Rico-Mayagüez.

-2. CIAT WB severity scale (1-9): 1=0% of foliage infected to 9=>80% of foliage infected. Grades and Categories: 1, 2, 3= Resistant; 4, 5, 6= Intermediate; 7, 8, 9= Susceptible.

Table 2. Mean percent leaf area infected and web blight score of three red mottled common bean lines from the VICARIBE trial and the susceptible check (ck) planted by August, 2001 at the UPR Isabela Substation and artificially inoculated with *Rhizoctonia solani* (Rs 012 isolate, AG-1).

Genotype	Origin	% leaf area infected	Grade [1]	Category [1]
DFA 76	CIAT	33.33	6	Intermediate
RAA 36	CIAT	36.67	6	Intermediate
RMA-3	CIAT	36.67	6	Intermediate
Morales (ck)	UPR	53.33	7	Susceptible
Mean		51.26		
L.S.D. (0.05)		16.68		
C.V. (%)		20.18		

CIAT= Centro Internacional de Agricultura Tropical; UPR= University of Puerto Rico-Mayagüez. [1] CIAT WB severity scale (1-9): 1=0% of foliage infected to 9=>80% foliage infected. Grades and Categories: 1, 2, 3= Resistant; 4, 5, 6= Intermediate; 7, 8, 9= Susceptible.

Table 3. Mean percent leaf area infected and web blight score of 12 common bean lines and the susceptible check (ck) from the UPR bean breeding program planted by August, 2001 at the UPR Isabela Substation and artificially inoculated with *Rhizoctonia solani* (Rs 012 isolate, AG-1).

Genotype	Origin	% leaf area infected	Grade [1]	Category [1]
VAX-6	CIAT	27.50	5	Intermediate
VAX-3	CIAT	30.00	5	Intermediate
MUS-N-8	Dom. Rep.	30.00	5	Intermediate
PRF 9702-84	EAP	32.50	6	Intermediate
HT 7719	CIAT	32.50	6	Intermediate
BAT 93	CIAT	32.50	6	Intermediate
G-13920	CIAT	35.00	6	Intermediate
MUS 181	CIAT	35.00	6	Intermediate
JB 569	Dom. Rep.	35.00	6	Intermediate
XAN 176	CIAT	35.00	6	Intermediate
PR 9806-37-2	UPR	37.50	6	Intermediate
Talamanca	CIAT	37.50	6	Intermediate
Morales (ck)	UPR	40.00	7	Susceptible
Mean		45.00		
L.S.D. (0.05)		16.29		
C.V. (%)		25.95		

CIAT= Centro Internacional de Agricultura Tropical; EAP= Escuela Agrícola Panamericana (Honduras); UPR= University of Puerto Rico-Mayagüez. [1] CIAT WB severity scale (1-9): 1= 0% of infected foliage to 9= >80% of infected foliage. Grades and Categories: 1, 2, 3= Resistant; 4, 5, 6= Intermediate; 7, 8, 9= Susceptible.

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SYNDROMIC ANALYSIS OF RESISTANCE OF WHITE BEAN TO FUSARIUM ROOT ROT

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Eighteen cultivars of white bean were assessed for resistance to Fusarium root rot caused by *Fusarium solani* f. sp. *phaseoli*. Plants were grown in a growth room with an 18-hour photoperiod and day/night temperatures of 20/17°C. A replication consisted of one 8.5 x 8.5 cm pot containing one seed planted 2.5 cm deep in fine vermiculite. Conidia from cultures of *F. solani* f. sp. *phaseoli* grown under laboratory conditions on potato dextrose agar were suspended in water and adjusted to a concentration of 10^3 conidia/mL. Ten days after sowing the seed, 10 mL of the spore suspension was delivered by pipette around each hypocotyl. The experiment was arranged in a completely randomized design with 6 replications. Four weeks after plants were inoculated, disease severity on the surface of the hypocotyl and on the surface of a cross-section of the hypocotyl was assessed on a scale of 0-4, where 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, and 4 = 76=100% surface discoloration of hypocotyl or cross-section of hypocotyl. Cultivar means within a trial and disease measure were compared by a protected LSD (P = 0.05). Mean disease severity for each cultivar was calculated as the average of four scores (two trials and two measures/trial). The syndromic score was the number of times across disease measures and trials that a cultivar occurred in the most resistant group (maximum 4).

The disease severity score of an entry varied with the measure of disease (surface or section) and trial (Table 1). Over all trials and attributes, mean disease severities ranged from 1.2 to 2.8, and syndromic scores ranged from 0 to 4. Four cultivars (AC Hensall, AC Trident, Aspen, and Centralia) were judged as most resistant both by syndromic score (4) and mean disease severity (1.2). Mean disease severity was not consistently related to syndromic score over the remaining 14 cultivars. Syndromic scores of 3, 2, 1, and 0 were associated with mean disease severities in the range 2.0-2.1, 1.8-2.1, 1.9-2.5, and 2.4-2.8, respectively. Using mean disease severity to provide an overall assessment of disease produced 9 different values over the 18 cultivars (1.2, 1.8, 1.9, 2.0, 2.1, 2.3, 2.4, 2.5, and 2.8). There are statistical procedures for comparing means over trials if the data can be considered as belonging to one experiment, but the final analysis often produces means not clearly separated from one another. Furthermore, means cannot be determined from attributes with different units. On the other hand, syndromic scores clearly separated the cultivars into 5 resistance groups. This separation had a statistical basis and provided a simple, direct and synoptic view of the relative resistance of the 18 cultivars to Fusarium root rot. Syndromic analysis can be used to assess resistance of plants to disease over a variety of disease measures and experimental conditions. Unlike the determination of means, syndromic analysis is not confined to measures that use the same unit. For example, syndromic analysis could provide a statistical basis for summarizing the effects of soilborne pathogens on disparate attributes of plant performance such as stand, shoot and root weight, visual disease severity, vigour, canopy density, phenotypic development, and yield. All that is required is to determine for each attribute which entries have the most desirable outcomes that are not significantly different. The result is a single, statistically-based score that measures relative performance of the entry over all attributes and trials.

Table 1. Disease severity[1] and syndromic score[2] of 18 white bean cultivars following inoculation with *Fusarium solani* f. sp. *phaseoli*.

Cultivar	Trial 1		Trial 2		Mean disease severity	Syndromic score
	Surface severity	Section severity	Surface severity	Section severity		
AC Hensall	1.2g[3]	1.2e	1.2de	1.2cd	1.2	4
AC Trident	1.3g	1.3de	1.2de	1.0d	1.2	4
Aspen	1.3g	1.2e	1.2de	1.2cd	1.2	4
Centralia	1.5g	1.2e	1.0e	1.0d	1.2	4
Avanti	3.3b-d	1.7b-e	1.8a-e	1.2cd	2.0	3
Navigator	3.5a-c	1.7b-e	1.8a-e	1.2cd	2.1	3
Envoy	2.3ef	1.5c-e	2.0a-d	1.2cd	1.8	2
Crestwood	2.2f	2.0a-c	1.7b-e	1.5b-d	1.9	2
AC Compass	3.7ab	2.0a-c	1.5c-e	1.2cd	2.1	2
Pilot	2.8de	1.7b-e	2.0a-d	1.2cd	1.9	1
Premiere	3.0cd	1.8a-d	2.0a-d	1.5b-d	2.1	1
Shetland	3.3b-d	2.2ab	1.7b-e	1.8b	2.3	1
Stingray	3.8ab	1.3de	2.7a	2.0ab	2.5	1
Vista	3.7ab	2.0a-c	2.0a-d	1.7bc	2.4	0
OAC Thunder	3.8ab	2.2ab	2.3a-c	1.7bc	2.5	0
OAC Gryphon	3.7ab	1.8a-d	2.5ab	1.8b	2.5	0
CDC Whistler	4.0a	2.2ab	2.2abc	1.7bc	2.5	0
OAC Laser	3.7ab	2.3a	2.7a	2.5ab	2.8	0

[1] Disease severity was rated on a scale of 0-4, where 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, and 4 = 76=100% surface discoloration of hypocotyl or cross-section of hypocotyl. Mean disease severity was calculated from disease severity scores from two trials.

[2] Syndromic score is the number of times the cultivar occurred in the most resistant group.

[3] Within a column, numbers followed by the same letter are not significantly different (LSD, P = 0.05).

REACTION OF BEAN GENOTYPES to *Fusarium* ssp. and *Rhizoctonia solani* IN CENTRAL MEXICO.

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In the highlands of Mexico bean yields are commonly reduced by the attack of pathogens. The bean root-rots induced by the fungi *R. solani* and *Fusarium* spp. are destructive diseases in the main producing areas (López, 1991). As for any other disease, the use of resistant varieties is one of the most promising methods for the control of the root-rots. The aim of this work was to identify resistant genotypes to both pathogens, *R. solani* and *Fusarium* spp, under greenhouse and field conditions.

Two experiments were carried out as follows: Experiment I: 15 bean genotypes were planted in the field under rainfed conditions during 1999 at Texcoco, Mexico (19º29′ N, 98º51′ W, 2240 masl, and 664 mm average yearly precipitation). Genotypes were chosen because of their known root-rot resistance: PI 203958, Negro Tacaná, Puebla 152, Canario 107 (susceptible check), Wisc. RRR, G 122, FR 266, Pinto Villa, Negro 8025, Negro Durango, BAT 477, Negro Cotaxtla 91, Negro INIFAP, Flor de Mayo Bajío (susceptible check), and G 12729.

Planting was done on july 24th in a naturally infested soil. Each genotype was sown in two three m rows with 30 plants, and replicated three times under a completely random design. The disease reaction was evaluated in consecutive phenological stages (V_3, R_5, R_7 and R_8). At each stage three plants in each replicate were dug out to score the percentage of incidence and disease severity induced by *R. solani* and *Fusarium* spp. Disease reaction was scored with a visual scale (Schoonhoven and Pastor-Corrales, 1987). During the growing season daily temperature and precipitation, were recorded.

Experiment II: Same genotypes were sown in the greenhouse on july 27th in two kg pots filled with soil from the site were the Experiment I was established. In each pot four seeds were sown and four pots per genotype. At the same phenological stages as in Experiment I, the disease reaction was evaluated on the four plants contained in a pot. Although both trials were carried in naturally infested soil, confidence of the results is supported for the high scores showed by some genotypes (>6 in the 1 to 9 scale).

In the field and the greenhouse experiments, root-rots ocurred during the whole cycle and none genotype was immune to both fungi. Genotype Negro Tacaná (DOR 390) resulted intermediate during the whole cycle to *Fusarium* spp. (data not shown) while G 12729, a wild genotype was resistant during the vegetative stages. The highest scores in the field were recorded at the R_5 and R_7 phenological stages. In the greenhouse, where growing conditions were relatively uniform, none genotype displayed a consistent resistant pattern during the whole cycle.

The level of *R. solani* severity was lower than the *Fusarium* spp. in the field, while in the greenhouse it was higher (Table 1). Among the 15 genotypes, only G 12729 (wild) displayed a consistent resistant response in the field to *R. solani*. Genotypes Pinto Villa, Wisc RRR, PI 203598 and BAT 477 displayed a consistent intermediate reaction to *R. solani* and *Fusarium* spp. The last three genotypes had been reported as resistant by Tu (1991), Abawi (1989) and Silbernagel (1990), and in the greenhouse trial they had a susceptible response to both fungi.

In the field *R. solani* severity was strongly related to average and maximum temperature during the growing cycle, while the incidence was related to all weather traits recorded. Fusarium severity was positively related to rainfall and both severity and incidence were negatively related to maximum temperature (Table 2).

Table 1. Average disease score on 15 bean genotypes to root-rots induced by *R. solani* and *Fusarium* spp. in four consecutive phenological stages.

Disease	Field conditions				Greenhouse conditions			
	20[x]/V3	45/R5	62/R7	97/R8	20/V3	45/R5	62/R7	97/R8
R. solani	4.0	3.5	3.1	3.1	4.1	4.0	4.8	5.6
Fusarium spp.	3.6	4.8	3.6	4.8	3.9	3.4	4.4	4.2

[x] Days after planting

Table 2. Relationship between average root-rots incidence of 15 bean genotypes and climatic factors registered during the growing cycle at Texcoco, Mexico, 1999.

	Fusarium spp. Severity	*R. solani* Severity	*Fusarium* spp. incidence	*R. solani* incidence
Max. Temp. ºC	-0.641	0.922	-0.839	0.714
Min. Temp. ºC	0.366	0.549	-0.005	0.797
Aver. Temp. ºC	-0.041	0.836	-0.418	0.920
Rainfall mm	0.762	0.162	0.417	0.564

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EFFECTS OF TRIAL CHARACTERISTICS ON DETECTING RESISTANCE OF WHITE BEAN TO WHITE MOLD

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In Ontario, resistance of white bean cultivars and breeding lines to white mold, caused by *Sclerotinia sclerotiorum*, is determined in field trials that rely on natural infection. Trials are considered acceptable if they have a reasonably low coefficient of variation and a reasonably high level of white mold in at least some entries. The objective of this study was to determine how the characteristics of trials affect the number of significant differences among entries.

Forty bean lines and cultivars (Hall et al. 1994) were evaluated for resistance to white mold in 9 trials conducted at Arkell, Ontario from 1992 to 2000. Six of the trials (1992, 1993, 1994, 1995, and 2 trials in 1977) were established in an area naturally infested with sclerotia of the pathogen. Plots spaced 72 cm apart were arranged in a randomized complete block design with 3 replications. Each plot contained 3 rows 4.5 m long and 18 cm apart. In 1996 and a third trial in 1997, plots designed for an artificial inoculation trial consisted of a single row 7 m long and 1 m apart and were arranged in a completely randomized design with 1 replication. In 2000, in a trial designed as an artificial inoculation trial, plots spaced 1 m apart between rows and 0.25 m apart within rows were arranged in a randomized complete block design with 3 replications. Each plot was a single row 4 m long. All trials were established in early June and were sown at a rate of 20 seeds/m. Severity of white mold was rated at harvest on a 0-4 scale, in which 0 = no disease and 1, 2, 3, and 4 represent 1-25%, 26-50%, 51-75%, and 76-100% of the surface of stems and branches affected by white mold. Fifty plants in 1992, 1993, and 1994, 30 plants in 1995, 1996 and the 3 trials in 1997, and 10 plants in 2000 per plot were rated for disease severity. In trials containing only 1 replication per entry (1996 and the third trial in 1997), each sampled plant was treated as a replication. Means for disease severity were compared by ANOVA and LSD (P=0.05).

The number of significant pairwise differences among entries (NSPD) was used to measure the ability of the trial to distinguish entries. NSPD increased with the mean disease severity of the trial but tended to a plateau beyond a mean severity of 0.4 (Fig. 1). NSPD increased linearly with the range of disease severity values in the trial (Fig. 2), and decreased as the coefficient of variation of the trial increased (Fig. 3). Disease severity scores for entries were significantly correlated in all pairwise comparisons of trials, except for comparisons involving one trial in 1997, and bore little relation to the mean disease severity of the trials being compared (Fig. 4). We conclude that evaluations of resistance of white beans to white mold will be more sensitive and more stable when based on trials with a mean disease severity >0.4 on a 0-4 scale, a wide range of disease severity values across entries, preferably >1.5, and a coefficient of variation <100. Of these, the range of disease severity values seems to be the most important factor affecting the discriminating power of the trial. The range of disease values in a trial could be widened by increasing the number of intervals in the severity scale, or by using disease incidence or maximum disease severity per entry.

Figure 1. Relation of the mean disease severity of the trial to the number of significant differences in severity of white mold in pairwise comparisons of 40 bean entries over 9 trials.

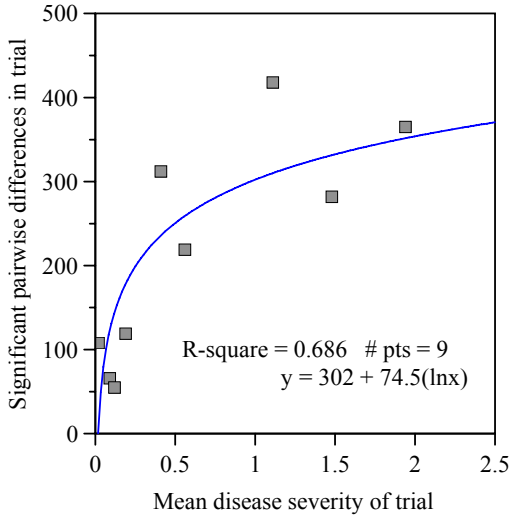


Figure 2. Relation of the range of white mold severity values in a trial to the number of significant differences in severity of white mold in pairwise comparisons of 40 bean entries over 9 trials.

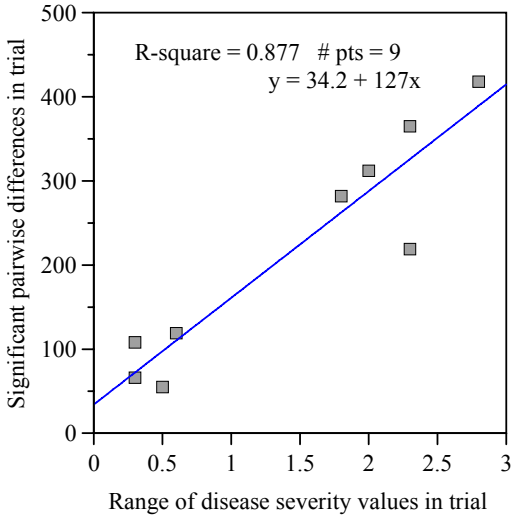


Figure 3. Relation of the coefficient of variation of the trial to the number of significant differences in severity of white mold in pairwise comparisons of 40 bean entries over 9 trials.

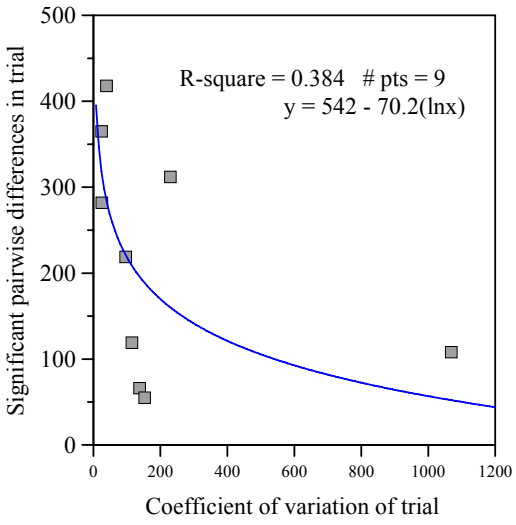
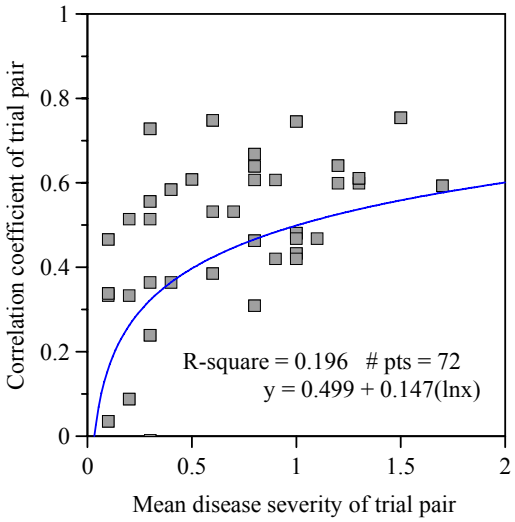


Figure 4. Relation of the correlation between trials in white mold severity values of 40 bean entries to the mean white mold severity of the two trials, assessed over all pairwise comparisons of 9 trials.



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COMPARISON OF SCREENING OF METHODS FOR AND IDENTIFICATION OF SOURCES OF RESISTANCE TO *Sclerotinia sclerotiorum* IN COMMON BEAN.

J.R. Steadman, Coordinator, K.M. Eskridge, Statistician, University of Nebraska. Data from C. Kurowski (California), R. Mainz (Minnesota), P. Griffiths (New York), J. Kelly (Michigan), J. Meyers (Oregon), and P. Miklas (Washington).

Developing resistant cultivars to *Sclerotinia sclerotiorum,* cause of bean white mold, is the most economical disease management strategy for the bean industry. However, only partial resistance has been identified to date. The objective of the study was to identify broadly-resistant bean genotypes by testing putative sources of resistance developed by bean breeders with greenhouse and field methods in different locations.

Field tests consisted of two rows of each entry and a common susceptible genotype resulting in a three-row plot 4.6m (15 ft) long replicated three times in a randomized complete block design. The greenhouse test was the straw test (Petzoldt and Dickson, 1996). Tests and locations were as follows: field - CA, MI, WA, OR; straw - OR, WA, MN, NY. Because of the differences in these data sets, e.g., in field disease incidence and severity, or length of stem affected and number of nodes affected, the entries were ranked from most resistant (1) to most susceptible (12) in each test. A Spearman's rank correlation was used to compare entry rankings in each test. Although no white mold developed in NE, D.P. Coyne recorded common bacterial blight (*Xanthomonas campestris* pv. *phaseoli*) reaction on the entries.

In general, there were significant ($p<0.05$) positive correlations among CA and WA field, and MN, NY, OR and WA straw. Other highly significant ($p<0.01$) positive correlations occurred among the straw tests in NY, MN, OR and WA. The three most highly associated tests were between NY straw and WA field ($R=0.872$, $p=0.0002$) and MN and OR straw tests ($r=0.879$, $p=0.0002$). All the straw tests produced similar rankings and were highly correlated even though different *S. sclerotiorum* isolates were involved at each state location.

The rankings for each test are found in Table 1. When an ANOVA was used on ranking, with each test as a block and bean genotype (entry) as a treatment, there were significant differences ($p=0.001$) among genotypes (Table 2). MO162 (J. Myers), G122, RedKanner (P. Griffiths) and NG8025 (J. Kelly) had the best mean rank, but I9365-25 and USWA-6 (P. Miklas) also were ranked significantly lower than Bunsi. CO75511, OT9743-4 and MO162 had the lowest common blight severity which also was equal to or lower than GN Beryl.

Acknowledgments

We wish to thank Kris Powers for technical assistance and Shayne Ortmeier for word processing.

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Table 1. Comparison of rankings of 12 bean lines for reaction to *Sclerotinia sclerotiorum* at 5 locations using field and straw tests in 2001.

	Straw				Field			
Entry	MN	NY	OR	WA	CA	MI	OR	WA
G122	1	1	1	5	4	3	8	2
I9365-25	3	5	6	3	5	7	7	10
OT9743-4	9	8	8	12	7	2	12	9
USWA-6	10	9	12	8	2	5	2	7
Beryl	8	12	11	11	12	11	11	12
NG8025	5	4	4	2	7	10	5	4
N97774	12	7	10	7	1	4	6	6
Bunsi	11	10	9	10	10	6	3	8
CO75511	6	11	5	6	11	12	9	11
OT9743-7	7	6	7	9	5	8	10	5
MO162	4	2	3	1	3	1	1	1
RedKanner	2	3	2	4	9	9	4	3

1 - 12 low to high disease.

Table 2. Mean ranking of bean lines for reaction to white mold from 8 field and greenhouse straw tests and one field evaluation of common bacterial blight severity in 2001.

Entry	Ranking (LSD @ 0.05=2.819)		% Common Blight (LSD @ 0.05=15.370)	
Beryl	10.375	A	13.330	DE
CO75511	8.875	AB	1.000	E
OT9743-4	8.375	ABC	6.333	E
Bunsi	8.125	ABC	26.670	CD
N97774	7.500	BCD	43.330	ABC
OT9743-7	7.125	BCDE	31.670	BC
USWA-6	6.250	BCDE	36.670	ABC
I9365-25	5.750	CDEF	35.000	ABC
NG8025	5.125	DEF	26.670	CD
RedKanner	4.375	EFG	51.670	A
G122	3.125	FG	44.670	AB
MO162	2.000	G	13.000	DE

Means with the same letter are not significantly different.

RESEARCH ON COMMON BACTERIAL BLIGHT AND HALO BLIGHT OF COMMON BEAN IN EAST AND CENTRAL AFRICA

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Introduction

Common bacterial blight (CBB) caused by *Xathomonas campestris pv. phaseoli* [XCP] (Smith) Dye and haloblight (HB) caused by *Pseudomonas syringae pv. phaseolicola)* are the two most important bacterial bean diseases in East and Central Africa. A variant of common blight pathogen, *Xanthomonas campestris pv. phaseoli var. fuscans* causes fuscous blight. The two strains frequently occur together. CBB is ranked the fourth most important bean disease in Africa, and HB the sixth. CBB causes losses of 220,000 t/year in Africa; of these 146,000 t are lost in Eastern Africa and nearly 70,000 t per year in Southern Africa (Wortmann et al, 1998). Annual losses due to haloblight are estimated at 181,000 t in Africa. Disease incidence and severity varies with country and also from season to season. Because of the economic importance of the CBB, a regional collaborative project was started in 1987. The project was led by Uganda where CBB is most severe, with national programs of Ethiopia, Kenya, Burundi, Rwanda, DR Congo and Tanzania as collaborators. Objectives of the sub-project were to identify suitable methods for germplasm evaluation; develop a regional CBB nursery; study variation, symptomatology and host range of Xcp and develop resistant cultivars. Our objective is to highlight some of the results from this sub-project, review briefly status of haloblight and discuss the relevance of these results to the regional breeding programs.

Results and Discussion

Results showed that CBB scores increased from R6 to R9 stages and spreaders increased disease pressure. Disease pressure was higher when the test materials were sown 1 to 2 weeks earlier than the spreaders than when they sown three weeks later. It was concluded that maximum CBB pressure can be obtained by (i) sowing the spreaders 1 to 2 weeks earlier, and (ii) sowing a spreader after every 6-8 test lines in single rows or box formation. XAN 159, XAN 112, G4399, PI 207262, GN Jules, GN Tara and GN`Sel.27 were found resistant to CBB at Kawanda. However progenies of crosses with XAN 112, XAN159 and G4399 succumbed to black root making transfer of CBB resistance to local cultivars difficult. A regional CBB nursery with 118 entries was created in 1989 from local collections, introductions, 30 lines from Ethiopia and six from Burundi-all with resistant reactions to CBB. Unfortunately, most of these materials succumbed to black root and were not used in further breeding work. At present the Regional CBB nursery has 70 entries from Ethiopia, Uganda, Kenya, Tanzania, Rwanda, Burundi and Malawi (Opio and Musaana, 1993). Of the 93 isolates collected from Uganda (68), Ethiopia (13), Tanzania (7), Rwanda (2) and Kenya (3), 75% were fuscous and 25% phaseoli. Pathogenic variation of Xcp was quantitative on *Phaseolus vulgaris* and qualitative on *P.acutifolius*. Differences existed in levels of resistance between leaves, stems, pods and seeds on the same plant. Gene action controlling resistance in most crosses was quantitative but was influenced by the stage at which data was recorded. Results showed that MCM 5001 and XAN 112 have resistance to seed transmission and can be used for breeding lines resistant

to both CBB infection and seed transmission. Additional resistance is found in VAX lines (Shree and Miklas, these proceedings).

Although haloblight is considered to be of high to moderate importance in Kenya, Tanzania, DR Congo, Rwanda and Burundi, limited work on halo blight has been done in Eastern Africa. However, work done by Teverson and Taylor (1994) with isolates from Africa identified nine races based on eight differential cultivars (Canadian Wonder, ZAA 54(A52), Tendergreen, Red Mexican U13, 1072, ZAA55(A53), ZAA12(A43) and Guatemala 196-B). All nine races occurred in Africa. Three frequent races (1, 4 and 6) accounted for over 60% of *P.s. pv.phaseolicola* isolates characterized. They reported that races 3 and 4 were confined to east and central Africa. Races 5 and 8 were dominant in Africa. Race specific and non-specific resistances were found in the 1048 accessions tested. However, race non-specific resistance occurred in only 1% of the accessions (GLP-X92, Urobonobono, Wis HBR 72, AFJ 29, Valliant, NAC 6S, NDM 14, GN*1 Sel 27, 2702/2, Pajuro, PI 150414, Gloriabamba, Jules and Poroto).

A sub-project to develop halo blight resistant cultivars was started at ISABU in Burundi in 1990 (Schmit, 1994). Crosses were made with the locally popular Dore' de Kirundo as a recurrent parent. Halo blight donors were Calima, Urubonobono, A410, A321, PVA 779, H75, Aroana and SM 1197. Additional 30 F_2/F_3 populations from CIAT were included in this program. Several advanced lines with a high level of resistance to halo blight have been selected from these populations. Populations derived from parents with I-gene (A321, A410 and Aroana) were severely affected by black root. Lines with I-gene were also highly resistant to race 3 of halo blight. Progress of this work has been severely hindered by civil strife in Burundi since 1994.

The current market led regional breeding programs in East and central Africa are focusing on multiple constraint breeding. Key to the success of these programs in knowledge of pathogenic diversity and reliable sources of resistance described in this paper. CBB is a priority constraint for red mottled, pinto, sugar, navy, yellow and brown/tan market classes. Halo blight is major constraint in red and white kidneys, climbers, large white, yellow and brown/ tan. New *P.s.pv.phaseolicola* differentials and sources of resistance identified by Teverson and Taylor (1994) should be incorporated in a regional halo blight nursery. The VAX lines, which have shown consistent resistance in many countries in Africa, need to be included in the CBB nursery and used in breeding programmes. Use of both race specific and race-non specific resistance appropriate to this region should provide an effective strategy for developing cultivars with improved and durable resistance to halo blight. The genetic map of the common bean seems to show that resistance genes for CBB and halo blight are closely linked (Miklas, 2002 personal communication). This implies that selection for resistance to Xcp may results to improved resistance to *P.s*.pv. *phaseolicola*. The recently formed African Bean CBB/HB working group is expected to coordinate activities addressing the two constraints.

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COMMON BACTERIAL BLIGHT OF BEANS RESEARCH IN UGANDA 1986-2001

Fina Opio and Annet Namayanja

Introduction

Common bacterial blight and fuscous blight of the common bean *Phaseolus vulgaris,* caused by *Xanthomonas campestris pv. Phaseoli* (Smith) Dye, XCP are the most important bacterial diseases of bean in Uganda. The two diseases are collectively referred to as CBB. The disease is more prevalent and severe in the low altitude areas of Uganda. In these zones CBB is still the most important disease of beans.

Since the identification of CBB as an important disease in Uganda in 1960 (Leakey, 1963), no particular attention was given to it until 1983. From 1983 – 1994 considerable effort was devoted to understanding the disease and its causal organism and developing resistant genotypes to the disease. This paper presents a summary of the results obtained from that effort.

Summary of the studies on CBB 1986-1994

The work during this period mainly focused on pathology and breeding.

Pathology

The emphasis in pathology studies was on determining the nature and extent of pathogenic variation of *Xanthomonas campastris pv. Phaseoli;* seed transmission, survival of the pathogen and crop loss associated with the disease. Results from this study indicated that:

(a). The XCP isolate differed in aggressiveness on *P.vulgaris* suggesting a quantitative variation while they differed in virulence on *P.acutifolius* suggesting a qualitative variation. Seven groups of XCP isolates were identified using eight genotypes of *P.acutifolius* (Opio *etal* 1996). Table 1

(b). Bean seed was the main source of primary inoculum. Seed infection level ranged from 0.3 to as high as 16.1%. (Opio *et al* 1993). There were significant differences in transmission efficiency among the genotypes.

(c). XCP was eradicated from soil after two consecutive seasons without beans. Fifteen species of weeds and two non host crops supported epiphytic population of XCP (Opio *etal* 1996).

(d). Yield losses in beans associated with CBB ranged from insignificant to 61.7% depending on susceptibility of the genotype.

Breeding

The breeding work focused on improving locally acceptable varieties (such as Kanyebwa, K20) for resistance to CBB. Donor parents included; PI 207262, IAPAR 16, BAC 6, GN Jules, GN Nebrasca selection 27, XAN 112 and XAN 159.The promising lines generated from these crosses were incorporated in the CBB nursery that was distributed to some countries in Africa. These lines have never been released because they lacked some attributes desired. However, further improvement on plant architecture and other characteristics can make them acceptable. In addition inheritance and heritability of resistance in *Phaseolus vulgaris* to CBB was carried out.

Activities on CBB 1994-2001.

In attempt to develop better detection methods for XCP in seed, some twenty isolates were used to develop a RAPD-PCR based technique at the Scottish Crop Research Institute in UK (SCRI). It was found that RAPDS could be used to identify the XCP and differentiate XCP from

XCP var. fuscans. In addition the breeder in Uganda has initiated a backcrossing programme to incorporate resistance for CBB in released varieties and poplar land races.

Conclusion

The results from the work emphasized an integrated approach to control CBB which involves resistance, low seed transmission efficiency, clean seed, rotation and proper seed certification. There is need for getting a quicker mechanism for detecting XCP in seed. Molecular tools provide a very good if not the best option. The use of molecular assisted selection should be incorporated in the breeding work for CBB not only in Uganda but the whole of Africa.

Table 1. Groups of *Xanthomopnas campestris pv phaseoli* isolates according to the leaf reaction of eight genotypes of *phaseolus acutifolius*.

Isolate	*Phaseolus acutifolius* genotypes							
		T1	T5	T8b	T19	T21	T22	PI 321-638	L242-45
1005	R[a]	R	R	R	R	S	R**	R	1
1010									
1032									
1069									
1024	R	R	R	R	S	R	S	R	2
1048									
1073									
1034	S	R	R	R	R	R	R	S	3
1038	R	I	R	I	S	R	S	R	4
1067	R	I	R	I	S	R	S	R	4
1065	R	I	R	R	R	S	I	R	5
1068	R	R	R	I	S	R	S	R	6
1083									
Rest of the17 isolates[b]									
	R	R	R	R	R	R	R	R	7

[a]Leaf reaction. R = Immune reaction, R** = Tolerant, I = Intermediate, S = Susceptible

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GRAM NEGATIVE BACTERIA DETECTED ON LEAVES AND SEEDS OF *PHASEOLUS VULGARIS* DURING YEARS 1994-2001

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A research project dealing with the study of the common bacterial blight and other diseases of beans had been developed from many years under the University of Puerto Rico, Mayaguez campus. During this period our main focus has been the study of the bacterial blight disease caused by *Xanthomonas campestris* pv. *phaseoli = X. axonopodis* pv. *phaseoli*. Along with this bacterium, some other gram negative bacteria have been commonly isolated from leaf lesions and seeds of different *P. vulgaris* genotypes collected on several countries of Central America, México and the Caribbean. Leaves and seeds represent an important mean for local and long distance dissemination of organisms such as bacteria. Distribution of germplasm using seeds as well as other plant tissues around the world increases the potential of microbes to be established in new localities. Besides the pathogenic potential there are microbes, which are common colonizers to certain plant parts. Gram negative bacteria which are common colonizers, or are in association with pathogenic bacteria of the leaf and seed of *P. vulgaris* are reported (Table 1).

Materials and Methods

Bacteria were isolated using ordinary procedures and surface disinfections with sodium hyperchlorite and three washes with sterilized water. Leaf tissue was cut in small pieces and let stand for around half an hour in nutrient broth before streaking. Seeds were macerated and let in suspension of nutrient broth for 2 hours at 4C. Serial dilutions were streaked on nutrient agar, and incubated at 28C for 3-5 days. Colonies representing different configuration, elevation, margin and pigmentation were separated. Purification procedures were conducted and identification with metabolic fingerprinting was performed using the BIOLOG method and pathogenicity tests under greenhouse conditions.

Results and Discussion

All bacteria identified within the *Xanthomonas campestris* group were pathogenic on bean leaves independently of the pathovar identified and caused common bacterial blight symptoms. Results indicate metabolic diversity within the pathovar *phaseoli*. It is recommended that researchers using the BIOLOG system create their own data base to get a better identification of the pathovar *phaseoli*. The other bacteria found were non-pathogenic on beans under our conditions. None of these have been reported in the literature as pathogenic with the exception of *Pseudomonas fluorescens* that has been found pathogenic on beans in very few cases (CIAT, 1994).

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Table 1. Gram Negative Bacteria associated with leaves and seeds of *P. vulgaris*

Identity	**Tissue**	**Year**	**Origin**
Agrobacterium rhizogenes	leaf	1994	Puerto Rico
Enterobacter agglomerans	leaf	1994	Puerto Rico
Kluyvera ascorbata	leaf	1994	Guatemala
Xanthomonas campestris pv. phaseoli	leaf	1994	Costa Rica
Xanthomons campestris pv. phaseoli	leaf	1994	Guatemala
Xanthomonas campestris pv. phaseoli	leaf	1994	Puerto Rico
Xanthomonas campestris pv. phaseoli (f)	leaf	1994	Costa Rica
Xanthomonas campestris pv. phaseoli (f)	leaf	1994	Guatemala
Xanthomonas campestris pv. phaseoli (f)	leaf	1994	Puerto Rico
Kingella denitrificans	leaf	1996	Puerto Rico
Pseudomonas cissicola	leaf	1996	Puerto Rico
Pseudomonas fulva	leaf	1996	México
Pseudomonas fragi	leaf	1996	Costa Rica
Xanthomonas campestris pv. phaseoli	leaf	1996	Dom. Rep.
Xanthomonas campestris pv. phaseoli (f)	leaf	1996	Costa Rica
Xanthomonas campestris pv. phaseoli (f)	leaf	1996	Guatemala
Xanthomonas campestris pv. phaseoli (f)	leaf	1996	Puerto Rico
Xanthomonas campestris pv. pelargonii	leaf	1996	Puerto Rico
Xanthomonas campestris pv. translucens	leaf	1996	México
Xanthomonas campestris pv. onion	leaf	1996	Puerto Rico
Xanthomonas oryzae pv. oryzae	leaf	1996	Costa Rica
Xanthomonas campestris pv. vesicatoria	leaf	1996	Costa Rica
Xanthomonas campestris pv. vesicatoria	leaf	1996	Guatemala
Xanthomonas campestris pv. vesicatoria	leaf	1996	Puerto Rico
Xanthomonas campestris pv. xanthosoma	leaf	1996	Costa Rica
Enterobacter agglomerans	seed	1999	Puerto Rico
Flavimonas oryzihabitans	seed	1999	Puerto Rico
Pantoea dispersa	leaf	1999	Puerto Rico
Pantoea agglomerans	seed	1999	Colombia
Pantoea agglomerans	seed	1999	Guatemala
Pantoea agglomerans	seed	1999	Honduras
Pantoea agglomerans	seed	1999	Nicaragua
Pantoea agglomerans	seed	1999	Puerto Rico
Pseudomonas fluorescens	leaf	1999	Puerto Rico
Sphingomonas paucimobilis	seed	1999	Honduras
Xanthomonas campestris pv. hederae	seed	1999	Nicaragua
Xanthomonas campestris pv. translucens	seed	1999	Nicaragua
Xanthomonas campestris pv. phaseoli	leaf	1999	Puerto Rico
Xanthomonas campestris pv. phaseoli (f)	leaf	1999	Puerto Rico
Pantoea agglomerans	seed	2001	Puerto Rico
Vibrio metschnikovii	seed	2001	Puerto Rico
Xanthomonas campestris pv. phaseoli	seed	2001	Puerto Rico
Xanthomonas campestris pv. phaseoli (f)	seed	2001	Puerto Rico

COMMON BACTERIAL BLIGHT STUDIES IN THE CARIBBEAN AND CENTRAL AMERICA

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In Central America and the Caribbean, common bacterial blight (CBB) of beans (*Phaseolus vulgaris* L.), caused by *Xanthomonas campestris* pv. *phaseoli* (E.F. Smith) Dowson (Xcp) can reduce both seed yield and quality. Studies on common bacterial blight were initiated in Puerto Rico in the 1970's as part of a USAID supported project. Virulent strains were purified and used for inoculation of leaves of potential sources of resistance and breeding lines. The use of multiple needles in the greenhouse and sand blasting in the field were the most common inoculation methods. Eventually, greenhouse inoculation was simplified by a flat clip-prick technique. Field techniques were improved by using bacterial inoculum that was purified and standardized in the laboratory and sprayed in the field with carborundum or sterilized sand (Zapata et al., 1985). Symptoms in the greenhouse and field were successfully graded using a 1-5 scale, where 1 = resistant and 5 = highly susceptible (Zapata et al., 1985).

High levels of resistance to CBB were developed in collaboration with Dr. R. Wilkinson by pyramiding minor genes for resistance from diverse sources. This was accomplished through the selection of resistant plants in the greenhouse at Cornell University and Puerto Rico and in field trials in Puerto Rico (Zapata et al., 1991). In 1990, Drs. G. Freytag, M. Zapata and R. Wilkinson released five CBB resistant germplasm lines (W-BB-1, W-BB-20-1, W-BB-3, W-BB-52, W-BB-II-56). The sources of resistance of these lines were GN-1 sel. 27 and lines with *P. coccineus* background (Zapata et al., 1991). XR-235-1-1 derives its CBB resistance from *P. coccineus* (Freytag et al., 1982). Miklas et al. (1999) released germplasm lines (ICB-3, ICB-6, ICB-8 and ICB-10) with resistance derived from *P. coccineus*. Freytag (1989) reported three linked dominant genes that conferred resistance in the yellow-seeded tepary bean line TL-40. When inoculated with specific strains of Xcp, an F_2 population from a cross between TL-40 and Mex-114 (susceptible) segregated 3 resistant: 1 susceptible as would be expected for single dominant gene resistance. However, there was evidence of linkage when the results from two or three strains were considered. Urrea et al. (1999) studied the inheritance of CBB resistance of three tepary lines. When inoculated with Xcp strain 484a, ratios in the F_2 generation showed that the resistance of Neb-T-6-s and PI321637 was governed by single dominant gene. Results suggested that resistance of Neb T-8a-s was conferred by two dominant genes with complementary effects. Lack of susceptible progeny in test crosses suggests that at least one locus conditioning resistance is shared among Neb-T-6-s, PI321637 and Neb T-8a-s. Dominican red mottled landrace varieties Pompadour 17 and Pompadour K have leaf and pod resistance when field tested in the Caribbean (Beaver et al., 1992). Early generation (F_4) evaluation of breeding lines derived from the cross Dorado/ XAN 176 were effective for screening for field resistance (Varela et al., 1995). The light red kidney germplasm line PR9443-4 had a highly resistant leaf reaction to three Xcp strains when evaluated under greenhouse conditions (Beaver et al., 1999).

Zapata et al. (1985) reported a strain specific reaction to Xcp in *P. vulgaris*. Based on several years of observation of pathogen dynamics, Zapata (1989, 1996a and 1996b) presented an explanation for variability of CBB reaction. Pathogen variability was studied through collaboration with researchers in Central America and the Caribbean where Xcp was prevalent throughout the year. A collection of the pathogen was made from several different countries in the region. Differential lines were identified as well as lines with resistance to multiple races from Puerto Rico, Dominican Republic, Costa Rica and Cuba. The differential lines are: XAN 159, NY 79-3939-1, A774, RAZ 50, L-81-61 and Jutiapa (Zapata, 1996). Other lines, XAN 309, G17341, VAX-2, GN #1 sel. 27, VAX 1 and W-BB-11 were identified to be resistant to isolates from Costa Rica, Dominican Republic, Honduras, and Nicaragua. Isolates from Honduras and Guatemala were the most virulent (Zapata et al., 1998). In 1996, Puerto Rico was the host for the first international workshop on bean common bacterial blight. A proposal was made at the workshop to establish minimum standards to designate Xcp pathogenic races through the use of differential lines and uniform inoculation methods (Coyne et al., 1996). Strong evidence supporting the existence different Xcp races in

Central America and the Caribbean has been found and reconfirmation of pathogenic races has been achieved in subsequent studies by M. Zapata (unpublished).

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EVALUATION OF *Phaseolus vulgaris* L. GENOTYPES FOR THEIR PERFORMANCE AGAINST "Common Bacterial Blight" (Xcp) FROM SALTA (ARGENTINA)

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Introduction

Argentinean production of *P. vulgaris L.* is concentrated in the northeast region (NOA) of which the province of Salta accounts for approximately 72% (178,000 t/year). Among the endemic diseases with a higher impact in the area "Common Bacterial Blight" particularly stands out which negatively affects performance and the quality of the grain by staining, reducing its size and shine. Falls in yield have been estimated at between 15 – 20%, which in turn have been estimated between values of 20 and 25 million dollars per year. Rejections due to stains in commercial type "large white" or "Alubia" can reach up to 10% (INTA estimation).

The wide diffusion of this pathogen, its association with the seed and the low effectiveness of chemical control has presented us with the need to consider an integrated management programme for the crop using better quality seeds and resistant varieties.

There are improved lines available for their resistance to this disease: VAX, developed in CIAT from an interspecific cross between *Phaseolus vulgaris L. x P. acutifolius L.* (Singh and Muñoz, 1999), which were evaluated for 20 isolates from Africa and Latin America showing good performance (Jara et al., 1999). From XAN 159, also derived from interspecific crossing between *P. vulgaris L. x P. acutifolius L*, HR lines were obtained from the "navy bean" type and improved in this aspect (Park et al., 1998).

The different genetic systems that can control the reaction to this disease in different parts of the plant have already been established. The identification of the sources of resistance to the local strains and the differentiation between them depending on the moment in the cycle and the part of the plant in which they are resistant are useful for the selection of parent stock that provide complementary resistance genes allowing widely resistant local varieties to be obtained.

Objectives

The objectives of this study were: a) to identify resistance sources to the isolates of Xcp from Argentina; b) to detect whether there is differential performance between these entries throughout the phenological cycle of the crop.

Materials and Methods

The materials were evaluated for an isolate of *Xcp var. fuscans (Ac)*, highly pathogenic, obtained from plants with symptoms from the variety "Alubia Selección Cerrillos INTA" from a crop grown in Cerrillos during 2001.

Eighteen lines of *Phaseolus vulgaris L.* were evaluated which had been tested in other regions of the world and behaving as resistant: CO-245-2/97, CO-261-2/97, CO-286-1/97, CO-311-4/97 (provided by Dr. Asensio); C-01-1, C-01-2, C-01-3, C-01-5, C-01-6, (VAX lines); C-01-7, C-01-8,C-01-9,C-01-23,C-01-25,C-01-26,C-01-27,C-01-28 y C-01-29 (provided by CIAT). HR-45 line (provided by Dr. Park) and C01-4 (VAX-4) (provided by CIAT) were used as resistant controls; and the local varieties with a known performance against the disease: Perla INTA and Paloma INTA (intermediate controls), and Alubia Selección Cerrillos INTA and TUC-180 (Estación Agroindustrial Obispo Colombres-EEAOC) (susceptible controls) were also used. The assay was carried out in a greenhouse in the area of Cerrillos (24º 54' S; 65º 29' W; 1250 masl),

Salta, Argentina. A random block design was made with 6 repetitions and three controls per material. The blocks were distributed throughout the whole greenhouse according to the radiation gradient and the temperature.

Seeding took place in individual plant pots on 5/10, and the inoculations on three different dates: 1) on the leaves, vegetative state (date: 25/10); 2) on leaves, before flowering (5/11); and 3) in pods during swelling with grains (5/12). The evaluations were made on the following dates: 5/11, 26/11 and 10/12 respectively.The leaves were inoculated using the multiple needles method, and the pods were punctured using histological needles with an inoculum of 1 x 10^7 ufc/ml obtained from a cultivar of Ac in nutrient agar medium (NA) for 40 hours at 28 ºC (Lienert and Schwartz,1994).

The following scale was used for evaluation: 1 Absence of symptoms. 2 Dry necrosis around the incision. 3 Oily halo around the incision. 4 Widespread necrosis. The results were analysed through an ANOVA, with the LSD test for the comparison of averages.

Results

Significant differences were found between the materials in each of the three phenological stages. Significant differences were only determined between blocks after the evaluation of the pods.

A slight variation in the reaction in the leaves to the disease from the vegetative through to the pre flowering stage was observed.

Of the nine materials that performed as immune in the leaf during the first evaluation, only two of them (C-01-6 and C-01-3) kept this immunity through to the second evaluation. The symptoms were slight for four in them (C-01-7, C-01-27, HR-45 and C-01-8), statistically equal to the two immune ones. VAX-4, C-01-5 and C-01-9 showed a more severe reaction and were statistically different from the first six.

Immun ity was observed in pods in the material CO-286-1/97.

The assay was less discriminatory in pods than in leaves. The difference observed between blocks 1 and 3 (extremes of the greenhouse) could be due to the higher temperature speeding up the ripening process of the pods making the interpretation of the symptoms more difficult.

Only entries C-01-8 and C-01-9 showed a good reaction in the three evaluated stages.

Acknowledgement

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SNAP BEAN PRODUCTION AND SOIL MICROBIAL BIOMASS IN FUNCTION OF PHOSPHORUS DOSAGES

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Introduction

Microbial biomass is a important component of the organic matter of the soil, since it is a reservoir of essential nutrients for the plants (Kaiser et al., 1995). The carbon content of the microbial biomass indicates the potential reserve of carbon in the soil that participates in the humification process (Gama-Rodrigues et al.,1997). The estimation of the microbial biomass of the soil supplies useful information about the changes in its biological properties due to several agricultural management pratices, such as addition of organic residues. According to Sparling et al. (1997), the microbial biomass can be used as a first indicator of alterations in biological processes in the soil, serving as a monitor for the fertility and polutional state of these. The assay was carried out with the purpose of evaluating the effects of phosphorus dosages in the carbon microbial biomass content of the soil (**Bio-C**) and the production of snap beans (cv UEL-1).

Materials And Methods

The trial was carried out in a greenhouse. It was used pots with 4 kg of soil colected from 0-20 cm horizon of a Oxisol (Latossolo Vermelho distrófico (EMBRAPA (1999)), clay texture, from the region of Londrina-PR, Brazil. All the pots received basic fertilization as indicated in Novais et al. (1991), except P. The treatments were: Control, 100, 200, 300, 400 and 500 kg P_2O_5 ha^{-1}, as triple superphosphate, applied at the depth of 5 cm. After the fertilization and during the experiment the soil moisture was maintained at 70% of the maximum water retention capacity. After emergence (8-10 days), 2 plants were left in each pot. The harvesting was carried out 50 days after emergence. The pods and root systems were collected from each pot and a soil sample, which was used to determine the carbon microbial biomass content (**Bio-C**), according Vance et al (1987). The experimental design was randomized with 5 treatments (doses of P) and 4 replicates. The data was used to obtain regression equations and correlation coefficients.

Results and Discussion

The correlations between **BIO-C**, root dry matter production (**RDMP**) and the fresh matter of commercial snap bean pods production (**FMPP**) were significant but negative (Table1).

Table 1. Correlation coefficients between **Bio-C**, root dry matter and raw matter of pods.

Bio-C	FMPP
RDMP	
-0,73*	- 0,72*

* Significant at 5%.

The root dry matter productions and pod fresh matter production, increased with the doses of P, adjusting to the square functions defined by the following equations: **RDMP** (g) = $1.6756 + 0.0199x - 0.00003x^2$ and **FMPP** (g) = $14.542 + 0.4927x - 0.007x^2$, respectively (Figure 1). The soil microbial biomass decreased with the increasing doses of P, adjusting to the equation: **BIO-C** (mg kg^{-1}) = $282.25 - 0.9097x + 0.0013x^2$ (Figure 2).

Figure 1. Pod Fresh Matter (FMPP) and root dry matter (RDMP) productions.

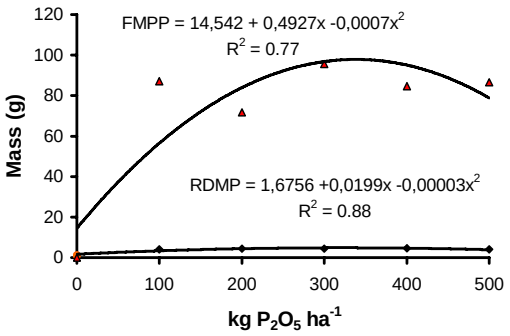
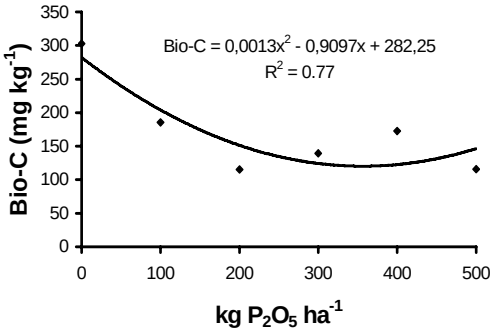


Figure 2. Soil Microbial Biomass Carbon (Bio-C)



Conclusion

The maximum yields of root dry matter and pod fresh matter were obtained with the doses of 331.67 e 351.97 kg ha^{-1} of P_2O_5, respectively. The correlation between **Bio-C** with the plant variables was significant, but negative. The minimum for **Bio-C** was defined in 349.88 kg ha^{-1} of P_2O_5.

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MINERAL AND ORGANIC FERTILIZATION OF SNAP BEAN CROP (cv. UEL-1)

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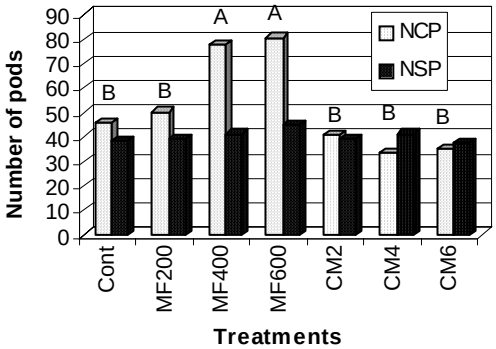
Now days in Brazil a great advance in organic agriculture is been held such in the crop area as in the new knowledge generation. Producing food free of agrochemicals is now a demand from many consumers. The use of mineral fertilizers solves the immediate need of nutrients for the crops, but the incorrect or abusive use of this input has resulted in the degradation of the soils and pollution of water resources. Organic fertilization improves the physical and chemical properties of the soils, increasing the supply of micro and macronutrients, reducing the effect of toxic substances and increasing its buffer capacity. The recommendations for snap beans crops are based mainly in the use of mineral fertilizers and the information about the use of organic fertilizers is scarce, specially for the tested cultivar in. This study was carried out with the purpose of evaluating the response of snap beans (cv UEL-1) to chemical and organic fertilization.

Material and Methods

The experiment was carried out in a Oxisol (Latossolo Vermelho Escuro (EMBRAPA,1999)), clay texture, at Campus of UEL, Londrina , PR-Brazil. For the organic fertilization cattle manure (**CM**) was used and for mineral fertilization (**MF**) a 04-14-08 fertilizer formulation was used. A complete randomized design with three replicates was used to test the following treatments: **Control, MF=200 k ha^{-1} , MF=400 k ha^{-1}, MF=600 k ha^{-1}, CM=2 t ha^{-1}, CM=4 t ha^{-1}** and **CM=6 t ha^{-1}**. At 36 and 42 days after sowing, the fertilizations were carried out, using a total of 2 t/ha of **CM** in the treatments with organic fertilization and 90 kg ha^{-1} of N (urea), in the mineral fertilization and control treatments (to avoid sub-optimal supply of N (ARAUJO et al. (2000)). At 62 days after sowing the harvest was carried out evaluating the number of small (**NSP**) and commercial pods (**NCP**) and the yield of commercial pods.

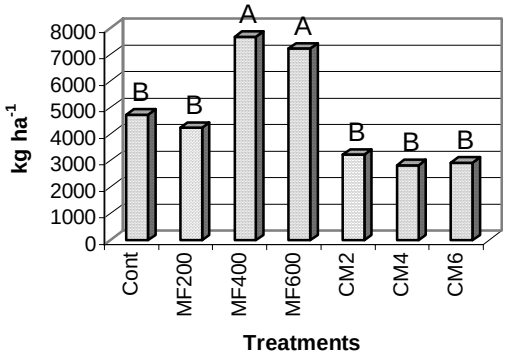
Results and Discussion

Figure 1. Number of pods / 10 plants[1]



[1] A, B are different by Tukey test at 5%.

Figure 2. Yield of commercial pods[1]



[1] A, B different by Tukey test at 5%.

The production of pods was influenced by the type of fertilization. The highest yields of commercial pods were obtained with the use of mineral fertilizers (Figure 1). The number of small pods (< 10 cm in length) wasn´t significantly influenced by the treatments but was higher than commercial pods in treatments with higher doses of CM (Figure 1).

Considering only yield of commercial pods isolated it can be see that the highest yields were obtained with the treatments MF400 and MF600 (Figure 2).

Conclusion

The mineral fertilization resulted in higher yields of snap beans in relation to the use of organic fertilization. The application of 400 kg ha^{-1} of 04-14-08, resulted in a higher yield of commercial pods. The highest doses of **CM**, resulted in a higher number of small pods than commercial pods, indicating a delay in the crop cycle.

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CHEMIGATION WITH FUNGICIDE ON BEANS USING THREE WATER RATES ON CONVENTIONAL AND NO-TILL SYSTEMS

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Introduction

The application of pesticides in irrigation water (chemigation) on a center pivot irrigation system can be an effective pest management system. The term fungigation has been used to describe fungicide applied through irrigation water. Vieira and Sumner (1999) showed that several fungicides applied through irrigation water can be effective, depending on crop, pathogen, disease severity, fungicide, and water volume. The objectives of this research were: 1) to evaluate the efficiency of a fungicide applied through irrigation water for control of bean diseases on conventional and no-till systems; and 2) to evaluate the influence of water volume on fungicide efficiency.

Material and Methods

A trial was carried out under a medium pressure center pivot irrigation system (2.9 ha) in Coimbra, Minas Gerais State, Brazil. In half of the area under the center pivot, beans were sown after maize in no-till system. In the other half, they were sown after soil preparation with plough followed by disc harrow (conventional system). The cultivar Pérola (carioca type) was planted in rows 0.45 m apart with 12-15 seeds/m on 28 July (winter). The fungicide epoxiconazole (0.1 liter/ha) was metered into the suction side of a water diaphragm pump in irrigation rates of 3, 5 and 7 mm. Each half area of the center pivot was divided into three sections where irrigation rates were tested. Before application, fungicide was diluted in 500 liters of water. Conventional spray treatment was applied in 300 liters of water/ha using a hand-held CO_2-pressurized sprayer. Epoxiconazole was applied at 45, 59, and 73 days after emergence. An untreated control was used for comparison. Each plot contained four rows 3 m long. Three replications of each treatment were placed in each section of the center pivot. No fungigated plots were covered with plastic. The coefficient of uniformity during applications varied from 84% and 88%. A diagrammatic scale (Godoy et al., 1997) was used for severity evaluation of diseases. Beans were harvested 107 days after emergence.

Results and Discussion

Two diseases were detected on beans: angular leaf spot (*Phaeoisariopsis griseola*) and alternaria leaf spot (*Alternaria* spp.). In untreated control angular leaf spot was low and alternaria leaf spot was low/moderate. In no-till system yield was 35% superior than in conventional one (data not presented). Severity of alternaria leaf spot was lower in the conventional system (4.6% vs. 7.1% on untreated control). Irrigation rates did not influence the performance of epoxiconazole applied through irrigation water (data not presented). Both fungigation and ground spraying reduced severity of diseases to very low levels (Tables 1 and 2) and increased 100-seed weight and yield (Table 3). Results indicate that epoxiconazole applied through irrigation in rates of 3 to 7 mm of water is efficient, regardless of the till system.

Table 1. Mean results of angular leaf spot severity in dry beans using two methods of fungicide application

Application methods	Foliar area affected by angular leaf spot[1] (%)		
	Evaluation 1[2]	Evaluation 2[2]	Evaluation 3[2]
Fungigation[3]	0.44 ± 0.05	0.92 ± 0.04	1.56 ± 0.13
Ground sprayer (300 /ha)	0.20 ± 0.05	0.40 ± 0.06	0.87 ± 0.08
Untreated control	3.15 ± 0.37	4.37 ± 0.42	6.00 ± 0.33

[1] Means cover no-till and conventional systems and standard deviation of means.
[2] Evaluations were made at 70, 77, and 84 days after emergence.
[3] Means cover three rates of water applied (3, 5, and 7 mm).

Table 2. Mean results of alternaria leaf spot severity in dry beans using two methods of fungicide application

Application methods	Foliar area affected by alternaria leaf spot[1] (%)		
	Evaluation 1[2]	Evaluation 2[2]	Evaluation 3[2]
Fungigation[3]	1.07 ± 0.29	2.04 ± 0.27	2.35 ± 0.35
Ground sprayer (300 /ha)	0.75 ± 0.17	1.28 ± 0.08	1.85 ± 0.29
Untreated control	4.42 ± 0.42	6.00 ± 0.25	7.08 ± 0.55

[1] Means cover no-till and conventional systems and standard deviation of means.
[2] Evaluations were made at 70, 77, and 84 days after emergence.
[3] Means cover three rates of water applied (3, 5, and 7 mm).

Table 3. Mean results of 100-seeds weight and yield using two methods of fungicide application

Application methods	100-seed weight[1]	Yield[1] (kg/ha)
Fungigation[2]	26.3 ± 1.30	2,199 ± 79.6
Ground sprayer (300 /ha)	27.0 ± 0.88	2,235 ± 56.2
Untreated control	24.9 ± 0.26	2,004 ± 47.3

[1] Means cover no-till and conventional systems and standard deviation of means.
[2] Means cover three rates of water applied (3, 5, and 7 mm).

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APPLICATION OF Mo IN COMMON BEAN INOCULATED WITH TWO *Rhizobium phaseoli* STRAINS[⊕]

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Introduction

N_2 fixation by symbiotic microorganisms allows less dependence on nitrogen fertilizing in crop production. *Rhizobium* - *Phaseolus vulgaris* symbiosis is limited by the incompatibility of the *Rhizobium* species as well as the lack of an adequate control in the limiting soil factors. Among these, more attention is paid to supplying macronutrients than to supplying Fe, Mo, and B. Vieira (1998a), points out that 40g foliar applications of Mo ha^{-1} applied after plant emergence increased the nitrogenase and nitrate reductase in the common bean, generating an increase in total accumulated nitrogen in the root. He also observed an increase in native Rhizobia efficiency as an effect supplying Mo, plus a 10% increase in the N concentration of the seed; and in certain soils the N fertilizer may be replaced by small amounts of Mo applied via foliage. Vieira (1998b), observed that nodulation of *Phaseolus vulgaris* L. diminished with the application of Mo via foliar, but in contrast, he found that Mo promoted an increase in the size of the nodule, diminished its senescence, and maintained a larger period of nodule effectiveness in the process of N_2 symbiotic fixing. Berry and Reisenauer (1967), indicate that in tomatoes the accumulation of Fe in the apical zone depends on the level of Mo in the nutrient solution. An adequate level of Mo improved Fe capture as far as the plants were concerned, and those with Mo deficiencies presented a lower capture and translocation of Fe. Mo absorption may diminish due to the presence of sulfur according to observations of Stout *et al*., (1951). Reisenauer (1963), observed that the $CaSO_4$ applications in Mo deficient soils diminish the yield and content of N in peas. The current work assessed the effect of Mo supply and the efficiency of two *Rhizobium* strains on the N, Mo, Fe content and the yield components in cv. Delicias.

Materials and Methods.

Work was established in the experimental agro-field at the School of Agronomy of the U.A.S.L.P., located on the San Luis Potosí –Matehuala Highway, km 14.5, in the Palma de la Cruz Ejido pertaining to the Municipality Soledad de Graciano Sánchez. The geographical coordinates are 22° 14' North Latitude and 100° 53' longitude, at an altitude of 1835 m. The soil is of medium texture, weakly alkaline pH and free of salts; it is poor in organic material content, rich in phosphorous, very rich in potassium and poor in iron. The Delicias variety with growth habit I was used. The experimental design used was an array of divided plots with a random block distribution and four replications. The sources of N (0 and 60 kg of N ha^{-1}, INIFAP strain and Biocampo- BIO-strain) were considered an "A" factor and (bl. 54 g of Mo ha^{-1} application and b2. without Mo application) the "B" factor, for a total of 8 treatments (Table 1). The *Rhizobium phaseoli* strains in peat stands, were applied at a dose of 1 kg ha^{-1}. In all cases, 60 kg of P_2O_5 ha^{-1} were applied. Urea at a 46% of N and ammonium molybdate were used as a source of N and Mo. The Rhizobia strain was applied prior to planting and the Mo application was done to the soil in bands ten days after the emergence. Five auxiliary water applications were given in all. The following variables were assessed. Samples of leaves without petiole were taken and the percentage of N (Kjeldahl), Fe and Mo ppm was determined at the flowering stage; and the percentage of N in grain and in straw was determined after the harvest. At the end of the growing cycle, the yield components number of pods per plant (NPP), number of grains per pod (GP), number of grains per plant (NGP), size of the seed in g $seed^{-1}$(SS), biomass production per plant in g $plant^{-1}$ (BPP), harvest index, (HI), and yield in kg ha^{-1} of grain were assessed.

Table 1. Treatments used in the experiment Mo application on common bean inoculated with two *Rhizobium phaseoli* strains.

1. 0 kg de N	2. 0 kg de N + Mo	3.*Rhizobium phaseoli* (INIFAP)	4.*Rhizobium phaseoli* (INIFAP)+ Mo
5.*Rhizobium phaseoli* (BIO)	6.*Rhizobium phaseoli* (BIO) + Mo	7. 60 kg de N	8. 60 kg de N + Mo

Results and Discussion

The percentage of N in leaves without petiole in the flowering stage was different among the N sources and the highest values observed with the *Rhizobium* application, with an average of 3.115% and 3.112% of N in the INIFAP and BIO strain, respectively. Fe ppm content increased significantly with inoculation and N application surpassing the

sample by more than 89 ppm. The average for the application of Mo and without Mo was statistically equal with values of 293.6 and 267.87 ppm of Fe, respectively. The content of Mo in leaves was different among the N sources, and increased significantly with the application of ammonium molybdate. The highest average was found with the application of INIFAP *Rhizobium*, and the application of Mo generated an increase of more than 2 ppm on average. The yield components number of pods per plant, number of grains per plant, number of grains per pod, production of biomass and harvest index did not respond significantly to the nitrogen sources and molybdenum application. SS was significantly affected by the N sources and by the effect of the source interaction N x Mo. The highest average was obtained with the BIO strain and application of molybdenum with 0,302 g seed^{-1} (Table 2). Grain yield responded to the different sources of N, and to the N x Mo interaction effect; the best yields were reached with the application of the BIO strain and 60 kg ha^{-1} of N, with application of ammonium molybdate. The N percentage in grain and straw increased significantly by 8.15 and 13.24%, respectively, with ammonium molybdate; among the N sources the BIO strain had the highest N percentage in straw and in grain, with an average of 0.952 and 3.05%, respectively.

Table 2. Significant levels of ANOVA and averages for variables studied in the experiment of Mo application on common bean inoculated with two *Rhizobium phaseoli* strains.

Source of N	Source of Mo	%NF	Fe ppm	Mo ppm	NPP	GP	NGP	SS	BPP	HI	Yield kg ha^{-1}	%NP	% NG
0 kg N	C-Mo	2.46	187.50	2.620 a	29.7	4.1	119.6	0.29 a	65.04	0.52	1666.67 b	0.795	3.02
	S-Mo	2.37	220.00	1.665 a	26.2	4.0	106.2	0.28 a	57.75	0.52	2000.00 a	0.840	2.78
	Average	2.42B	203.75B	2.14 B	28.0	4.1	112.9	0.29 A	61.40	0.52	1833.33 B	0.817 B	2.89
Strain	C-Mo	3.19	321.50	6.85 a	37.4	3.8	143.8	0.27 b	76.43	0.51	1800.00 b	0.925	2.96
INIFAP	S- Mo	3.04	265.00	1.84 b	33.6	4.2	142.9	0.28 a	73.71	0.54	2066.67 a	0.760	2.87
	Average	3.12 A	293.25A	4.35 A	35.5	4.0	143.4	0.27 B	75.07	0.53	1933.33 AB	0.842 B	2.91
Strain	C-Mo	3.00	325.50	4.305 a	24.9	4.3	107.8	0.30 a	61.01	0.53	2133.33 a	1.010	3.27
BIO	S- Mo	3.23	300.00	2.800 a	29.1	4.3	127.2	0.29 b	66.97	0.54	2000.00 a	0.895	2.82
	Average	3.11 A	312.75 A	3.55 AB	27.0	4.3	117.5	0.29 A	63.99	0.53	2066.67 A	0.952 A	3.05
60 kg N	C-Mo	2.73	340.00	3.645 a	32.9	4.4	145.9	0.28 a	74.31	0.55	2133.33 a	0.965	2.97
	S- Mo	2.63	286.50	1.590 b	33.0	4.2	141.7	0.28 a	72.95	0.55	1966.67 b	0.765	2.84
	Average	2.68AB	313.25 A	2.620 B	32.9	4.3	143.8	0.28 AB	73.63	0.55	2050.00 A	0.86 AB	2.91
Average													
Factor	*C-Mo*	*2.84*	*293.6*	*4.35 A*	*31.2*	*4.1*	*129.2*	*0.285*	*69.18*	*0.52*	*1933.3*	*0.923 A*	*3.05 A*
Mo	*S-Mo*	*2.81*	*267.8*	*1.97 B*	*30.5*	*4.2*	*129.4*	*0.282*	*67.84*	*0.53*	*2008.3*	*0.815 B*	*2.82 B*
S.V													
S. de N		*	*	*	Ns	Ns	Ns	*	Ns	Ns	*	*	Ns
S de Mo		Ns	Ns	*	Ns	Ns	Ns	Ns	Ns	Ns	Ns	*	*
Interaction N x Mo		Ns	Ns	*	Ns	Ns	Ns	*	Ns	Ns	*	Ns	Ns
DSH_N		0.48	49.03	1.6				0.017			201.18	0.1075	
DSH_{Mo}				0.818								0.0876	0.201
$DSH_{N\ x\ Mo}$				1.63				0.009			174.07		

**=significant difference in ANOVA, Ns=non significant difference in ANOVA; averages with same small letter are statistically the same with DSH_{NxMo} by interaction NxMo. Averages with the same capital letter are statistically the same with DSH_N for the N sources and with DS_{Mo} for the application of Mo*.

Conclusions

The yield components did not exhibit response to the addition of the different sources of N, with the exception of the seed size variable. The N, Fe and Mo content in mature upper leaves at the flowering stage, grain yield, seed size, % of N in straw and grain was positively affected by inoculation and the application of f nitrogen fertilizer; the BIO strain was shown to be more effective. The addition of Mo improved the N content in the straw and grain, as well as the accumulation levels of Mo in leaves.

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THE RESPONSE OF FIVE DRY BEANS MARKET CLASSES TO NITROGEN PLACEMENT AND TIMING

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The response of dry beans to nitrogen fertilizer has not been studied in Ontario in over 20 years, and yet it is an integral part of the cropping practices for Ontario growers. Growers typically use between 30-50 kg N/ha to increase yield and combat root rot problems. Over time, significant changes have occurred in the cultivars grown, crop management practices, nitrogen fertilizer products and the application equipment available. A three year study was initiated to update the response of six cultivars from five dry bean market classes to various nitrogen fertilizer rates, timing of application and placement methods. Nitrogen fertilizer was applied at five rates (0, 25, 50, 75 and 100 kg N/ha) preplant incorporated, two rates (25 and 50 kg N/ha) in a 5 x 5 cm band at planting, and two rates (25 and 50 kg N/ha) applied just prior to flowering and incorporated into the soil. White pea, cranberry and dark red kidney market classes were evaluated since they are the dominant market classes in Ontario. The last two dry bean market classes evaluated (Otebo and Kintoki), are a recent addition to Ontario's coloured bean acreage, and are grown under contract for export to Japan.

Nitrogen fertilizer applied at planting, either by preplant incorporation or banding beside the crop row, gave a significant yield increase in two of three years of the study. In most years, the yield increase would be too small to pay for the nitrogen fertilizer applied for all the market classes except for the specialty niche market classes, which typically command higher prices. Root rot disease pressure was low in each year of the study. Under moderate to severe root rot disease pressure, the yield response to nitrogen fertilizer should be sufficient to cover the fertilizer and application costs. The preflower nitrogen timing caused a significant yield decrease in 1998 and 1999, compared to any of the nitrogen treatments applied at planting. To maximize yield, this data suggests that nitrogen is needed early in the season to develop the plant 'factory' and not at flowering or pod filling.

Applied nitrogen did not impact plant maturity. This disagrees with other work, and conventional wisdom, which suggests that nitrogen application tends to delay maturity. Abnormally hot, dry harvest weather in 1998 and 1999 at the research location probably contributed to this finding by speeding the plants towards maturity at a quicker pace than normal.

The application of nitrogen resulted in a small increase in seed size. The differences in seed size were due to the amount of nitrogen fertilizer, not due to the nitrogen placement method or timing. The increases in seed size were not enough to provide a meaningful economic return to cranberry bean or kidney bean market classes; two of the market classes where growers receive premiums for larger seed size.

The seed protein content of three cultivars (OAC Gryphon, OAC Laser and Otebo) was significantly increased by applying nitrogen, while in the other three cultivars (Montcalm, Cran 34 and Kintoki) the response was less. The nitrogen placement method or the timing of nitrogen application did not impact seed protein content for any cultivar. The economic importance of increased seed protein content is unknown for most of the market classes tested. There appears to be a need to investigate niche markets for higher protein white pea beans, based on the results from this study. Preflower nitrogen application does not influence seed size, compared to nitrogen applied at planting. This may lead growers to rethink the timing of nitrogen application in specialty market classes such as Kintoki beans.

A complete copy of the report can be downloaded from the Ridgetown College website by going to *http://www.ridgetownc.on.ca/Research/Reports/Subject/ediblebeans.htm.*

PRELIMINARY TESTS WITH SUNFLOWER (*Helianthus annuus L.)* PRODUCT FOR WEED CONTROL IN BEANS

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Introduction

Weeds are among the problems facing farmers the world over. The importance of weeds is often underestimated. However, weeds can be sufficiently serious to cause crop losses in the order of 30 – 100% (Nieto *et al*., 1968; Phillips, 1991). In many Eastern and Southern African countries, the traditional method of weeding is hand hoeing. This method is laborious and time consuming (Gondwe, 1991). Chemical weed control can be effective. However, it has many limitations especially to the poor resource farmers. A search for alternative methods is considered important. Sunflower product which is known to control growth of various weed species (Mayorquīn *et al*, 2000) can offer such an alternative. The selectivity of the sunflower product on control of growth of food crops is not known. The objective of this study was to evaluate the effect of sunflower product on growth of bean and naturally occurring weed species.

Materials and Methods

The study was carried out in the greenhouse at the Agricultural Research Institute, Uyole, Mbeya, Tanzania. Sunflower product was prepared by crushing wastes of the receptacle in a mortar and pestle. The product was tested at rates of 7.5g and 10.0g per 50 and/or 100g of soil.

Three methods of applying the product were employed (a) Whole amount of product was placed on top of 100g of soil, (b) Whole amount of product was mixed thoroughly with 50g of soil and the mixture placed on top of 50g of soil, (c) The whole amount of product was completely mixed with 100g of soil.

The tests were done using 10cm diameter plastic pots. The soils used were collected from three different sources. (a) Natural forest – this was sterilized before use, (b) Field previously grown with maize, (c) Field previously grown with bean.

One hundred grams of soil from each source and 100g of sunflower product served as controls. There were 22 plot treatments. The experimental design was randomized block with three replications. The plastic pots containing the soil/sunflower product treatments, were placed in small deep plates which served as water reservoirs. Four seeds of the bean cv. Uyole ‘96 were planted in each pot. Bean seedlings emergence and weed seeds (natural) that germinated were recorded at 14, 21 and 28 days after planting (dap).

Results and Discussion

Results are summarized in Table 1. Growth of beans in sunflower product treatments was delayed and generally poor. Placing sunflower product on top of soil appeared to lead to poor growth of beans. Results also show that weeds were comparably lower in sunflower product treatments than in controls – indicating the potential of the product to control growth of weeds. Because sunflower is grown widely in Tanzania, investigations on utilization of its products would benefit the farmer. Further research on proper and acceptable rates and application methods are considered to be of great importance.

Table 1. Mean number of bean and naturally occurring weed seed emergence

Treat	Treatments	Mean No. of plants emerged				
		Beans			Weeds	
		14d.a.p.	21 d.a.p.	28 d.a.p	BL	GR
1.	10g sunflower product placed on top of 100g forest soil.	0.00f	0.66de	1.66cdef	0	0
2.	10g sunflower product mixed with top 50g forest soil	3.00a	3.33ab	3.33ab	0	0
3.	10g sunflower product mixed completely with 100g forest soil.	2.33abc	2.00abcde	3.66ab	0	0
4.	7.5g sunflower product placed on top of 100g forest soil	1.33cde	2.00abcde	2.33abcde	0.16*	0
5.	7.5g sunflower product mixed with top 50g forest soil	2.00abcd	3.33ab	3.33ab	0.33*	0
6.	7.5g sunflower product mixed completely with 100g forest soil	2.66ab	3.00abc	3.00abc	0.16*	0
7.	10g sunflower product placed on top of 100g ex maize field soil	1.00def	0.67de	1.66cdef	0	0
8.	10g sunflower product mixed with top 50g ex maize field soil	2.00abcd	1.00de	1.66cdef	0.08*	0
9.	10g sunflower product mixed completely with 100g ex maize field soil	1.33cdef	3.66a	3.66a	0.74	0
10.	7.5g sunflower product placed on top of 100g ex maize field soil	0.33ef	0.33e	2.33abcde	0	0
11.	7.5g sunflower product mixed with top 50 ex maize field soil	1.33cde	0.33e	0.33f	1.24	0
12.	7.5g sunflower product mixed completely with 100g ex maize field soil	2.33abc	1.66bcde	1.66cdef	0	0
13.	10g sunflower product placed on top of 100g ex bean field soil	0.33ef	1.00de	2.00bcde	0.33*	0
14.	10g sunflower product mixed with top 50g ex bean field soil	2.33abc	2.00abcde	2.00bcde	0.66	0.33
15.	10g sunflower product mixed completely with 100g ex bean field soil	1.66bcd	0.33e	1.33de	0	0.33
16.	7.5g sunflower product placed on top of 100g ex bean field soil	0.33ef	1.66bcde	1.00ef	0.16*	0
17.	7.5 sunflower product mixed with top 50g ex bean field soil	1.66bcd	1.00de	1.00ef	0.08	0.16
18.	7.5g sunflower product mixed completely with 100g ex bean field soil	1.00def	0.66de	0.33f	0.24	0.41
19.	100g forest soil (control)	1.67bcd	2.33abcd	2.33abcde	0	0
20.	100g ex maize field soil (control)	0.33ef	1.33cde	1.00ef	4.9	1.08
21.	100g ex bean field soil (control)	1.66bcd	2.00abcde	1.66cdef	1.41	0.33
22.	100g sunflower product (control)	0.00f	2.00abcde	2.66abcd	0	0

* = Sunflower; BL = Broad Leaved; GR = Grasses; Values in the same row with different letters differ significantly ($p<0.05$) as separately by Duncan's multiple range test

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SELECTION OF BEAN LINES TOLERANT TO LOW SOIL FERTILITY CONDITIONS IN AFRICA

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Introduction

Low soil fertility is the main factor that constrains bean crop yield to a great extent in most bean production areas of the Central, Eastern and Southern Africa. The major soil fertility related problem are low available N and P, and soil acidity.

Bean is generally grown by small scale farmers with very little input to improve soil productivity. Genetic approach to identify bean genotypes adapted to soils with inadequate nutrient supply and low pH associated nutritional disorders as a complement to soil management is considered to be a sound strategy for a better bean crop productivity.

During the last decade attempt to identify genotypes adapted to low soil fertility through a Pan-African effort by a working group called Bean Improvement for low Fertility Soils in Africa (BILFA) indicated considerable genetic variability in bean germplasm for adaptation to low soil fertility (Wortmann *et al*. 1995). However, most tolerant lines selected were not often of the regionally preferred seed types, hence focus in BILFA III was major market classes, particularly large seeded Andean types.

Materials and Methods

BILFA III, initiated in 1998 had originally 200 lines, which were evaluated for low fertility soil adaptation. These materials were evaluated at several sites, each with a specific stress:

- Low N: Namulonge/Uganda, Selian and Maruku/Tanzania, Bembeke/Malawi
- Low P: Rubona/ Rwanda, Kakamega/Kenya and Misafu/Zambia
- Low pH: Mulungu/Congo and Antsirabe/ Madagascar.

For two seasons, all entries were evaluated at moderate stress, when a well adapted control variety under stress performs at 40 to 50 % of its normal unstressed performance and the best top 50 percent lines are selected. The 50 selected lines are then evaluated further under moderate and no stress conditions for two or three seasons. Lines having performed well under limited N and P supply and acid soil conditions are considered tolerant.

Results and Discussion

Among 200 original set of materials evaluated at different locations under different stresses, several lines gave consistently higher yield compared to local checks and previously selected tolerant checks. Twenty-eight lines were found tolerant to low N and 29 tolerant to low P and acid soils conditions (Table 1). Large seeded materials with 100-seed weight of 40 g or better, are found in all tolerance groups, although most were in the low N tolerant group. Calima types seeds were also common.

Table 1: Low soil N, P and acidity tolerant lines selected from BILFA III nursery.

Low N			Low P			Low pH		
Line code	Seed Size	Seed color	Line code	Seed size	Seed color	Line code	Seed size	Seed color
A 286	17	Carioca	AFR 619	34	Red	37/66/6	23	Tan
AFR 675	24	Navy	AFR 675	24	Navy	A 286	17	Carioca
AFR 699	40	Red	AFR 708	44	Calima	A 344	27	Cream
AFR 714	23	Navy	AFR 714	23	Navy	AFR 708	44	Calima
AND 871	35	Calima	AND 871	35	Calima	AFR 714	23	Navy
CAL 143	50	Calima	ARA 4	21	Cream	ARA 4	21	Cream
CAL 150	50	Calima	CIM 9314-36	41	Calima	BRB 119	31	Calima
CIM 9314-33	42	Red	CIM 9314-37	34	Calima	DB 201/77/1	19	Navy
CIM 9314-36	41	Calima	CIM 9331-1	25	Red	CIM 9314-3	37	Calima
CIM 9315-1	24	Pink	CIM 9331-2	29	Pink	CIM 9331-1	31	Red
CIM 9315-3	27	Calima	CIM 9331-3	23	Red	CIM 9415	38	Calima
CIM 9318-4	27	Calima	DB 196	20	Navy	CNF 5520	44	Calima
CIM 9331-3	23	Red	DOR 663	17	Black	DFA 53	28	White
DB 196	20	Navy	FEB 192	19	Cream	FEB 197	22	Black
DOR 715	18	Red	FEB 196	20	Carioca	G 12489	44	Calima
FEB 192	19	Cream	G 2858	21	Tan	G 2910	21	Calima
FEB 196	20	Carioca	G 5889	15	Cream	G 3480	15	Black
G 5889	15	Cream	LSA 32	32	Carioca	G 5889	15	Cream
LSA 32	32	Carioca	MORE 92018	49	Tan	HM 21-7	45	Red
MORE 92018	40	Tan	PAN 150	24	Carioca	LRK 34	45	Pink
PAN 150	24	Carioca	RWR 1873	35	Calima	LSA 144	22	Red
PRELON	20	Navy	RWR 2075	44	Red	PAN 150	24	Carioca
RAB 482	17	Red	RWR 2091	37	Red	RAB 482	17	Red
REN 22	21	Navy	SDDT 49	20	Carioca	RWR 1742	22	Red
RWK 10	40	White speckled	SDDT 54-C5	31	Pink	RWR 1873	35	Calima
SDDT 55-C4	42	Calima	UBR(92)24/11	15	Navy	UBR(92)11	18	Carioca
UBR(92)25	18	Navy	VEF 88(40)L1PYT6	24	Red	VEF88(40)L1PYT6	24	Red
VEF88(4O)L1PYT6	24	Red	XAN 76	18	Calima	XAN 76	18	Cream
			ZAA 5/2	33	Cream	ZAA 5/2	33	Calima

Many lines showed multiple tolerance, having performed well under at least two stresses. These were: ARA 4, A 286, AFR 675, AFR 708, AFR 714, AND 871, CIM 9314-3, CIM 9314-36, CIM 9331-1, CIM 9331-3, DB 196, FEB 192, FEB 196, G 5889, LSA 32, PAN 150, RAB 482, RWR 1873, VEF 88(40)L1PYT6.

Conclusion

The results show the existence of tolerance to low soil fertility in market classes bean types. These materials need to promoted and disseminated for wider impact. The promising varieties identified have opened a new opportunity for higher bean productivity on acid soils and those with limited N and P supply especially for resource poor farmers with limited ability to apply other soil amendments. Some of tolerant varieties have performed well on-farm and have been released in some collaborating countries. Others are been included as parents in national and regional breeding programs.

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COMMON BEAN PRODUCTION ON TOP OF FIELD CROP RESIDUES IN NO TILL SYSTEM AND RESPONSE TO NITROGEN APPLICATION IN OXISOL OF BRAZILIAN SAVANNAH

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Research evidences have indicated economic bean responses to nitrogen top applications up to 130 kg/ha presenting grain yields over 3000 kg/ha (Barbosa Filho et al. 1996, Yokoyama et al. 1996). Nutritional factors may cause yield losses in irrigated beans under direct seeding especially related to nitrogen deficiencies.

Nitrogen fertilization must be better understood if studied in parallel with crop residue stored at the soil surface in no till system. Generally, a C/N imbalance are observed in any field researches cropped with dry bean (*Phaseolus vulgaris* L.). Nitrogen deficiencies are observed in these crops due the high increasing of carbon came from the dry matter production of previous crops (Oliveira et al. 1996)

Maize and sorghum are high residue producer crops similar to Brachiaria grass in single crop. Rice and soybean can be considered low residue producers (Figure 1).

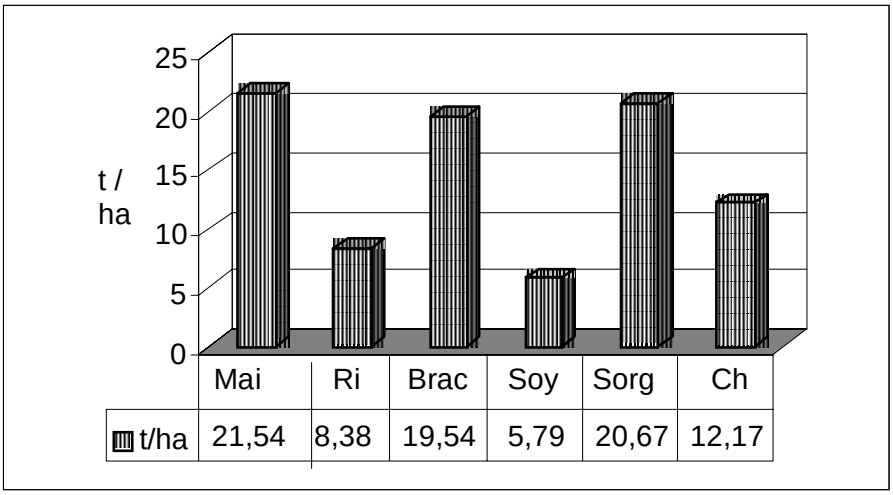

Figure 1. Dry weight of crop residues in no till system, at Fazenda Santa Fé, Municipality of Santa Helena de Goiás, Brazil.
(May = maize, Ri=rice, Brac = *Brachiaria brizantha*, Soybean, Sorg=sorghum and Ch=check).

In order to evaluate the common bean response to nitrogen doses when cultivated over plant residues. a field experiment was carried out using Pérola cultivar subjected to nitrogen top applications of 45, 90, 135, and 180 kg/ha. The oxisol used was a Oxisol classified as Purple Latosol of Santa Helena from Fazenda Santa Fé, Goiás State.

Table 1. Mean production of dry bean (kg/ha), Pérola cultivar, in no till system during winter season in response to increasing doses of nitrogen applied as ammonium sulfate at Fazenda Santa Fé, Municipality of Santa Helena, GO.

	CROP RESIDUE				
N. kg/ha	**Maize***	**Rice***	**Brachiaria**	**Soybean***	**Sorghum***
45	3.374	2.484	3.047	3.139	3.198
90	3.455	2.833	3.294	3.185	2.901
135	3.781	2.467	3.404	3.604	2.900
180	3.421	2.159	3.275	3.162	3.542
Mean	**3.508 a**	**2.486 b**	**3.255 a**	**3.273 a**	**3.135 ab****

Bean on crop + brachiaria residue. **The means in the line followed by the same letters are not significantly at P<0.05.

Results obtained did not indicate significant differences among doses of nitrogen and it was concluded that adequate soil management in addition to proper soil fertility as well as the maintenance of soil humidity provided by the soil protection due to the presence of plant residues favor the application of low nitrogen amounts. Significant differences were observed, however, in relation to differences in crop residues. The highest yield was observed when the dry bean was cultivated over residues from maize inter-cropped with Brachiaria (3508 kg/ha) followed by soybean (3273 kg/ha), Brachiaria (3255 kg/ha) and rice (2486 kg/ha) in single crops (Table 1).

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pH AND HYDROGEN + ALUMINUM CONTENTS IN OXISOL OF BRAZILIAN SAVANNAH CROPPED WITH DRY BEAN IN THE NO TILL SYSTEM

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The strip of soil pH that permits the best conditions for the development of the dry bean (*Phaseolus vulgaris L*) locates among 5,8 to 6,2 (soil :water 1:2,5). In this strip, almost all the nutrients are in the maximum availability to the plantas or in enough concentrations for the development and production for most of the more explored cultures in the Brazilian Center West Region. In lower pH, it has been observed low concentrations of phosphorus, calcium, magnesium and other nutrients. At the same time the presence of aluminum in the soil and other toxicant ions are observed in condition of low soil pH.

Most of the savanna soil is worldwide known by its low natural fertility. Those soils when not well amended or intensely cultivated present low exchangeable bases that are removed by the grains or parts of the plants. In response of calcium, magnesium and potassium withdrawing the soil pH is reduced resulting in low productivity due to the low availability of several essential nutrients to the plant.

Table 1. Bean productivity and parameter of production, Pérola cultivar, on crop residues in no till system at Fazenda Santa Fé, Municipality of Santa Helena de Goiás, Goiás State, Brazil.

Crop residue	**Plant/m^2**	**Pod/plant**	**Grain/pod**	**Weight of 100 grains**	kg/ha
Maize /Brac.	22,8 ab	12,3 ab	4,4 a	26,09 b	3.508 a
Rice	21,9 ab	12,0 ab	3,0 b	28,01 a	2.486 b
Brachiaria	25,3 a	11,98 ab	4,3 a	23,11 c	3.255 a
Soybean	22,2 ab	11,5 b	4,5 a	25,99 b	3.273 a
Sorghum	21,2 b	13,4 a	4,5 a	23,10 c	3.136 ab
CV %	25,26	17,42	24,40	11,44	21,02

The means in the column followed by the same letters are not significantly at P<0.05.

With the objective of knowing the effect of the high dry bean productivity in the soil acidity in no till system an experiment carried out using the Pérola cultivar, at Fazenda Santa Fé in Santa Helena of Goiás, State of Goiás during the years 1999 and 2000. In the first year, the area was occupied with maize + brachiaria, rice, soybean and sorghum and in the second year the dry bean was cultivated in these same areas where the residues of those different crops were stored. The dry bean production presented the following order: corn + braquiária (3 508 kg/ha) > soy (3 273 kg/ha)> brachiaria (3 225) > sorghum (3 136)> rice (2 486) (Table 1).

The high productions of dry bean obtained in the parcel where maize + brachiaria residues were storeded can be explained by the high production of vegetable residue of both crops besides the control that the brachiaria exercises on several dry bean diseases. Other good productions were obtained in the parcels where the single brachiaria was cultivated. Besides its well-known qualities it presents high mass productions without the competition between the crops. Although the soybean not be a high vegetable mass production, the dry bean produced well on parcel with soybean residue is due to the improvement in the soil fertility and nitrogen incorporation thought biological fixation.

The good performance the dry bean cultivated on sorghum residue is due to its high mass production besides the high capacity to recycle nutrients from the deepest layers for the soil surface.

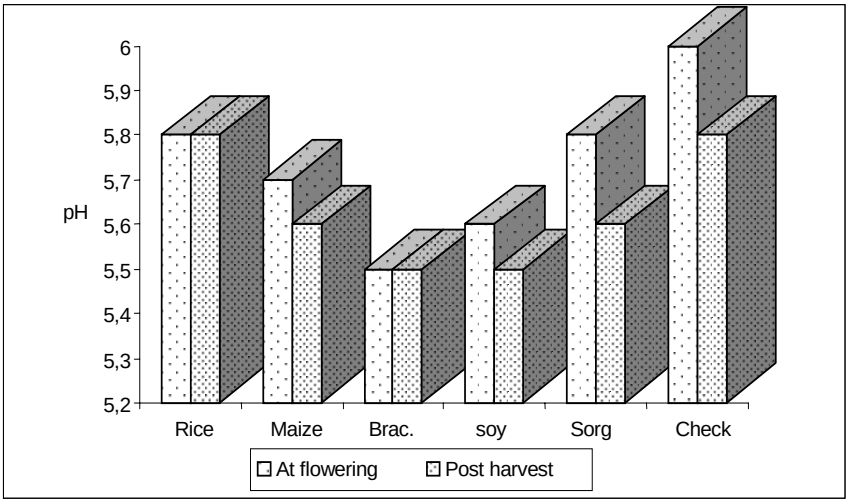

Figure 1. Variation of soil pH during the flowering and post harvest in areas cultivated with dry bean, Pérola cultivar, on residue of maize in consortium with *Brachiaria brizantha* and in residue of single crops of rice, soybean and sorghum. Fazenda Santa Fé, Santa Helena de Goiás, GO, 2000. (Brac=brachiaria and Sorg=sorghum)

The pH of the soil varied with the culture (Figure 1). The most exhausting cultures removed the highest amounts of exchangeable bases, contributing to the decreasing of soil pH or increasing of soil acidity. The lowest variation in soil pH was observed in areas where the rice and the braquiária were grown; the rice for demanding small amounts of nutrients in relation to the other crops and the braquiária for coming back to the soil high amounts of nutrients. The other crops studied removed the nutrient through the grains and parts of plant influencing the variation of soil acidity.

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YIELD OF BEANS IN RESPONSE TO CHEMICAL FOLIAR APPLICATIONS AT FLOWERING STAGE

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Introduction

Abortion of up to 85% of developing flower buds is a major contributor to low productivity in common bean. Since fertilization of flowers occurs within a few hours of pollination, even short-term environmental stresses at this critical time could cause reduced flower set and pod retention. Minerals, vitamins, and other nutritional constituents, which in vitro improved pollen germination and pollen tube growth, have not been investigated extensively under field environments (Weaver et al., 1985). These authors verified that sprays of calcium nitrate, boric acid, different sugars and other products at first flower opening stage altered pod retention and seed yield, but response varied with beans source. In Brazil, some farmers of sprinkler irrigated areas of beans cultivated during fall-winter are applying solutions of sugar or calcium, of different concentrations, during the flowering of beans. Increase in bean yields have been reported by some farmers. This study was undertaken to determine if foliar-applied chemicals could increase seed yield of beans, mainly under irrigated conditions.

Material and Methods

Three trials were installed in Coimbra, Minas Gerais State, Brazil, on 17 July 2000 (trial 1), 7 March 2001 (trial 2), and 24 July 2001 (trial 3). These trials were sprinkler irrigated. Another trial was installed in Viçosa, Minas Gerais, on 20 October 2001 (trial 4). This trial was carried out during the rainy season when temperatures are higher. The cultivar Pérola (carioca class) was used in Coimbra and the cultivar Ouro Negro (black), in Viçosa. Both cultivars are type III and were sown in rows 0.5 m apart with approximately 15 seeds per meter. Plants were sprayed (450 liters/ha) when 20% to 100% of plants had at least one open flower with the following chemicals: 1) control (tapwater); 2) calcium nitrate (300 mg/liter); 3) boric acid (100 mg/liter); 4) sucrose 1.0% (w/v); 5) combination of 2 and 3; 6) combination of 2 and 4; 7) combination of 3 and 4; and 8) combination of 2, 3 and 4. Each plot contained four rows 5 m long. A randomized complete-block design with seven replications was used. Fertilizer (8-28-16), at a rate of 350 kg/ha, was banded 4-5 cm deep at planting. Between twenty and twenty-eight days after emergence 100 kg/ha of urea were distributed along the bean lines. Weeds were controlled with hoe and with the postemergence herbicides fomesafen + fluazifop. A broad-spectrum fungicide was applied, preventively, three times. Insects were controlled when necessary.

Results and Discussion

Only in trial 1 there was significant difference among treatments (Table 1). Yield of treatment with spray of boric acid + sucrose (2,546 kg/ha) was superior to yield of treatments 1 (2,197 kg/ha), 2 (2,258 kg/ha), and 8 (2,257 kg/ha). Treatments 5, 6, and 7 also differ significantly from control. In this trial, there was a problem with the irrigation pump during parts of stages R6 (flowering) and R7 (pod development) of beans. Consequently, those plants suffered a period of water stress, which did not happened in trials 2 and 3. During trial 4, rains were more constant and intense than normal and temperatures lower than normal. This situation meant no significant stress for beans in trial 4. These results show that chemical foliar applications at flowering are not efficient when beans do not suffer stress.

Table 1. Mean seed yields of dry beans in four trials, Minas Gerais State, Brazil.

Foliar treatments[1]	Seed yield (kg/ha)			
	Trial 1	Trial 2	Trial 3	Trial 4
1. Control	2,197 c[2]	3,365	2,728	2,278
2. Calcium nitrate (300 mg/l)	2,307 abc	3,314	2,385	2,142
3. Boric acid (100 mg/l)	2,258 bc	3,553	2,622	2,313
4. Sucrose (1%)	2,437 abc	3,276	2,750	2,310
5. Calcium nitrate (300 mg/l) + Boric acid (100 mg/l)	2,481 ab	3,370	2,782	2,342
6. Calcium nitrate (300 mg/l) + Sucrose (1%)	2,481 ab	3,196	2,691	2,236
7. Boric acid (100 mg/l) + Sucrose (1%)	2,546 a	3,314	2,907	2,209
8. Calcium nitrate (300 mg/l) + Boric acid (100 mg/l) + Sucrose (1%)	2,257 bc	3,390	2,923	2,404

[1] All treatments included 0.05% Tween 20 surfactant.
[2] Means separation within columns by Duncan's multiple range test, 5% level.

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INFLUENCE OF SOIL DROUGHT ON GROWTH AND BIOMASS PARTITIONING IN YOUNG BEAN (*Phaseolus vulgaris* L.) PLANTS

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Introduction

Drought is a stress situation which frequently affects plant growth and productivity. Approximately 60% of bean production regions suffer serious drought conditions. The main way for reaching high and stable yields is breeding of drought tolerant bean genotypes. Breeders have developed tolerant bean genotypes better adapted to specific environmental stress conditions, although the nature of the acquired tolerance remains usually unknown. Drought tolerant genotypes combine different tolerance mechanisms, and in specific conditions the ones are leading. The determination of these leading mechanisms is of importance for breeding process.

The objective of the present work was to study the effect of soil drought on growth and biomass partitioning in bean plants and select genotypes differing in degree of RGR inhibition.

Material and Methods

Seeds of five bean genotypes (Plovdiv 10, Plovdiv 11M, Dobrudjanski 7, A 195 and BAT 477) were sown into plastic pots with 1.5 kg of sandy soil. Plants were grown in a phytostatic chamber with a day/night air temperature of 24/18 ºC, 14 h photoperiod and an average photosynthetic photon flux of 125 μmol m^{-2} s^{-1}. A pot with a plant constituted one replication. The plants were watered twice a week with a modified Hoagland solution and the remaining days with distilled water, so as to maintain the soil to field capacity. 14 days after the emergence plants were divided into two groups - control and droughted. The second group was subjected to a 10-day drought by withholding watering until a soil moisture of 30 % field capacity was reached. By the end of treatment, this soil moisture was maintained. Control plants were grown at field capacity. At the end of the drought period, the plant dry weight and leaf area were estimated. Based on these values the plant growth analysis parameters and the parameters describing biomass partitioning were determined (Beadle, 1993).

Results and Discussion

The results in Table 1 show that the growth in all five bean genotypes, subjected to drought, were inhibited. The main parameter of plant growth analysis-relative growth rate (RGR), was reduced to the greatest extent in plants of A 195 and BAT 477. In Plovdiv 11M, the reduction was less. Our results corroborated the studies of Van den Boogard *et al*. (1997) and White *et al*. (1990) who established a significant decrease in RGR in wheat and bean plants when grown under water stress conditions. RGR is determined by two parameters - "physiological" - net assimilation rate (NAR) and "morphological" - leaf area ratio (LAR). NAR was inhibited to the greatest extent in A 195 and BAT 477 showing that these changes were most important in growth inhibition. In Plovdiv 11M and Plovdiv 10 the decrease of NAR was insignificant and it was LAR that played a leading role in the growth inhibition. NAR is determined by net photosynthetic rate, dark respiration intensity and the relative ratio of non-photosynthetic organs - roots. According to Van den Boogard *et al*. (1997) biomass accumulation in roots is an advantage for plants grown under water-stressed conditions, increasing their water uptake capacity. The costs of a larger root system are the costs of construction (possibly at the expense of construction of photosynthetic tissue) and the increased respiratory losses associated with their maintenance. In all genotypes tested, RWR changed insignificantly. Hence, the NAR inhibition was mainly due to the reduced photosynthesis and/or the increased dark respiration

intensity. LAR is determined by two components - leaf weight ratio (LWR) and specific leaf area (SLA). In all cultivars studied, SLA decreased to a greater extent as compared to LWR. This was associated with the more significant decrease in leaf area as compared to that in LWR. An insignificant increase of SLA was shown only in A 195. Plant dry weight in BAT 477 and A 195 decreased to a greater extent - 29.9% and 25.2%, respectively.

Table 1. Mean relative growth rate (RGR [g g^{-1} d^{-1}]) and net assimilation rate (NAR [mg cm^{-2} d^{-1}]) over the period of soil drought and parameters describing biomass partitioning after ten days of soil drought of five bean genotypes. DW-plant dry weight [g]; LAR-leaf area ratio [cm^2 g^{-1}]; RWR-root weight ratio [g g^{-1}]; SWR-steam weight ratio [g g^{-1}]; LWR-leaf weight ratio [g g^{-1}]; SLA-specific leaf area [cm^2 g^{-1}]. Values are the means SE of five replicates. *, **, ***, indicate significant difference at P<0.05, P<0.01, P<0.001, respectively, between control and soil drought for each cultivar.

	Plovdiv 11M	Plovdiv 10	Dobrudjanski 7	A 195	BAT 477
			control		
RGR	0.0983±0.004	0.1161±0.006	0.1213±0.006	0.0932±0.004	0.1177±0.008
NAR	0.212±0.011	0.351±0.016	0.341±0.017	0.282±0.014	0.344±0.016
DW	0.8444±0.032	1.1884±0.085	1.1109±0.092	1.4456±0.106	0.8321±0.071
LAR	287.7±12.3	301.7±14.2	307.9±15.6	283.7±12.9	315.7±16.3
RWR	0.243±0.012	0.251±0.014	0.243±0.013	0.262±0.015	0.230±0.014
SWR	0.244±0.012	0.214±0.015	0.236±0.014	0.217±0.013	0.209±0.012
LWR	0.513±0.026	0.535±0.027	0.521±0.025	0.521±0.026	0.561±0.029
SLA	560.8±21.3	563.8±22.4	590.9±24.1	544.6±22.9	562.8±23.4
			soil drought		
RGR	0.0796±0.002 **	0.0861±0.004 **	0.0863±0.005**	0.0570±0.002***	0.0740±0.003**
NAR	0.195±0.009	0.303±0.012	0.265±0.015*	0.175±0.008**	0.239±0.012**
DW	0.7667±0.029	0.9342±0.074	0.8390±0.069	1.0825±0.086*	0.5865±0.030*
LAR	218.1±11.4 **	206.5±11.2 **	255.2±21.7	261.3±20.9	279.9±15.6
RWR	0.240±0.012	0.262±0.014	0.249±0.012	0.281±0.015	0.231±0.014
SWR	0.277±0.013	0.252±0.014	0.253±0.014	0.224±0.011	0.250±0.014
LWR	0.483±0.022	0.486±0.019	0.498±0.021	0.495±0.024	0.519±0.020
SLA	473.5±17.8 *	404.3±20.7 **	502.5±21.6 *	548.2±23.9	524.8±23.1

On the basis of our results, we could suggest that bean genotypes Plovdiv 11M and A 195, which differ in degree of RGR inhibition and biomass partitioning, are suitable for further model investigations.

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SEED YIELD AND BIOMASS WERE ASSOCIATED WITH EVAPOTRANSPIRATION AND GROWTH DEGREE DAYS IN *Phaseolus vulgaris* L.

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Introduction

In Mexico, the common bean (*Phaseolus vulgaris* L.) is a crop of major importance for human consumption. The cultivar Michoacan 12A-3 was collected and cultivated in Central Mexico for several years.

The growth of a crop depends on diverse environmental factors; i.e., solar radiation, rainfall, temperature, and evapotranspiration (Etc) (Dirks and Bolton, 1981). Several studies have found a positive relationship between Etc and yield in some crops such as corn, wheat and bean (Liang *et al.*, 1991; Musik *et al.*, 1994; Escalante *et al.*, 2001). Growth degree days (GDD) calculated from temperature have also been found to correlate positively with the yield (Muchow *et al.*, 1990; Escalante *et al.*, 2001). The objective of the present study was to determine the relationship of Etc, GDD, and rainfall to biomass production and seed yield in a temperate region of Central Mexico, where cv. Michoacan 12A-3 was cultivated for several years.

Materials and Methods

Data was collected on biomass production and seed yield of *P. vulgaris* cv. Michoacan 12A-3 from several sowings carried out in Chapingo, Central Mexico (98º54' N, 19º48' W, 2250 above sea level, with a temperate climate). Ten sowings were carried out in Chapingo from 1973 to 1993 in the spring-summer cycles under rainfed conditions. Meteorological data (temperature, precipitation, and Etc) were collected, corresponding to the sowing date and the physiological maturity of each year.

Results and Discussion

The ten sowings had different plant densities and diverse fertilization levels, and little relationship was found association between biomass production and seed yield and Etc, precipitation and GDD (r ranged from 0.06 to 0.32). However, when only some years were considered, the relationship increased as shown in Figure 1 and Table 1. The association among Etc with biomass production (5 years) and seed yield (7 years) was significant at $p \leq 0.01$ in both cases. Similar results have been found in bean in hot and temperate weather (Escalante *et al.*, 2001).

Table 1. Correlations between meteorological and yield parameters.

Parameter	Biomass	Seed yield
	r[†]	r[‡]
Evapotranspiration	0.87	0.88
Precipitation	0.32	-0.12
GDD	0.75	0.68

[†](1973, 1974, 1977, 1978 and 1980)

[‡](1973, 1974, 1977, 1978, 1980, 1985 and 1986)

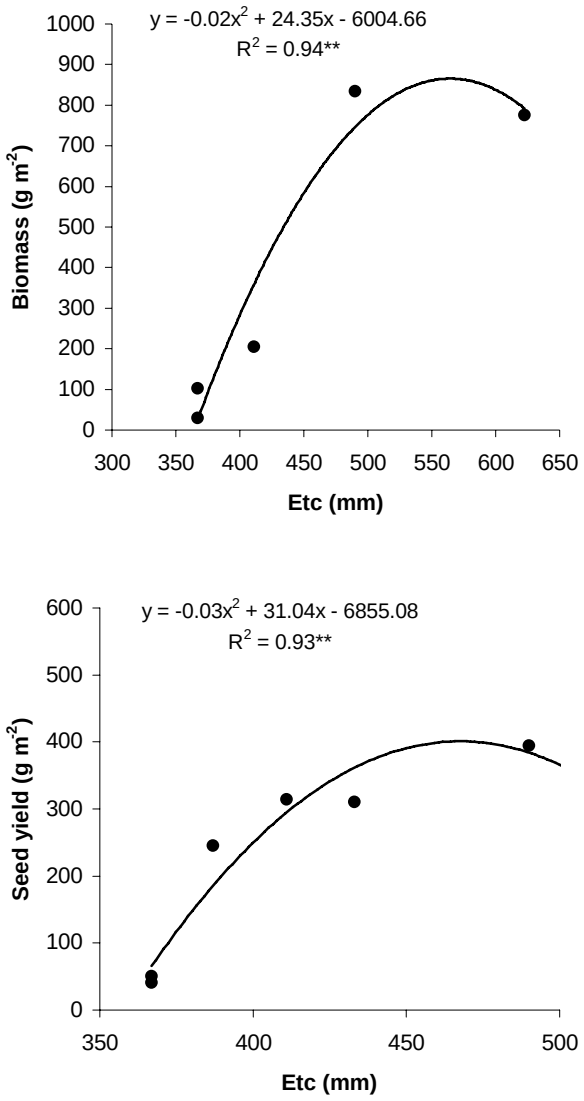

Figure 1. Relationship of biomass and seed yield with evapotranspiration (Etc) (mm).** level of significance p≤0.01.

There was no association of biomass and seed yield with precipitation; this may be due to auxiliary irrigation applied during the first growth stages. GDD was significant (p≤0.05) for biomass production (r^2=0.76), but not for seed yield (r^2=0.42).

The most important parameter associated with yield was Etc for the cv. Michoacan in the different sowings of Chapingo.

Conclusion

There was a high relationship between Etc and biomass (5 years) and agronomic yield (7 years). GDD showed a significant association only for biomass.

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INHERITANCE OF DROUGHT RESISTANCE TRAITS IN DRYBEAN (*Phaseolus vulgaris* L.): RWC AND LWP

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Introduction

Dry beans in Kenya are predominantly produced under rainfed conditions in the semi-arid areas that cover over 60% of the total area that receive low erratic and unpredictable rainfall such that they are prone to early season, mid-season and end-season drought episodes. Earliness has been used to avoid end season drought (Ronno and Shakoor, 1990; Muigai and Ronno, 1991). For an optimum bean production system in these areas, breeding and selection of drought resistant genotypes and development of cultural practices to manage the crop to suit the environment is essential. New varieties could be developed through identification of traits related to underlying mechanisms of adaptation to water deficit (White *et al.,* 1994); and incorporation of the traits into dry bean varieties having desirable agronomic characters.

Evidence for genetic variation in various components of dehydration avoidance is ample in dry beans but studies of mode of inheritance of drought predictive characters are limited.

This study investigated the inheritance of two indicators of drought resistance namely, relative water content (RWC) and leaf water potential (LWP) using known sources.

Materials and Methods

The study was conducted at Kiboko, a semi-arid site during the off season of 1997 (August – October) to allow for controlled water application with little interference from the rains. The site is located at 975m above sea level and coordinates of 2^{o} 12′ S and 37^{o} 43′ E .

The materials for the study were obtained from crosses involving the following selected bean genotypes. The RWC was studied using high value parent P4 (Ex-Embu) and low value parents P10 (Ex-Nyeri) and P28 (Ex-Kisii). Two crosses P4 x P10 and P4 x P28 were generated. LWP was studied using high value parents P2 (Ex-Kitui) and P4 (Ex-Embu) and low value parent P20 (Ex-Nyeri). Two crosses P2 x P20 and P4 x P20 were generated.

For each cross, the parental genotypes, F_1, F_2 and backcrosses to either of the parents were grown in the field in randomised complete block design with three replications. Seeds were sown 45 cm x 15 cm in each plot. The experimental plots were watered to field capacity immediately after planting and only upto emergence to facilitate uniform germination

Estimates of midday RWC were made between 1200 and 1400 h at 22 and 36 days after emergence (DAE) using methods described by Turner (1980). Ten leaf discs, 7 mm in diameter were punched out from recently most expanded leaf on each of the three randomly selected plants in each plot. Measurements were recorded in %RWC. LWP was determined in each plot from fully expanded young trifoliate leaves using a portable pressure chamber (model PMS Instrument Co.; Oregon, USA) 22 and 36 DAE. Three samples from different plants were measured per plot. The readings were recorded in Mpa units.

The generation mean analyses using six parameters were conducted for each cross following the model of Hayman (1958) and Mather and Jinks (1977). Estimates of the genetic effects were calculated for each cross by least squares from matrices. An F-test was used to determine significance of variation attributable to a specific gene effect. The model was corrected for the mean (m) effect and replication x generation mean square was used as an error term.

Percent genetic variability for each gene effect was calculated by using its mean square value and the total generation mean square, corrected for the mean (m) effect. By doing so, the magnitude of the different genetic effects within and among the crosses and traits measured was easier to compare. All statistical analyses were carried out using PROC GLM SAS routines (SAS, 1988).

Results and Discussion

The percentages of total genetic variability for RWC and LWP are presented in Figure 1 and 2 respectively. Generation mean analyses showed that RWC was predominantly influenced by additive (d) and additive x dominance (j) genetic effects at pre-flowering (22DAE) and at pod development (36DAE). The presence of significant digenic interactions implies that selection procedures that exploit epistatic genetic effects may be used to improve RWC. Delayed selection beyond F_3 generation and then use of bulk-pedigree breeding method is suggested. In comparison, LWP was predominantly controlled by highly significant additive (d) genetic effects both at pre-flowering and at pod development in both crosses. These results suggest the use of pedigree and backcross breeding methods for improvement of LWP in beans.

Figure 1. Percent genetic variability due to the gene effects in each cross for RWC at 22 DAE and 36 DAE at Kiboko

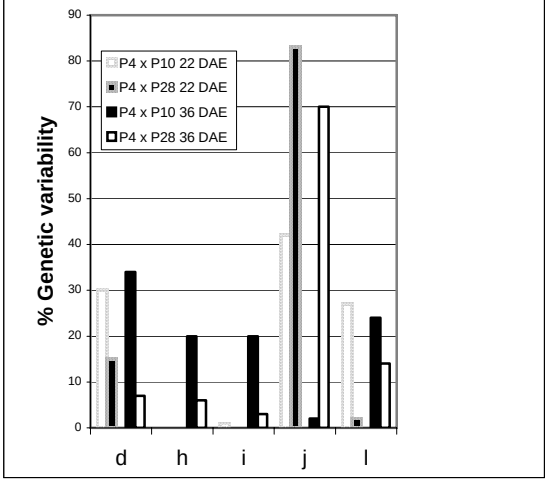
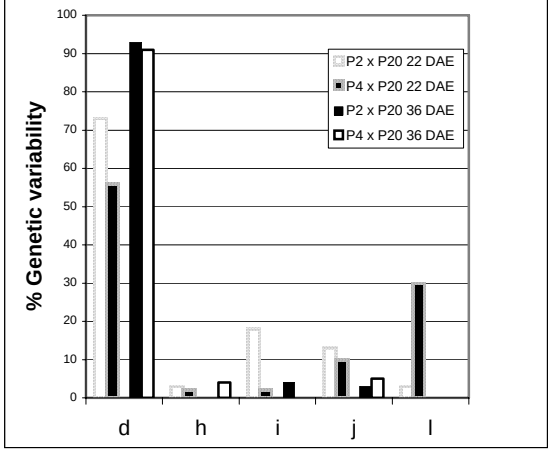


Figure 2. Percent genetic variability due to the gene effects in each cross for LWP at 22 DAE and at 36 DAE at Kiboko.



Where, d = additive, j = additive x dominance interaction, h = dominance, l = dominance x dominance interaction, i = additive x additive interaction effects

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INHERITANCE OF DROUGHT RESISTANCE TRAITS IN DRYBEAN (*Phaseolus vulgaris* L.): TAPROOT LENGTH AND ROOT DRY WEIGHT

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Introduction

Availability of drought resistant varieties and development of appropriate cultural practices are a prerequisite to an optimum bean production system for semi-arid areas. Progress in developing drought resistant varieties could be enhanced through identification and incorporation of traits related to mechanisms of adaptation to water deficits.

An increase in taproot length and root weight may indicate a greater density of roots or a greater depth of the roots. Greater root proliferation would also allow a greater soil volume exploration (Runkulatile *et al*., 1993). Both are important morphological adaptations to water deficits in dry beans that enable a greater degree of extraction of soil water (White and Izquierdo, 1991). Significant variability exists for root traits in dry bean germplasm under water deficit (Ronno, 1999). Varieties identified include BAT 477 CIAT, (1985), Ulonzo, White Haricot, and GLP1004 (Runkulatile *et al.*, 1993). However, breeding for these traits in dry bean is limited. Genetic understanding and incorporation of superior root traits in addition to other mechanisms of drought tolerance such as earliness in commercial dry bean varieties may enhance and stabilise yields.

This study attempted to elucidate the mode of inheritance of taproot length (TRL) and root dry weight (RDW) under field water deficit.

Materials and Methods

The study was conducted at Kiboko in Kenya during off-season of 1997 to allow for controlled water application with little interference from the rains. The site is located at 975m above sea level and co-ordinates of 2^{o} 12′ S and 37^{o} 43′ E.

The study involved use of crosses between genotypes earlier identified as part of this study under field water deficit. Two high value parents P2 (Ex-Kitui) and P8 (ex-Kirinyaga) and a low value parent P23 (Ex-Meru) were used to generate two crosses P2 x P23 and P8 x P23. In each cross, the parental genotypes, F_1, F_2 and backcrosses to either of the parents were grown in troughs in randomised complete block design with three replications. Each trough comprised a replicate. Seeds were sown 30 cm x 15 cm in each plot.

The troughs were constructed using baked bricks filled with field soil. Each trough measured 10.8m x 3.5m of 1.2m high. Each trough was irrigated to field capacity prior to planting. The amount of water required was determined following procedures developed by Doorenbos and Pruitt (1977). No additional irrigation water was applied after emergence.

Generation mean analyses was conducted by fitting six parameters (m, d, h, I, j and l) following the models of Hayman (1958) and Mather and Jinks (1977). Estimates of the genetic effects were calculated for each cross by least squares from matrices. An F-test was used to determine significance of variation attributable to a specific gene effect. The model was corrected for the mean (m) effect and replication x generation mean square was used as an error term.

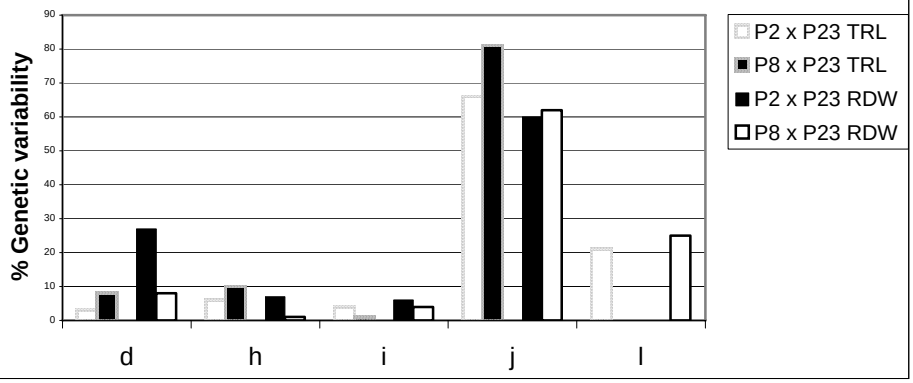
Percent genetic variability (PGV) for each gene effect was calculated by using its mean square value and the total generation mean square, corrected for the mean (m) effect. By doing so, the magnitude of the different genetic effects within and among the crosses and traits measured was easier to compare. All statistical analyses were carried out using PROC GLM SAS routines (SAS, 1988).

Results and Discussion

Figure 1 shows results of PGV due to gene effects for root growth.

Generation mean analyses showed that taproot length (TRL) and (RDW) were predominantly controlled by additive x dominance (j) genetic effects in both crosses and traits at pod development (36 DAE). The presence of significant digenic interactions implies that selection procedures that exploit epistatic genetic effects may be used to improve the root growth traits. Delayed selection beyond F_3 generation and then use of bulk-pedigree breeding method is suggested for improving root traits in a dry bean breeding programme for adaptation to water deficit areas of Kenya.

Figure 1. Percent genetic variability due to the gene effects in each cross for TRL and RDW at pod delopment (36 DAE) in Kiboko.



Where, d = additive, h = dominance, i = additive x additive interactions j = additive x dominance interactions, l = dominance x dominance interaction effects

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YIELD AND PHENOLOGICAL ADJUSTMENT IN FOUR DROUGHT-STRESSED COMMON BEAN CULTIVARS

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Introduction

Common bean (*Phaseolus vulgaris* L.) is an important rainfed crop in Mexico. In this country, drought is one of the most limiting factors in common bean. Drought, defined as a plant and/or soil moisture deficiency that affects crop growth and development might be intermittent (when it affects different developmental stages and its effect varies on intensity, incidence opportunity, duration, rhythm of establishment and plant preconditioning) in the highlands, or terminal, observed in the tropical lowlands mainly affecting the crop at the end of the growing cycle when soil moisture is gradually receding. The objective of this trial was to assess the role of phenological adjustment and biomass accumulation on the seed yield of four bean varieties grown under intermittent and terminal drought.

Material and Methods

An experiment was established at two locations in México: Cotaxtla, Veracruz in the lowlands, and Texcoco, State of Mexico in the highlands. Four varieties were tested, two type I growth habit: G4523 (drought resistant) and Rayado Rojo (drought susceptible) from the Nueva Granada race; another two of prostrate type III growth habit, Pinto Villa (drought resistant) and Bayo Madero (drought susceptible) from the Durango race. A randomized complete block design with an split plot arrangement and three replications was used at both locations.

Terminal drought (TD) was studied in Cotaxtla, which consisted on the suspension of irrigation at 38 days after planting (DAP). In Texcoco, the experiment was planted under a rainshelter and the following treatments were studied: 1) intermittent drought (ID), which included two drying cycles before physiological maturity was reached. This treatment consisted of the suspension of irrigation at the first compound leaf stage (18 DAP) and rewatering at the plant wilting condition (50 DAP). Afterwards it was left to mature without irrigation. 2) terminal drought, in which the treatment was similar to that of Cotaxtla. In both locations an irrigated (I) treatment was included as control. The experimental plot consisted of two 5 m rows in Cotaxtla; and two 4 m rows in Texcoco, 60 cm apart (18 plants m^{-2}).

In Texcoco, four destructive aboveground biomass samples were done: before starting the intermittent drought treatment, at flowering, after irrigation and at physiological maturity (17, 45, 61 and 102 DAP, respectively). In Cotaxtla three samplings were carried out: at flowering, seed filling and physiological maturity (43, 64 and 85 DAP, respectively). At each sampling date, three plants were taken per plot. Individual plants were dissected by phytomer and its components (stem, leaves, petioles, pods and seeds). Seed yield, days to flowering (DF) and to physiological maturity (DPM) were recorded. The data from each location were analyzed separately.

Results and Discussion

In both locations highly significant differences ($P<0.01$) were observed among moisture conditions and cultivars for all the evaluated traits. Drought intensity index (Fischer and Maurer, 1978) varied from 0.37 (terminal drought), in Cotaxtla, to 0.49 (terminal drought) and 0.63 (intermittent drought) in Texcoco. Pinto Villa showed the highest seed yield in all moisture conditions at both locations (Table 1). Significant modifications in number of days to physiological maturity (DPM) were observed under drought, mainly in the high yielding cultivars G4523 (type I) and Pinto Villa (type III). The latter showed the largest reduction for DPM from 93 under irrigation (I) to 80 in terminal drought and 77 under intermittent drought (Figure 1). This phenological adjustment, in Texcoco, combined with a high daily average of seed yield per plant per

day (g plant^{-1} day^{-1}) of 0.50 under I, 0.34 in TD and 0.30 for ID contributed to an overall high seed yield. A similar trend was observed for G4523. The response of the four varieties under irrigation and terminal drought was similar at the two locations.

In Texcoco, the susceptible cultivar, Bayo Madero (type III), exhibited a significant reduction in the harvest index (HI) from 0.34 under I to 0.29 under ID, while in Rayado Rojo (type I) the reduction was from 0.33 under I to 0.26 under ID. These results were related to the larger pod and seed number reduction observed in susceptible cultivars. Similar results were obtained at Cotaxtla**.**

Biomass production was related to seed yield across moisture conditions with R^2 values of 0.99** in Texcoco, and 0.93** in Cotaxtla. Earliness to flowering and to PM showed negative and significant relationship with seed yield. Cultivars with phenological adjustment, of the period from flowering to maturity, showed high yield. In both locations biomass partitioning was similar in all cultivars despite growth habit differences. Across moisture treatments larger values for plant biomass accumulation, pods and seed number were registered at the six basal phytomers. Pinto Villa showed the lowest variation in individual seed weight among phytomers.

The above results suggest that earliness to flowering and the adjustment in days to maturity, are important traits in the adaptation of common bean under limited moisture environments. All the evaluated varieties showed basal dominance in biomass accumulation, pod set, seed number and individual seed weight. High aerial biomass production and a high rate of biomass partitioning to pods and seeds, in tolerant cultivars, allowed the stabilization of yield across moisture conditions.

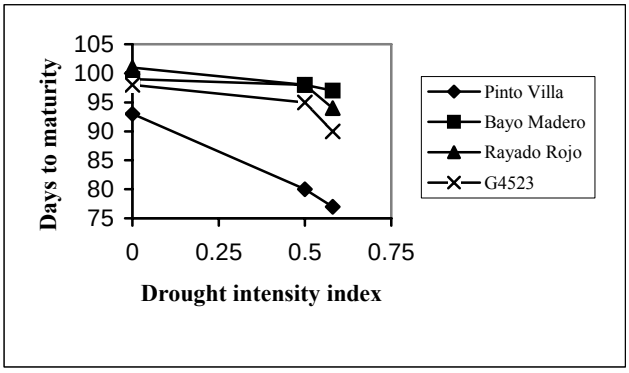

Figure 1. Days to physiological maturity in four common bean cultivars under drought intensity index of 0 (irrigated), 0.5 (terminal drought) and 0.58 (intermittent drought). Texcoco, State of Mexico. 2001.

Tab

different moisture conditions[1], at two locations in Mexico. 2001.

Cultivar	Growth Habit	Cotaxtla, Veracruz.			Texcoco, State of México.			
		DF	TD Yield	I Yield	DF	TD Yield[1]	ID Yield	I Yield
G4523	I[2]	37	14	20	44	16	15	28
Rayado Rojo	I	39	8	17	48	10	8	33
Pinto Villa	III	32	17	21	40	26	23	47
Bayo Madero	III	35	9	18	42	21	15	35
Mean		36	12	19	44	18	15	36

[1] TD = terminal drought, ID = intermittent drought and I= irrigated (control); [2] I = determinate bush and III=Indeterminate prostrate.

Reference

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GROWTH RESPONSE OF BEAN PHYTOMERS TO WATER STRESS

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Plants might be considered as formed by phytomers of different developmental stages. A phytomer consists of one internode, the leaf in its upper node and the bud subtended by the leaf (*sensu* Aitken, 1974; Bell and Bryan, 1993).

Plant water stress due to soil water deficit diminishes plant growth. However, the reduction has not been compared in different phytomers of the same plant. The present work was based on the hypothesis that a phytomer will show a differential response to a stress according to its developmental stage. The objective was to determine in three early vegetative developmental stages of the bean plant, the effect of water stress on the growth of the individual phytomers and its parts (internode, petiole, leaf lamina).

Materials and Methods

Seeds of *Phaseolus vulgaris* L. type III cvs Pinto Villa (PV, tolerant to water stress) and Bayo Madero (BM, susceptible) were sown one per plastic pot with 2.250 kg of sandy soil. One plant per pot represented the experimental unit. Plants were grown in a controlled-environment cabinet, with 12 h light/25 °C and 12 h dark/20 °C with a photosynthetic photon flux density averaged 670 μmol m^{-2} s^{-1} (photosynthetically active radiation). The experiment was a completely randomized design with five replications. A treatment consisted of the combination of the two cultivars, two water regimes (unstressed, water stressed) and three developmental stages. The plant water stress was induced by withholding water at each developmental stage: 1) unfolded simple leaf (V2), 2) unfolded first compound leaf (V3) and 3) unfolded third compound leaf (V4). These leaves were designated reference leaves. Unstressed controls were provided in which the soil was maintained close to field capacity. The leaves (especially the reference ones) were observed daily as to the occurrence and progress of the wilting condition.

A destructive sampling was performed three days after a reference leaf reached the permanent wilting condition. A leaf in such condition displays bent borders and does not rehydrate during the pre-dawn period. Laminar area and dry weight (dried at 80 °C, 72 hrs) of parts of individual leaves, and of internodes, were recorded and compared to those of the control.

Results and Discussion

The symptoms of the permanent wilting condition of individual leaves of a plant aroused acropetally. For example, in V4, these symptoms appeared first in the simple leaf, and progressed upwards as the duration (days, d) of the suspension of watering increased. In the three stages both bean cultivars had fewer number (30 to 50 %) of phytomers under stress than their controls (Fig. 1) and also fewer offshoots.

Water stress also diminished the dry weight of the phytomers and their parts (internode, petiole, leaf lamina) with respect to unstressed of comparable parts. In the three developmental stages the inhibition was higher in the younger phytomers, (especially in V3) and in BM than in PV. The values for most of the variables in unstressed plants were slightly higher in BM than in PV.

The stress inhibited the leaf area of the phytomers (Fig. 1) in a similar fashion as the dry weight. However the inhibition of dry weight was higher than that of the area.

Conclusion

Water stress reduced differentially: the dry matter of phytomers, of their parts and offshoots as well as the leaf area, depending upon the ontogenetic age (position along the stem) and the plant

developmental stage, as shown for the reference leaves. Even thought the water stress was induced at the same developmental stage (of the reference when the leaf just unfolded), their response differed according to their position along the stem (V2, V3, V4) The dry matter and leaf area reduction of the shoot depends upon: a) the inhibition of differentiation of new phythomers (from apical buds) and of offshoots (from auxiliary buds) and b) the differential inhibition of the dry weight and leaf area of homologous phytomers of the main axis. Differences were found between cultivars under irrigation and water stress. PV was less affected by the stress than BM.

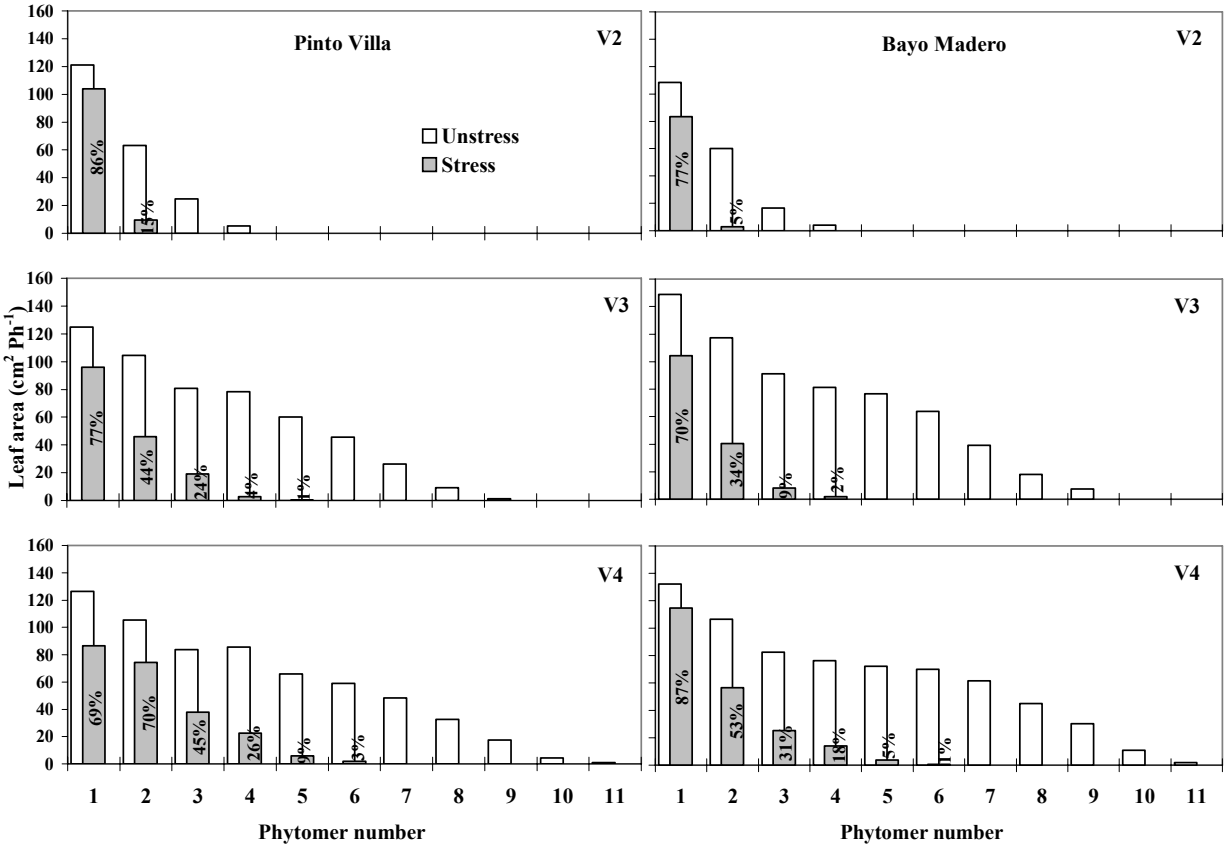

Figure 1. Average area of a leaf lamina of each phytomer (Ph) of Pinto Villa & Bayo Madero bean at three developmental stages (V2, simple leaf; V3, first compound leaf; V4 third compound leaf) of water-stressed and unstressed plants. The values are an average of five replications. The numbers in bars represent the percentage of the stressed compared to the unstressed control. Leaf area for phytomer 1 corresponds to the sum of the two simple leaves.

Acknowledgements

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LEAF GAS EXCHANGE AND WATER RELATIONS IN YOUNG BEAN (*Phaseolus vulgaris* L.) PLANTS UNDER DROUGHT CONDITIONS

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Introduction

Water stress affects negatively the functional status of plant organism. Most affected are the water exchange, growth and development, uptake and assimilation of mineral nutrients and the photosynthetic carbon assimilation. Special attention is paid to the investigations of the plant water relations, the functional activity of the photosynthetic apparatus, the changes in the activities of main enzyme systems under drought. Ones of the promising methods for assessing drought tolerance of plants are the growth analysis and monitoring of changes in leaf gas exchange and water relation.

The objective of the present study was to establish the effect of soil drought on leaf gas exchange and water relations in two bean genotypes, which were previously selected for their different degrees of inhibition of the main growth analysis parameter - relative growth rate (RGR) - Plovdiv 11M and A 195.

Material and Methods

The plants of two bean genotypes - Plovdiv 11M and A 195, were grown into plastic pots with 1.5 kg of sandy soil. 14 days after the emergence plants were divided into two groups - control and droughted. The second group was subjected to a 10-day drought by withholding watering. The conditions of growing and the drought treatment have been previously described (Berova and Zlatev, 2002). The leaf gas exchange parameters - net photosynthetic rate (A), transpiration rate (E) and stomatal conductance (gs) were determined at the end of the drought period on the petiole of the central leaflet of the first compound leaf. The analyses were conducted with a portable photosynthetic system LCA-4 (ADC, Hoddesdon, England) under the following conditions - light intensity (PAR) - 750 μmol m^{-2} s^{-1}, CO_2 concentration - 350 μmol mol^{-1} and temperature of 26 °C. Relative water content (RWC) was determined according to Morgan (1986). Leaf water potential was determined using a pressure chamber (Turner, 1988). Transpiration per unit plant weight (T) was calculated as the ratio of water use on the last day before sampling and plant dry weight. Plant water use efficiency (WUE) was calculated as the ratio of biomass increase and water use over the drought period.

Results and Discussion

The results in Table 1 show that after ten-day drought period, the leaf gas exchange rate in the plants of both genotypes was significantly reduced. In cv. Plovdiv 11M, E and gs were reduced to a greater extant than A, while in cv. A 195, A was suppressed more than E and gs. The photosynthetic water use efficiency, expressed as the A/E ratio, increased significantly in cv. Plovdiv 11M, while in cv. A 195 it decreased insignificantly. Stomatal closure is a well-known plant response to water stress, restricting water losses. Here, the photosynthetic rate is undoubtedly reduced. By the end of the drought period, the plants of cv. Plovdiv 11M restricted their transpiration to a greater extent than did the plants of cv. A 195. Plants differ in the stomatal role in maintaining the functional activity of photosynthetic apparatus during periods of drought (Chaves, 1991). In certain plants, the stomatal control is of dominant importance and these plants are characterised by increased water use efficiency. In others, that keep their stomata relatively open,

due to either being able to compensate for water losses or to a loss of stomatal control, the water use efficiency could remain unchanged or insignificantly reduced. Our studies related the young bean plants of cv. Plovdiv 11M to the first group, and those of cv. A 195 - to the second.

By the end of the drought period, the changes in the relative water content (RWC) of both cultivars were significant. A greater RWC reduction was established in Plovdiv 11M. Ψ_w decreased significantly in both cultivars. The changes in RWC and Ψ_w were probably due to some structural and functional changes, ensuring plant adaptation to the drought treatment (Paleg *et al*., 1984).

Table 1. Leaf gas exchange parameters in two bean genotypes (Plovdiv 11M and A195) after ten days of soil drought. A-net photosynthetic rate [μmol m^{-2} s^{-1}]; E-transpiration rate [mmol m^{-2} s^{-1}]; gs-stomatal conductance [mmol m^{-2} s^{-1}]; A/E-photosynthetic water use efficiency [μmol $mmol^{-1}$]. Values are the means ± SE of five replicates. *, **, ***, indicate significant difference at P<0.05, P<0.01, P<0.001, respectively, between control and soil drought for each genotype.

parameters	Plovdiv 11M control	Plovdiv 11M Drought	A195 Control	A195 drought
A	12.78±0.49	9.73±0.37 **	12.94±0.43	8.33±0.32 ***
E	4.11±0.14	2.58±0.11 ***	3.97±0.14	2.83±0.12 **
gs	175±12	115±12 *	180±14	140±11
A/E	3.10±0.09	3.77±0.10 **	3.26±0.10	2.94±0.08

Table 2. Changes in the leaf relative water content (RWC [%]), leaf water potential (Ψ_w [MPa]), transpiration per unit plant weight (T (g g^{-1} day^{-1}]), and plant water use efficiency (WUE [mg g^{-1}]) of two bean genotypes (Plovdiv 11M and A195) after ten days of soil drought. Values are the means ± SE of five replicates. *, **, ***, indicate significant difference at P<0.05, P<0.01, P<0.001, respectively, between control and soil drought for each genotype.

parameters	Plovdiv 11M control	Plovdiv 11M Drought	A195 Control	A195 drought
RWC	92.40±0.80	77.30±0.70 ***	94.50±0.90	82.60±0.70 **
Ψ_w	-0.43±0.03	-1.60±0.06 ***	-0.36±0.02	-1.30±0.05 ***
T	16.70±0.90	8.70±0.50 ***	17.20±0.40	10.10±0.80 ***
WUE	11.40±0.60	11.80±0.80	11.10±0.50	9.30±0.40 *

The plants of tested genotypes showed identical response in terms of the water relation parameters - relative water content and water potential. RWC decreased to a lesser extent, while the Ψ_w reduction was by more than two times. Water use efficiency (WUE) increased slightly in cv. Plovdiv 11M and decreased significantly in cv. A 195.

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LOW AND HIGH RADIATION EFFECTS ON MORPHOLOGY AND BIOMASS ACCUMULATION IN SNAP BEAN (*Phaseolus vulgaris* L.)

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Introduction

The architecture of plants is dependent on the quantity, direction, duration, and quality of light. Photosynthesis is dependent on light for energy and for induction of enzymatic processes (Nilsen and Orcutt, 1996).

A plant submitted to high radiation levels generally has thicker and smaller leaves with a thicker cuticle than those plants grown in low-light environments (Nilsen and Orcutt, 1996). Low-light environment plants have a large leaf area and chloroplast number (high chlorophyll content) (Fitter and Hay, 1987).

The aim of the present study was to determine the influence of low and high radiation levels on the morphology, biomass production, chlorophyll content and leaf area in a snap bean (Phaseolus vulgaris L.) cv. Black Valentine.

Materials and Methods

The study was carried out in Montecillo, Mexico (19ºN; 98ºW, 2250 above sea level, and a temperate climate). Snap beans cv. Black Valentine seeds (indeterminate growth) were planted in pots with soil and grown in a greenhouse. The experimental design was a complete randomized block with four replicates. The treatments were a) plants under high radiation levels at midday (1100 μmoles m^{-2} s^{-1} of photosynthetically active radiation) and 37ºC, and b) plants under low radiation levels (500 μmoles m^{-2} s^{-1}) and 30ºC.

Results and Discussion

There were no differences in number of nodes and branches, and shoot diameter. The low radiation plants showed a higher number of reproductive structures (buds, flowers and pods) and leaflets and were taller than the plants under high radiation.

Environmental temperature is influenced by the net radiation, and the high radiation plants were subjected to high temperature (37ºC) induced by the high levels of radiation. High temperatures have complex impacts on photosynthesis, principally thylakoid membrane malfunction; leaves increase respiration (Nilsen and Orcutt, 1996). The high temperature induced abortion and abscission of reproductive structures, and low seed yield, but it also accelerated plant senescence and induced low leaf area and chlorophyll content (Table 1).

Table 1. Leaf area, chlorophyll content, biomass production, seed yield and harvest index (HI) of bean plants cv. Black valentine grown under high (94 DAS) and low radiation (122 DAS).

Treatment	Leaf area (Cm^2)	Chlorophyll (SPAD units)	Biomass (g per plant)	Seed yield (g per plant)	HI
High-light radiation	121 a†	24.0 a	2.47 a	0.18 a	0.07 a
Low-light radiation	841 b	38.1 b	6.66 b	1.95 b	0.29 b

†Tukey p≥0.05; DAS= days after the sowing

In contrast, the low radiation plants grew under a lower temperature (30ºC), and they had a larger number of reproductive structures. They also produced more biomass and seed (Table 1).

Moreover, there was a positive relationship between the biomass and the evapotranspiration in plants grown under low and high radiation (r^2=0.95 and 0.92, respectively) (Figure 1). The slope in Figure 1 represents the water use efficiency (WUE), and the plants grown under low radiation had a higher WUE than the plants under high radiation. Several authors have found a positive

association between biomass and Etc, which had a linear relationship with the yield in maize and bean (Howell et al., 1998; Escalante et al., 2001).

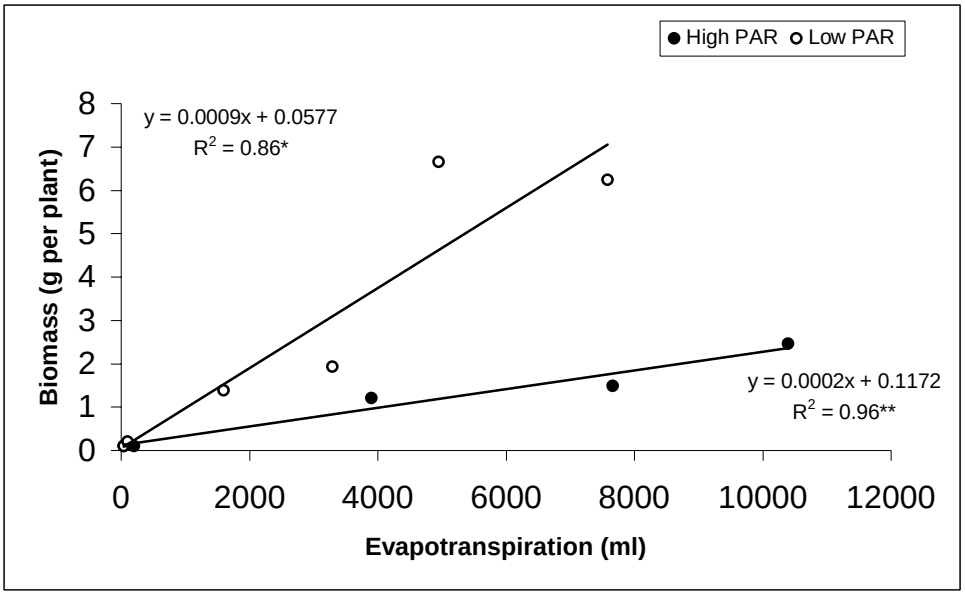

Figure 1. Relationship between biomass production and Etc in bean plants cv. Black valentine grown under high radiation (94 DAS) and low radiation (122 DAS). **,* level of significance $p \leq 0.01$, and 0.05.

Conclusion

The plants grown under low radiation produced more biomass, higher seed yield, larger leaf area and higher chlorophyll content than the plants grown under high radiation.

There was a positive relationship between evapotranspiration and biomass in both treatments.

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DRYBEANS DEVELOPMENT AND YIELD SIMULATION BY CROPGRO

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Introduction

CROPGRO is a generic growth model of DSSAT for simulation of development and growth of grains legumes soybean, drybeans, peanuts and chickpeas (Hoogenboom et al., 1999). It is a deterministic and process oriented model that simulates, in a main time step of one day, several processes including soil and nitrogen balances, duration of growth stages, biomass and yield accumulation for a specific cultivar, under different soil types, weather conditions and crop management. In CROPGRO, development and growth depend on coefficients classified in groups related to species, ecotype and cultivar. The last include coefficients that differentiates cultivars and are required to be calibrated.

The objectives of this research was to calibrate genetic coefficients for two bean cultivars and use those coefficients as inputs in CROPGRO to test model's performance for predicting crop development and yield in Paraná State, Brazil.

Materials and Methods

Measured data were obtained from field experiments conducted in Londrina, Paraná (Latitude 23° 27', Longitude 51°57'). Two widely planted cultivars (IAPAR14 and IAPAR57) were sown at different dates during fall (PD1 and PD2) and spring (PD3), and submitted to different water regimes, obtained by irrigation at different soil water tension (I_0 = non irrigated; I_1 = irrigation at 25 kPa; I_2= irrigation at 70 kPa) or by suppression of water supply from irrigation and rain during different times over the growing cycle (S_0 = full irrigation; S_1 = 0 to 20 days; S_2 = 21 to 40 days; S_3 = 41 to 60 days; S_4 = 61 days to physiological maturity).

Crop growth was simulated for the same periods that experiments were conducted using soil and climatic inputs obtained locally. Calibration of the genetic coefficients was performed using data from treatments with no water stress (I_1 and S_0) and test of the model was accomplished with remaining data.

Results and Discussion

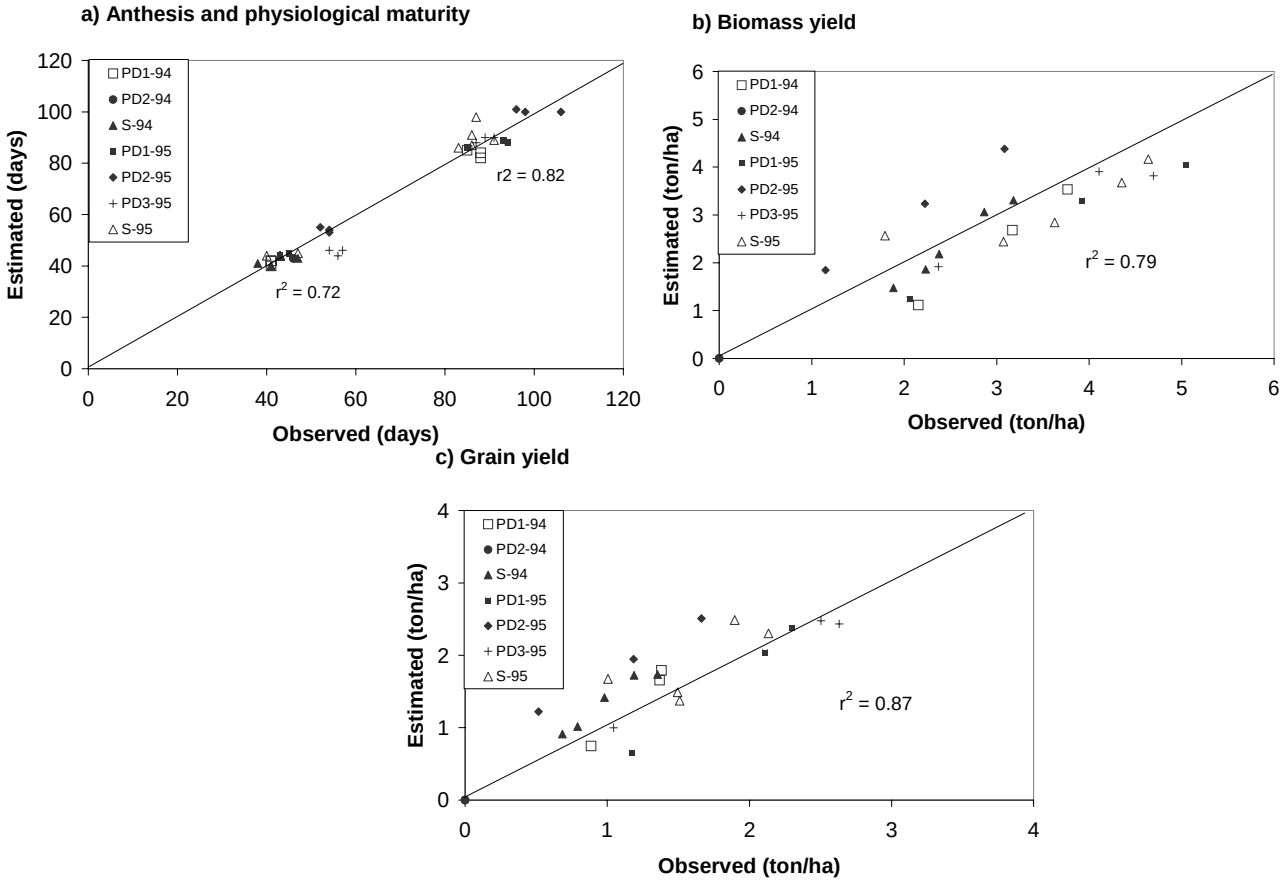
In the calibration of the genetic coefficients, it was assumed that both cultivars are insensitive to photoperiod, present similar thermal requirements for the period emergence-flowering (EM-FL = 27 thermal days) and have the same leaf characteristics (specific leaf area = 295 $cm^2.g^{-1}$ and maximum leaf area = 133 cm^2). Differences between cultivars were defined by coefficients related to the reproductive period, assuming pod and seed appearance earlier in IAPAR14 (FL-SH = 3 thermal days e FL-SD = 11 thermal days) than IAPAR57 (FL-SH = 5 thermal days e FL-SD = 13 thermal days). The earlier growing cycle of IAPAR57 was attributed to a shorter grain filling period (SD-PM = 23 thermal days) than the one of IAPAR14 (SD-PM = 28 thermal days). A higher value for maximum photosynthesis rate was assigned to IAPAR57 (LFMAX =1.0 mg $CO_2.m^{-2}.s^{-1}$), as compared to IAPAR14 (LFMAX = 0.9 mg $CO_2.m^{-2}.s^{-1}$), to express its higher yield attained in an earlier growing cycle. The remaining genetic coefficients were taken from the agronomic traits of each cultivars, as measured in the field, such as seed weight (0.2 g for both) and seeds per pod (3.5 for IAPAR14 and 4 for IAPAR57).

The comparison between estimates and experimental data revealed that CROPGRO predicted satisfactorily dates for anthesis and physiological maturity, as well as biomass and grain production for both cultivars, in different planting dates and water regimes (Figure 1).

Conclusion

CROPGRO simulated reasonably drybeans development and yield using calibrated genetic coefficients for a wide range of environmental conditions in Parana. This allows the use of the model in further studies involving crop zoning and irrigation requirements in the region.

Figure 1. Simulated and observed periods for emergence to anthesis and emergence to physiological maturity, and biomass and grain yield production in different planting dates for the cultivar IAPAR14 in Londrina, Paraná



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COEFFICIENT OF INTERFERENCE – A NEW TOOL FOR INTERPOPULATION STUDIES.

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The concept of interference, developed by Muller (1969), comprehends the reciprocal influence of each the components of a given pair of plants in a community, on each other's behavior. According to Rice (1984), such influence comprises two kinds of effects: those from competition and those from allelopathy. Allelopathy is the result of biochemical interactions among the distinct groups of plants, including microorganisms. The term covers both inhibitory and stimulatory interactions. Competition effects, in another hand, result from removal or reduction of some environment component that is required by some other co-habitant plant. Among these environment conponents, are water, minerals, light and food.

From these concepts, it was designed a new coefficient, the Coefficient of Interference (CI), that can be used to compare the behavior of a given plant population under pure stand with its behavior under mixed stand The limits of the mixed stand concept can vary from a mixture of cultivars within a given species, to the mixture of different species, such as in bean-maize intercropping. The term of comparison will always be the behavior of the considered population under pure stand.

The Coefficient of Interference can be expressed as:

$$CI_i = \frac{Wmi}{Wpi}$$, where Wmi = i population behavior in mixture and

Wpi = i population behavior in pure stand

CI values higher than unity (1,0), result from positive effects of the mixture on the behavior of the considered population. CI values equal to the unity (1,0), reveal neutral effects of the mixture and CI values below 1,0, reflect negative effects of the mixture on the behavior of the considered population.

An illustration on the use of CI can be observed from experiments that were carried out, in the locations of Canguçu and Passo Fundo, in the state of Rio Grande do Sul. The common bean cultivars Carioca, Guateian 6662 and Tayhú, have ben cultivated in pure stand and as a mixture. Plant population was equivalent to 240.000 plants/ha. Table 1 shows CI values obtained for grain yield. Tayhú displayed greater-than-one values, what reflects the stimulatory effects of Guateian 6662 and Carioca on its grain yield. It's CI mean value (CI = 1.24) differed from 1.0, and represents a mean increase of 24% in yield as compared to its pure stand cropping. Carioca and Guateian 6662 have shown mean values below 1.0, although not statistically significant.

In the present example, the ideal situation would be the one in which all the components would display CI values greater than one. This would mean that the mixture would yield more than any of the cultivars in pure stand. This picture, associated to the common phenomenon of greater yield stability of genotype mixtures, would point out to the use of such mixture, with all the positively derived effects.

The Coefficient of Interference also, might be used to compare the behavior of a given population in mixtures under different environments. One of the main requirements to its determination, is the establishment of pure stand plots to be used as check.

Table 1 – Coefficients of Interference (CI) for grain yield of the common bean cultivars Carioca, Guateian 6662 and Tayhú, in Canguçu, RS, in 1992/93 and 1993/94 and Passo Fundo, RS, in1993/94.

Cultivar	Canguçu		Passo Fundo	Mean CI
	1992/1993	1993/1994	1993/1994	
Carioca	1,09 AB	0,79 A	0,81 A	0,90 ns B
Guateian 6662	0,75 B	1,06 A	0,93 A	0,91 ns B
Tayhú	1,40 A	1,22 A	1,12 A	1,24** A

CI values followed by a same letter, do not differ, at $\alpha = 0,05$, by Duncan's test.
ns and **: not significant and significant at $\alpha = 0,01$ respectively, by t test for difference from 1.0.

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DRY BEAN YIELD COMPARISONS ACROSS FOUR PLANTING DATES IN CENTRAL WASHINGTON

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Introduction

In Central Washington, bean crops are most commonly planted late in May through early June because the soil temperature is higher than 10 C (50° F); this encourages quick germination to avoid the damage from toxicity of pre-plant herbicides. Beans are short seasoned, with some bean lines taking as little as 75 days to reach maturity. If the bean crop can be harvested early, the opportunity exists for double cropping to increase farm income, protect the soil from winter erosion, or to facilitate crop rotation as a part of best management practices for soil nutrient management, interruption in pest cycles, and to increase bio-diversity in the soil profile. Most of the bean lines grown in Central Washington mature from 75 to 110 days.

Materials and Methods

Six bean lines with early, mid and late season maturity characteristics were used in two-year experiments conducted on Shano silt loam soil at Othello Research Farm. LeBaron and Othello (early maturity lines), Burke (mid season line) USWA-33, 70 (mid to late maturity lines) Rojo Chiquito and USWA-39 (late maturity line) were planted at 14 day intervals starting early May through mid June. The experimental site received a pre-plant irrigation, commercial fertilizer inputs to adjust soil nutrients to 112 kg ha^{-1} of N and 56 kg ha^{-1} of $P_2 O_5$ and an application of 4.6 l ha^{-1} Eptam 7E + 2.3 l ha^{-1} Sonalan prior to planting. Beans were planted in a randomized complete block design with 4 replications using a cone seeder mounted on a John Deer Flex 71 planter. Irrigation water was supplied through out the season as needed by furrow applications to maintain good soil moisture and was cut off when 50% of pod turned brown. Bean lines and planting date treatments are presented in Tables 1 and 2.

Results and Discussion

Generally as soil temperature increased, days to germination and days to flowering decreased. The earliest planting date, first week in May, emerged in 14 to 15 days, planting date 2 emerged in 9 to 10 days, planting date 3 in 7 days, and planting date 4 (mid June) in 5 days. Days to flowering in the earliest planting date treatment was germination time plus 32 day for early maturity line and 41 d for late maturity one. All other planting date treatments had days to flowering at germination time plus 34-35 d regardless of early, medium or late maturity bean lines.

Late planting date treatment's yields tended to be the higher than the early planting dates treatments regardless of the bean line's length of growing season requirement. Seed fill duration did not vary much by planting date treatments. The differences were only the time between planting to germination.

In 2000, LeBaron, USWA-70, Burke, and USWA-33 produced more seed at later planting dates (Table1), but only LeBaron and USWA-70 yields were significantly different from other treatments. In 2001, all lines yielded more in later planting dates, but only USWA-33 and USWA-70 yield response was significant (Table 2). Overall yields of 2001 were much lower than in 2000, this was due to the field selection and environmental differences among two years.

Results of these two years showed that planting bean in Central Washington should be done late in May or early in June to achieve optimum yield. Damage to bean crops from early fall frost is unlikely due to the 150 day average planting season in Central Washington. Early planting does not increase seed yield but does lengthen the growing season of all bean lines tested in the area.

Table 1. Planting Dates Effects on Dry Bean Yield in Central Washington. 2000.

	Planting Date					
Entry	May 4	May 18	Jun 1	Jun 15	Mean†	Pr>F
	----------kg ha^{1}----------					
LeBaron	2612c	3105bc	3419ab	4018a	3289	0.02
Burke	3758a	3856a	3700a	4426a	3935	ns
Othello	3069a	4043a	3810a	3370a	3586	ns
USWA-33	2706a	3188a	2549a	3809a	3013	ns
USWA-39	2004a	2088a	2792a	1979a	2229	ns
USWA-70	2098ab	2566ab	1825b	2996a	2407	0.08
Mean	2708	3141	3016	3433	3076	

Table 2. Planting Dates Effects on Dry Bean Yield in Central Washington. 2001.

	Planting Date					
Entry	May 4	May 18	Jun 1	Jun 15	Mean†	Pr>F
	----------kg ha^{1}----------					
LeBaron	1444a	1766a	1776a	2040a	1757	ns
Burke	1517a	2199a	2291a	2585a	2148	ns
Othello	1370a	2082a	1790a	2764a	2001	ns
USWA-33	541c	1128b	1506ab	1657a	1207	0.002
Rojo Chiquito	1043a	1871a	2078a	2224a	1855	ns
USWA-70	581b	1347a	1194a	1407a	1132	0.022
Mean	1083	1732	1772	2113	1683	

†Means in a row followed by the same letter are not significant different at P = 0.05 according to the least significant t-test.

PLANT CONFIGURATION AND DENSITY EFFECTS ON DRY BEAN PRODUCTION IN CENTRAL WASHINGTON

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Introduction

Approximately 18,000 hectares of dry bean are planted every year in Central Washington for seed and export to international and domestic markets. Under current management practices in Central Washington, beans are seeded at 7.5 cm within the row and on 56-cm row spacing. The wider row spacing, as a management practice, decreases the incidence of root rot, allows mechanical cultivation and furrow irrigation. Breeders continue to work on disease resistant lines but are also developing lines that have compact and upright plant growth habit and/or more determinate lines. In this series of experiments, population configurations were tested against current management practices. More plants per unit area may increase bean seed yield.

Materials and Methods

Plant configuration and population trials were conducted at Othello Research Farm of Washington State University in 1998, 1999, 2000 and 2001.

- 1998 - Lines in 5 market classes, with different plant architecture, i.e., upright or prostrate growth habit were seeded at 28-cm and 56-cm row spacing.
- 1999 – In two separate trials - One trial repeated initial trial with ten cultivars from three market classes seeded at 28- and 56-cm row spacing. The second trial was seeded at 56-cm row with 5.7 and 7.5 cm in-row seed spacing using black and navy bean lines.
- 2000 and 2001 – Lines from four market classes were seeded on 56-cm row spacing at five in-row seed spacing levels.

All experimental plots were planted on Shano silt loam. Row spacing treatments were sprinkler irrigated in 1998 and 1999 and planting density plots were furrow irrigated in 1999, 2000 and 2001. Soil nutrient levels were adjusted to 112 kg ha^{-1} of N and 56 kg ha^{-1} of P_2O_5 and Eptam (4.7 l ha^{-1}) and Sonalan (2.3 l ha^{-1}) were applied pre-plant during land preparation for all years. All seed was treated with Captan 4 L slurry and planted with a cone seeder. The middle 2 rows of each plot were harvested for yield comparisons. The experimental design was randomized complete block design with four replications.

Discussion

In 1998, average yields of 28-cm or 56-cm between-row spacing treatments did not differ significantly. However, the bean lines with upright architecture tended to yield more when seeds were planted closer together with-in the row. Pinto and small red varieties (more prostrate architecture) tended to produce more seed when planted at 56-cm row spacing while black, navy and pink varieties (more upright architecture) were more productive in the narrower 28-cm row spacing (data not shown). In 1999, pinto and red lines responded to plant configuration with significant yield response and although the black lines yielded higher with 56-cm row spacing, it was not significant (data not shown).

Black and small white lines were more productive if planted on 56-cm row spacing with higher seed density within the row in 1999. The mean yield of 22 small white lines (Table 1) was 3415 kg ha^{-1} in higher density plots (5.7 cm seed spacing); an average 382 kg increase over the standard populations (7.6 cm seed spacing). The black beans responded with similar results; the

higher seed density plots had yields that were 382 kg ha $^{-1}$ than the lower density treatment (Table 1).

In 2000 and 2001, a study was conducted to look at five planted seed densities on 56-cm row spacing. In 2000, only Burke (pinto) and USWA-39 (dark red kidney) produced seed yields significantly higher than lower seed density plots (Table 2). All other lines showed an increase in yields at closer seed spacing but the increase was not significant. In 2001, the yield response across all density treatments showed no difference (data not shown).

Conclusion

Each market class has compact or prostrate plant species. The small and compact species are suitable to less space between plants in the row and there was a lower percentage of yield loss during direct combining. Higher plant density may decrease yield in root rot infested soil. In many cases used in this experiment, higher plant densities in narrow row spacing or standard spacing increased seed yield. Seed size and other agronomic factors were very similar in narrow or standard row spacing and plant configuration. Field cultivation with current equipment can be a problem in narrower row spacing during the growing season if pre-plant herbicides are not very effective.

Table 1. Row Spacing and In-Row Seed Density Effect on Small White and Black Bean Yields. 1999. †

	5.7 x 56	7.6 x 56			5.7 x 56	7.6 x 56	
Small White	**cm**	**cm**	**Avg**	**Black**	**cm**	**cm**	**Avg**
	--------kg ha^{-1} --------				**--------kg ha^{-1} --------**		
Norstar	4110	3280	3695bc	Midnight	4158	3606	3882abcd
AC Compass	3802	3185	3494cde	T-39	3834	3504	3669bcdef
ISB 1256	3611	3162	3387cde	Shinny Crow	3606	3561	3584cdefg
Vista	3593	3114	3354cdef	Onyx	3927	3044	3486defgh
ISB 1814	3323	2981	3152efghi	UI 911	3200	3571	3386efgh
Mackinac	3331	2887	3109efghi	Shadow	3325	2907	3116hi
Mean	3415a	3033b	3224	Mean	3688a	3306b	3497
LSD (.05)	127	127	426	LSD (.05)	118	118	401
CV (%)	13.1	13.1	13	CV (%)	11.5	11.5	12

Table 2. Plant Density Effect on Dry Bean Yield. 2000. †

	7.62	6.35	5.43	4.75	4.24	Line	LSD	
Bean Line	**cm**	**cm**	**cm**	**cm**	**cm**	**Avg**	**-0.05**	**Pr>F**
	----------kg ha^{-1} ----------							
LeBaron	3267a	3135a	2872a	3198a	3215a	3138d	703.56	Ns
Burke	3992bc	3762c	4151ab	4444a	4275ab	4125b	364.79	0.014
Othello	4210a	4210a	4332a	423a	4481a	4331a	534.36	Ns
USWA-33	3065a	3180a	2975a	3145a	3521a	3178d	515.79	Ns
USWA-39	2272b	2231b	2147b	2659a	2717a	2403e	372.59	0.016
ICB10-5	2784a	2343a	2271a	2728a	2678a	2519e	680.8	Ns

†Data not included showed no significant differences between treatments. Complete data tables are available by contacting An Hang <ahang@tricity.wsu.edu> -

EFFECT OF POPULATION SIZE AND SPATIAL ARRANGEMENT IN A NEW ERECT COMMON BEAN GENOTYPE (*Phaseolus vulgaris* L.).

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In breeding projects, erect type genotypes have been systematically eliminated in competition with prostrated type genotypes. This happened because erect and prostrated types have been tested with the same population size and spatial arrangement whatever plant ecotypes.

The aim of this project is to maximize yield on a new erect genotype (type II) with closed angle in branches insertion stem.

A factorial field experiment was carried out in split randomized blocks with five replicates. The treatments were: distance between rows: 45 or 22,5 cm; seed density: 10 or 20 seeds per linear meter; fertilization: 500 or 1000 kg/ha (8-28-16).

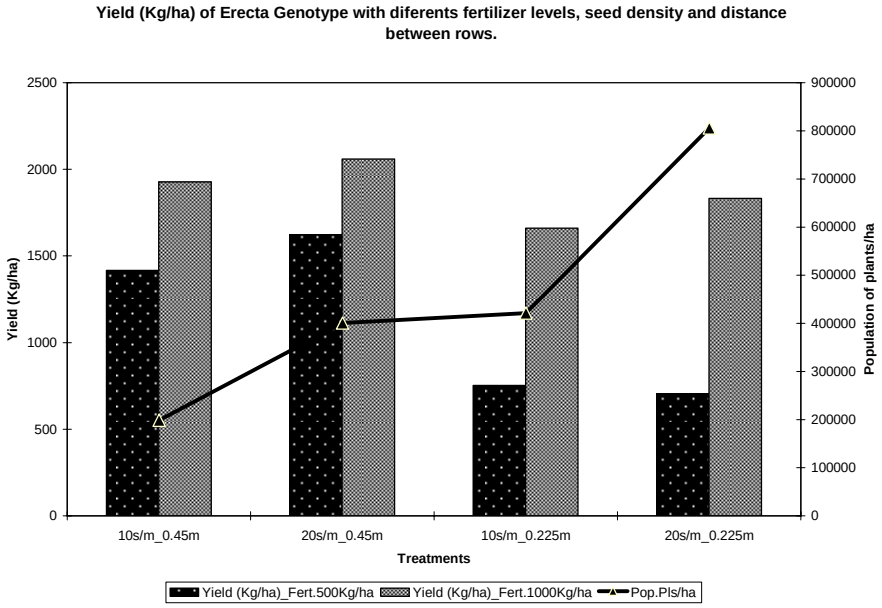
The erect genotype was developed by the brazilian company "FT-Pesquisas e Sementes Ltda" (FT –Research and Seeds Ltd).

According to Table1, fertilizer was the main limiting factor, mainly at 22,5 cm between rows, because of the competition among plants.

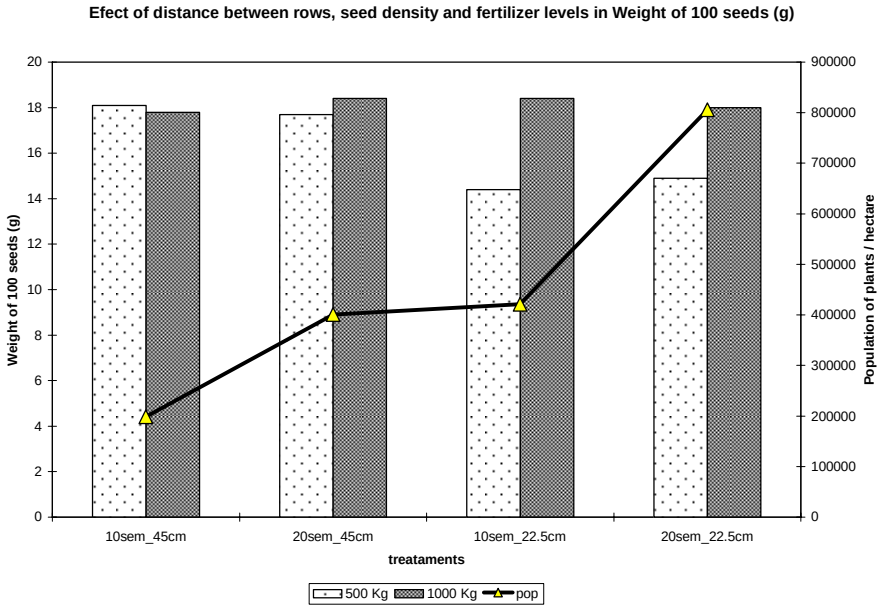
Table 1. Seed density, distance between rows, fertilizer level, yield and weight of 100 seeds of an erect genotype of common.

Treatment	Seed density (Seed/meter)	Distance between Rows (meter)	Fertilizer level (Kg/ha)	Yield (Kg/ha)	Weight of 100 seeds (g)
1	10	0.45	500	1416	18.1
2	10	0.45	1000	1928	17.8
3	20	0.45	500	1621	17.7
4	20	0.45	1000	2060	18.4
5	10	0.225	500	753	14.4
6	10	0.225	1000	1662	18.4
7	20	0.225	500	704	14.9
8	20	0.225	1000	1832	18

Considering the same population size with about 400.000 plants per hectare (treatments 3, 4, 5 and 6), it is possible to maximize yield changing spacial arrangement. The maximal yield was obtained with 20 seeds per meter and 45 cm between rows. This means that, beside fertilization level, the productivity is linked to the number of seeds sowed per linear meter. The lowest productivity was obtained with low distance between rows and low fertilization level (Figure 01).



Whatever the spatial distribution, the weight of 100 seeds was the same for highest fertilization (Figure 2). Similarly to yield, the low distance between rows decreased the weight of 100 seeds (Figure 2).



According to the results, in the environmental conditions tested, the new erect genotype produced more with high level of fertilization and 20 seeds per linear meter. However, additional spatial arrangement must be tested for this genotype.

SEED LOT AFFECTS STAND ESTABLISHMENT, FINAL PLANT STAND AND YIELD IN EARLY SNAP BEAN PLANTINGS

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A decrease in seed quality (vigor) resulted in decreased yields in many crops harvested in the vegetative or early reproductive stages (reviewed by Tekrony and Egli, 1991). The question asked; does this relationship also exist for snap beans (*Phaseolus vulgaris* L) that are harvested in the edible-pod stage and harvested in an once-over manner. Earlier research conducted by our group studied carry-over samples of the same seed lot of four snap bean varieties in 1999 (Taylor et al., 2000). The carry-over samples were simulated by mild aging in the laboratory. Germination results, field emergence and yield declined with severity of aging. The purpose of the present study was to examine the effect of seed lot differences on germination, stand establishment, final plant stand and yield. Research was focused on 'Hystyle', a major commercial variety used for processing. The objectives of these studies were to 1) test commercial seed lots of 'Hystyle', 2) perform seed quality tests on each lot and 3) conduct field studies to obtain seedling emergence and yield in 2000 and 2001.

All seed samples were equilibrated at 50% relative humidity, resulting in a seed moisture content of about 10% (fresh weight basis), (Taylor, 1997). Two seed quality tests were performed on each sample: standard germination test at 25 °C on roll towels, and chilled germination test with saturated, cold roll towels. The chilled germination test uses the same procedure as the standard germination test, except seeds are placed on chilled towels at 10 °C that were saturated with water. The seeds were kept chilled for 3 days and then transferred to 25 °C, an optimal temperature for germination, for another 7 days. This test provides both a cold temperature and wet condition stress on seeds. Field studies were performed in 2000 and 2001 at the Vegetable Crop Research Farm, NYSAES, Geneva, NY. In 2000, a preliminary test was conducted on two seed lots. Plots were sown on May 9, 2000, and the growing season was excessively wet. In 2001, four seed lots were tested and represented one high and three medium quality seed lots, all of acceptable quality. An acceptable seed lot, as determined by the Federal Seed Act, has a minimum germination of 70%. Plots were established on May 9, 2001, and the growing season was warm and dry.

Germination and field data for 2000 are shown in Table 1. Both seed lots had good germination levels; however, seed lot 2 had a lower percentage chilled germination than lot 1. Parallel to the lower lab stress test result was lower field emergence, reduced plants per meter of row at the end of the season and total yield.

In 2001, four seed lots were studied in the laboratory and field studies. The standard AOV revealed that only seed lot 1 was different from seed lot 3; however, seed lot 1 had greater chilled germination than the other three lots. Furthermore, seed lot 1 was superior to the other three lots for the percentage field emergence and final plant stand (plants/meter). No significant differences were measured in yield when comparing seed lots. Comparing seed lot 1 with all other lots (2, 3 +4) was analyzed as an orthogonal contrast. Seed lot 1 was better than all other lots for each laboratory and field parameter.

Table 1. Comparison of two seed lots of 'Hystyle' on laboratory and field parameters in 2000.

	Percent				
Seed lot	Standard germination	Chilled germination	Field emergence	Plants / meter	Yield Mg / ha
1	94	91	72	17.5	7.50
2	88	75	65	16.2	6.81

Table 2. Comparison of four seed lots of 'Hystyle' on laboratory and field parameters in 2001.

	Percent				
Seed lot	Standard germination	Chilled germination	Field emergence	Plants / meter	Yield Mg / ha
1	94 a	96 a	89 a	25.4 a	11.9 a
2	87 ab	65 b	70 c	22.1 b	10.5 a
3	78 b	63 b	75 bc	23.1 b	9.8 a
4	84 ab	75 b	80 b	23.1 b	9.9 a
contrast 1 vs others	0.0146	<0.001	<0.001	<0.001	0.0497

Based on these findings, seed quality is important for early plantings as the high quality seed lot resulted in enhanced stand establishment, final plant stand and yield. This data is consistent with our previous research on simulated carry-over samples, that a reduction in seed quality is related to decreased field performance (Taylor et al., 2000). Moreover, this data supports the general hypothesis that a decrease in seed quality (vigor) results in decreased yields in many crops harvested in the vegetative or early reproductive stages (Tekrony and Egli, 1991).

Acknowledgment

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DETERMINATION OF CAROTENE CONTENT IN YELLOW-SEEDED COMMON BEAN VARIETIES

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Introduction

Yellow-seeded beans are a minor commercial grain type from the Andean gene pool that are eaten in a subset of Latin American bean-producing countries, notably in Peru, Mexico and Brazil. Recently, they have become embroiled in a controversy in the United States where a patent was claimed on the marketing of yellow beans. In the meantime, the scientific basis for the yellow color is not known. In this study we decided to analyze whether the yellow color was derived from carotenoids or not. To do this we selected a group of accessions representing all the major types of yellow beans that are found in primary and secondary centers of diversityfor common beans and analyzed them for seed carotenoid content to determine if there is genetic variability for the trait.

Materials and Methods

A total of 10 common bean genotypes with various tones of yellow-colored grain (from canary to sulphur yellow) were selected for this study (Table 1). Three parts of the grain were analyzed: 1) whole seed, 2) peeled seed (embryo) and 3) seed coat. In the case of seed coat tissue, we were interested in studying the effect of seed storage and oxidation on carotenoid content of the seed coat and therefore extracted the seed coat of both fresh and stored dry grain samples. The tissues were ground into a fine powder with a coffee mill and a tungsten ball bearing mill. Extraction began by resuspending 0.3 g (for the stored seed coat and embryo) or 1.0 g (for the whole seed) of ground tissue in 10 ml of petroleum ether and 5 ml of water at 35-60 °C. The mixture was homogenized before centrifugation at 3000 rpm for 5 min at 10 °C. The ether phase was removed to a new tube while the aqueous phase was re-extracted with an additional 10 ml of petroleum ether and combined with the previous ether phase. Both of these aliquots were dried down with sodium sulfate in a vacuum centrifuge. The extracts were then resuspended in 1 ml of petroleum ether and used immediately for the analysis. The samples were analyzed in a UV-visible spectrophotometer at 455 nm, using petroleum ether at 30-60 °C as a blank. A standardization curve was made from β-carotene in a petroleum ether solvent and used for the quantification of β-carotene in the sample. The linear regression of the calibration curve was C(ppm) = K x Abs +B, where K= 6.6707 and B= 0.2084. To confirm the presence of β-carotene in each group of samples, readings were made for the UV-visible spectra in the range from 200-600 nm and compared to the commercial standard (Sigma).

Results and Discussion

Table 1 and 2 summarize the amount of carotenoid detected in the different tissues analyzed. The amount of β-carotene found in the stored and freshly-ground seed coats was similar and relatively low, averaging 0.131 mg per 100 g of tissue. Although the seeds ranged from light to dark yellow and had different tones ranging from canary to sulphur yellow, the amount of carotenoid was not associated with the intensity of the yellow seed coat color. Whole seed contained lower amounts of β-carotene (averaging 0.042 mg per 100 g of tissue) than the embryo tissue alone (averaging 0.147 mg per 100 g of tissue) indicating that most of the carotenoid is in the embryo tissue not in the yellow seed coat. This carotenoid concentration was within the same range as that found for the dissected seed coat in the previous experiment, however these would be comparisons across experimental conditions given that 1 g of embryo and whole seed tissue was used versus only 0.3 g of seed coat tissue, an amount that is at the lower limit of detection. In these experiments absorbance levels at 455 nm were very low and no additional readings were taken at other wavelengths. Therefore to confirm that the measurements were detecting carotenoid and not some other substance at this absorbance spectra, the spectra of a larger sample of 10 g of fresh whole bean seed tissue, processed as described above, was measured at wavelengths from 200 to 600 nm and was compared to that of the β-carotene standard. In this analysis, there generally was a good fit between the data for the sample and the standard, indicating that β-carotene was indeed the pigment being measured at 455 nm absorbance and that this carotenoid is present in the fresh bean seeds. These results suggest various avenues for future

research. First, given that carotenoid levels are very low in both the dry bean embryo, the optimal amount of tissue to analyze for future β-carotene experiments in beans, should be increased to 10g. Second, since the yellow color in yellow seed coats is unlikely to be due to carotenoids given their low concentrations, but rather is probably due to some other pigment, it will be important to read the absorbance wavelength of 490 nm as well as at 455 nm. Also, to confirm the presence of β-carotene, more sensitive tests using MS/HPLC can be undertaken. Mass spectrophotometry especially should be able to determine the identity of xanthophylls that have been suggested as the major pigment in seed coat of yellow beans. The amount of B-carotene in various stages of bean seed maturation will be another area of interest. Especially in the Andes and certain areas of the Caribbean, bean seeds are commonly consumed at physiological maturity but before they dry down. To do this beans are shelled while the pods are still green or just starting to turn yellow. These beans are often referred to as green-shelled beans and command a premium price because of their fast cooking time. In future experiments, we will look at carotenoid levels during grain development, their stability upon cooking and whether this is different in mature dry grain versus green-shelled beans.

Table 1. Analysis of carotene concentration (expressed in mg carotente/100g tissue) in stored and fresh seed coat (SC) tissue from 10 common bean genotypes with yellow grain color.

Genotype	Name	Origin	Weight (g)	mg carotene /100g tissue[1]	
				Fresh SC	Stored SC
G-5703	Canario Corriente (LM2-57)	Peru	0.30	0	--
G-57	Swedish Brown	USA	0.30	0.10	0.19
G-2288	Maragwe Oga	Kenya	0.30	0.10	0.18
G-4547	Liborino de Mata	Colombia	0.30	0.09	0.16
G-11035	Bayo Regional	Mexico	0.30	0.12	0.20
G-13094	Mayocoba	Peru/Mexico	0.30	0.08	0.18
G-14253	Peru 13	Peru	0.30	0.09	--
G-19833	Caucha Chuga	Peru	0.30	0.07	0.20
G-21715	Dore de Kirundo	Burundi	0.30	0.09	0.31
G-22041	Garbancillo Zarco	Mexico	0.30	0.09	0.10

Table 2. Analysis of carotene content of whole seed and fresh embryo tissues in 10 common bean genotypes.

Genotype	Whole seed			Embryo		
	Weight (g)	Absorbance (λ 455nm)	mg carotene /100g tissue	Weight (g)	Absorbance (λ 455nm)	mg carotene /100g tissue
G-5703	1.0	0.013	0.03	0.30	0.017	0.10
G-57	1.0	0.019	0.03	0.30	0.028	0.13
G-2888	1.0	0.017	0.03	0.30	0.027	0.13
G-4547	1.0	0.024	0.04	0.30	0.037	0.15
G-11035	1.0	0.085	0.08	0.30	0.028	0.13
G-13094	1.0	0.029	0.04	0.30	0.047	0.17
G-14253	1.0	0.027	0.04	0.30	0.094	0.27
G-19833	1.0	0.041	0.05	0.30	0.043	0.15
G-21715	1.0	0.022	0.04	0.30	0.022	0.11
G-22041	1.0	0.022	0.04	0.30	0.026	0.13

1/ carotene extracted from 0.3 g and 1.0 g of tissue for tissues described in table 1 and 2, respectively

CORRELATION OF IMMATURE (*IW*) AND MATURE SEEDCOAT COLOR OF DRY BEANS (*PHASEOLUS VULGARIS* L.)

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Introduction

Food legumes are grown and consumed in nearly all parts of the world. Seedcoat color is an important trait affecting marketing of dry beans. Environmental conditions and disease can cause seedcoat discoloration. Dry bean cultivars need to have the typical color of a particular market class and be free from of-type seeds and seed discoloration. Hail, high temperature, rain, and sprinkler irrigation may influence the development of bean seedcoat yellowing (Ergun et al. 2001).

Immature white seedcoat trait consisting of reduced green color in immature seedcoats and early development of white color in white-seeded cultivars, is determined by a single recessive gene (*iw*). This gene is independently inherited from *P* locus for mature seedcoat color and from *Y* locus for green versus wax pod color (Baggett & Kean 1984a and 1984b; Dean 1970; Singh 1991).

The seeds of some cultivars of common bean are covered with yellowing, when the weather is hot and dry during pod-filling stage (especially 10-14 days before ripening). The yellowing is not infectious (Genchev & Kiryakov, 1994b).

Materials and Methods

In order to determine the strength of the correlation at high temperature and low relative air humidity between the time of change of seedcoat color during seed formation and the percent of seedcoat yellowing, 20 bean cultivars were used: Abritus, Prelom, Sanilac, G 2883, Dobroudjanski 7, Dobroudjanski 2, Seafarer, HR 45, Plovdiv 164, Dobroudjanski ran, Ternovo 13, Trudovetz, Declivis Romulus, Dunav 1, A 195, Oreol, Zornitza, IIRR 7585, Mx 1834 and WA 1921.

Reading of seedcoat color was done according to Genchev & Kiryakov (1994a) under field conditions during 2000 and 2001; these years were characterized by high temperatures and low relative air humidity. The yellowing of seedcoat in accessions with white seeds and the change of normal color in accessions with colored seedcoat was read as percent of seedcoat area.

The correlation strength was determined by calculating the biserial coefficient (r_b) (Kalinov, 2001).

Results and Discussion

Change of seedcoat color from green to white or some other occurs at different stages of seed formation - from a half to 90 % of the weight of the mature seed. From the accessions given in Figue 1, the following have immature white seedcoat (IW): Abritus, Prelom, Sanilac, G 2883, Dobroudjanski 7, Dobroudjanski 2, Seafarer, HR 45, Plovdiv 164 and Dobroudjanski ran, and with normal green seedcoat (NG) were Ternovo 13, Trudovetz, Declivis Romulus, Dunav 1, A 195, Oreol, Zornitza, IIRR 7585, Mx 1834 and WA 1921. This normal immature green seedcoat color is not to be confused with the persistent green character, described by Dean (1968). In the first group (IW) change of color occurs at higher seed moisture and takes more time in comparison to the second group (GN). Ergun et al. (2001) point out to hails, high temperatures, rains and sprinkler irrigation as the most closely related causes for yellowing of seedcoat. In our opinion, each environmental deviation from the crop norms in the GN accessions causes this phenomenon.

There is an evident close correlation between late change of seedcoat color and the percent of seedcoat area with yellowing, which is definitely undesirable from a market viewpoint. In the

accessions we investigated the correlation between the time of seedcoat color change and the percent of yellowing was $r_b = 0.747$ at a rate of significance $\alpha = 0.001$.

In the future our breeding program will be aimed at selection of new cultivars with immature white seedcoat.

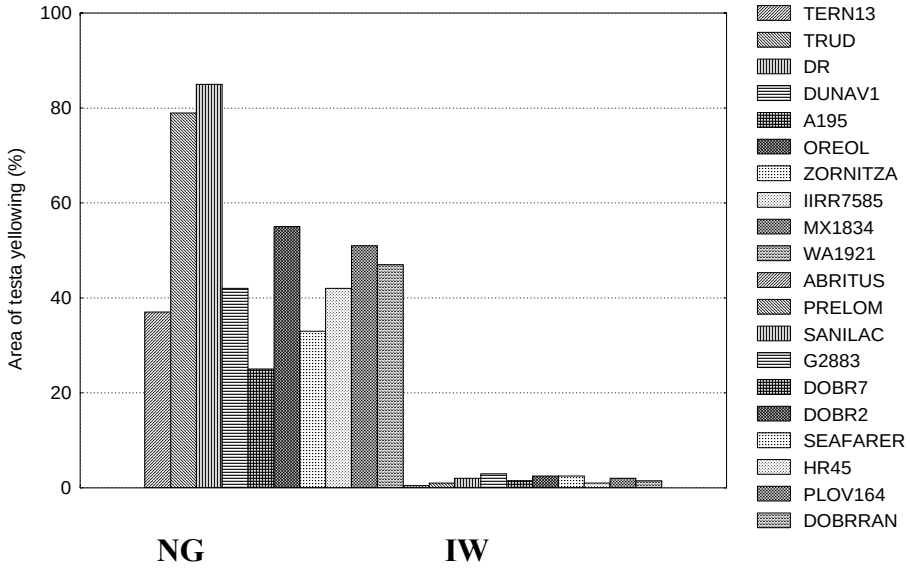

Figure.1 Percent of yellowing of seedcoat in bean accessions with c NG (normal green seedcoat color) [TERN13 (Ternovo 13), TRUD (Trudovetz), DR (Declivis Romulus), DUNAV 1, A 195, OREOL, ZORNITZA, IIRR 7585, Mx 1834, WA 1921] and with IW (immature white seedcoat color) [ABRITUS, PRELOM, SANILAC, G 2883, DOBR7 (Dobroudjanski 7), DOBR2 (Dobroudjanski 2), SEAFARER, HR 45, PLOV164 (Plovdiv 164) and DOBRRAN (Dobroudjanski ran)].

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FOOD QUALITY OF DRYBEAN (*Phaseolus vulgaris* L.) LANDRACES FROM DIFFERENT STATES OF MEXICO

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Introduction

Bean landraces are a valuable genetic resource of México. In order to face the growing food demand and also the malnutrition problem of a big part of the country would be important to use this germplasm as a source of genes that may improve both yield and food quality traits that determines consumer´s preferences. For these propose, it is necessary to know the genetic diversity that encompass its agronomic adaptation and variability in food quality traits such as cooking time, protein content, and digestibility, antinutritional factors among others.

In Mexican improved bean varieties a range of protein content from 16 to 26 % has been found (Jacinto *et al*., 1993), this is an expression of native variability, as long as selection for high protein content has never done. Data about protein content and other grain components of Mexican bean landraces is scarce. An important commercial quality parameter is cooking time which in improved varieties varies between 49 and more than 200 min (Jacinto *et al*., 1993) while in bean landraces there are no enough information about variability in cooking time. The objective of this study was to characterize food quality traits of Mexican bean landraces.

Material and Methods

Food quality of 184 bean landraces from different states from the high valleys of Mexico was evaluated. The genotypes were sown at the Valle de Mexico Experimental Station, Texcoco Mexico, during the 2000-growing season. The plots were hand threshed as they matured, seeds were kept at 5 ° C until the tests were performed.

One hundred grain weight, water absorption capacity, seed coat, solids in broth and protein contents were determined in replicated samples. Cooking time was measured according to sensorial method in a sample of 25 grains previously soaked in water for 18 hours.

Results and Discussion

Bean landraces showed a wide variability in the food quality parameters (table 1), they exhibited highly significant differences among accessions for color, grain weight, and size as well as for cooking time, water absorption capacity, seedcoat content, thickness of broth, and protein content.

The accessions evaluated represent only a fraction of the total bean landraces present in the different localities. In general, it was found that the size of grains was similar among accessions independently of the origin of the genotype with exception of some accessions from Michoacán, Chiapas, and Oaxaca that tend to show small grains (< 25 g per 100 seeds). Protein content ranged from 17.8 to 26.5 %, those values were similar to the ones detected in breed cultivars that were reported from 17 to 26% (Jacinto *et al*., 1993).

Those results enable us to say that it is possible to use this variability in breeding programs and also suggest that even though selection for agronomic adaptation to specific areas, as well as for

cooked grain flavor has been carried out by bean producers, in protein content there is no a definite tendency which could had occurred as a result of indirect selection.

Table 1. Simple Statistics of quality grain variables from 184 bean landraces from different states of Mexico

Variable	Mean	Std dev	Minimum	Maximum
100 grain weight	29.46	7.33	8.56	57.68
Volumn (mL)	23.50	5.82	10.00	48.00
Water absorption (%)	80.35	21.93	6.00	117.00
Seedcoat (%)	8.96	0.57	7.60	11.20
Cooking time (min)	84.15	19.68	54.00	155.00
Solids in broth (%)	0.37	0.08	0.21	0.62
Protein (%)	21.88	1.57	17.75	26.52

Cooking time exhibited a wide range from 54 to 155 min that in general is considered from medium to good cooking quality compared to improved breed bean cultivars that ranged from 40 to more than 200 minutes (Jacinto *et al*., 1993). We found an association between cooking time and water absorption (r= -0.58**) indicating, that in some degree accessions with higher water absorption capacity were faster to cook.

Landraces from Zacatecas Jalisco y Querétaro states tend to be harder to cook, while the ones from México and Puebla States tend to be faster to cook. This behavior could be in part a genetic expression or also be related to the ability of the landraces to adapt to the conditions of Texcoco, the growing locality.

The amount of solids in broth defines its flavor and consistence; thicker broths contain more solids and are preferred for the consumers. Amount of solids in broth was quite variable among landraces, ranging from 0.33 to 0.60 %. In comparison improved cultivars had showed a wider range (0.20 to 0.60 %) showing thinner broths in some cases (Jacinto *et al*., 1993). There existed an association between grain volume and the solids content in broth (r=0.55**) meaning that bigger grains tend to produce thicker broths.

From the analysis by locality it was evident a wide diversity of colors: solids stripped and mottled. It was remarkable the presence of many landraces with yellow seeds some of them shiny and others dull coat. In general yellow color is an unusual color in seeds of breed varieties. It was also remarkable the presence of gray-mottled landraces that tend to segregate towards purple tonalities.

The variability found in the food quality traits of bean landraces could be used for breeding of bean cultivars taking in account the preferences of several regions.

Reference

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NICHE MARKET DRY BEAN VARIETY TRIAL

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Dry beans are generally considered a large-scale bulk commodity crop yet they are also well suited to small-scale production for niche markets. Dry beans are harvested in the fall, are easily stored over the winter, and can be a good addition for direct market farmers at the beginning and end of the growing season. A primary criterion for variety suitability in the maritime Pacific Northwest is early maturity. Cool summer temperatures in the region result in a low number of growing degree days (1900 GDD in Vancouver) and most varieties are harvested 15 or more days later than in the mid-west. This report presents some of our preliminary findings, and a full copy of our report can be viewed at http://eastafricacrsp.wsu.edu/beans/BeanVarietyReport2001.pdf

Materials and Methods

Thirty-three dry bean varieties were planted at WSU Vancouver REU on June 6 in a randomized complete block design with four replications. Plots were 2 rows wide and 10 feet long, spacing between rows was 2 feet, and spacing in the row was 2 inches. Three runner varieties were included, however they were not staked or trellised. The field was certified organic and was maintained accordingly. Plants were harvested from the center 5 feet of both rows for a total harvest area of 10 feet per plot. Whole plants were placed in burlap bags, and dried in field ovens for 16 hours at 68° C, until seed moisture was approximately 12%. Beans were threshed and cleaned by hand and total marketable bean weight (g) was measured. 100 beans were randomly selected and weighed from each plot, and length and width (cm) of 25 beans were measured.

Results and Discussion

Beans emerged June 17-21, 11-15 days after planting (DAP) (Table 1). Flowering began on July 15 (39 DAP), and all varieties had reached the 50% flowering stage by August 9 (64 DAP). Harvest was from September 24 to October 11 (110-127 DAP), and rain prevented further harvesting. Mean yield for 10 feet of row was 446 g, and yield differed significantly among varieties. In this trial, *pinks* (588 g) and *cranberry* (490 g) were the highest yielding types of beans. Varieties that yielded greater than 600 g per 10 feet of row were G18689 (681g), NW-63 (655 g, 1.4 g), and Etna (640 g). The three climbing-types of beans, Scarlet Emperor (156 g), Painted Lady (167 g), and Kentucky Wonder (197 g) were very low yielding as can be expected as they were not trellised.

Mean weight of 100 beans was 47 g and differed significantly among varieties. Painted Lady (105 g) and Scarlet Emperor (89 g) were twice as large as most other varieties. Varieties with 100-bean weight greater than 60 g were Etna (69.7 g), Trout/Jacob's Cattle (66.5 g), Old Fashioned Soldier (66.2 g), Cardinal (63.8 g), and CELRK (63.0 g). Length and width of 25 beans and the length of 10 pods were measured in order to further characterize bean size. We have developed a web page, http://eastafricacrsp.wsu.edu/beans/BeanVarieties.html, that includes this information for niche market bean varieties to facilitate variety selection by farmers. The page includes a brief description of each variety based on our data and a color photograph as the color and pattern of the beans play a large role in variety selection for niche markets.

Table 1. Days after planting (DAP) to emergence, flowering, and harvest; bean yield (g) for 10-feet of row; length and width (cm) of 25 beans; and pod length (cm) of 33 varieties at WSU Vancouver REU in 2001.

Type Variety	1st Flw DAP	50% Flw DAP	Harv DAP	T Bean Wt	Wt 100 Beans	Lth 25 Beans	Wth 25 Beans	Pod Lth
Dark Red Kidney								
Montcalm	43.7	46.5	112.2	431.2	51.89	39.3	19.2	11.65
Light Red Kidney								
CELRK	40.7	42.5	110	437.9	63.03	42.3	20.8	12.8
Kardinal	45.7	47	116.5	479.9	57	42.2	21.8	13.15
White Kidney								
Cannellini	40.3	42.5	115	320.4	59.26	38.5	20.1	13.45
Small White/Navy								
Norstar	49.3	52.7	111.7	465.6	17.79	21.1	14.4	8.45
Arthur	46.3	50.7	112.2	481.5	14.71	20.9	14	9.25
Small Red/ Red Mexican								
LeBaron	42.7	48.5	110	344.4	34.68	28.2	18.7	9.5
Montezuma Red	45	47	111	401	36.28	26.5	16.7	9.5
NW-63	40.7	45	116.2	655	34.64	28.4	19.2	9.9
Pink								
UI-537	47.7	49.7	115.2	592.9	36.42	28.1	18.3	9.35
PR95-055-2-1-16	49.3	52.5	112	581.9	36.83	31.3	19.2	9.95
Black								
UI-911	43.7	45.5	118	487.1	20.1	22	14.9	9.9
Midnight Black Turtle	54	56.7	118.2	517.4	17.91	22.3	14.7	10.13
Black Coco (*Johnny's*)	42	43	118	317.1	53.58	30.3	21.7	11.6
Black Coco (*Territorial*)	43	45.2	115.5	516.4	55.15	30.9	21.9	10.8
ICB-10-5-14	52	55	118	406.7	21.47	23.7	16.1	8.9
Cranberry								
Vermont Cranberry	42.3	48	111.7	457.1	48.82	30.6	19	13.35
Etna	42	44.7	113.5	639.5	69.67	35	22.3	14.1
Thort	43.3	46.7	114	452.2	55.33	33	22.6	13.4
Speckled Bays	45	46.7	116.2	357.1	49.48	28.3	20.1	10.4
Andrew Kent	43	45.5	119.7	572.6	58.26	31.3	27.4	13
Tongue of Fire	42.3	43.5	116.3	502.9	58.83	32.6	22.9	11.08
Cardinal	43	45.5	118.7	452.8	63.76	34	22.7	12.9
Yellow Eye/ Partially Colored								
Maine Yellow Eye	42.3	44	113.5	285.5	43.49	28	19.6	10.2
Old Fashioned Soldier	42.7	46	113.5	575.9	66.16	40.2	20	13.95
Trout/Jacobs Cattle	40.7	43.2	113.7	394.3	66.54	40.1	19.9	15.45
USWA-27	51	56.7	117	435.2	32.46	28.2	18.1	9.45
Other								
G18689	55	58	115.2	681.3	23.58	24.6	15.8	10.5
Scarlet Emperor	40	44.5	127	155.9	88.49	47.4	26.4	16.45
Painted Lady	39	42.7	127	167.4	104.48	49.5	29.3	15.3
French Flageolet	50.5	54	114.5	518.1	25.39	31	15	12.7
California Blackeye 46	67	-	-	-	-	-	-	14.75
Pole								
Kentucky Wonder	61	64	127	197	38.85	34.9	18	15
Mean	**46**	**48**	**116**	**446**	**47**	**32**	**20**	**12**
P Value	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0091**	**-**

CORRELATIONS BETWEEN CHEMICAL COMPONENTS IN TWO GREEN BEAN VARIETY TYPES

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The total sugars, starch and cellulose are part of the chemical composition, which forms the nutrient properties of the green bean. The change in the values of these characters has been studied both in the process of breeding and in sterilization and freezing of pods (Lu and Chang, 1996; Gonzalez et al., 1998).

Components of the chemical composition of 5 cultivars with flat and 5 cultivars with oval pods were studied during 2000 and 2001 in the Institute of Horticulture and Canned Foods, Plovdiv. The harvesting was performed at two degrees of maturity. The dry matter content by Manuelyan (1966), total sugars content by Shoorl-Regenbogen, starch and cellulose content by Heneberg-Shtoman (Genadiev, 1968) were analyzed using mean sample of 0.500 kg. The degree of maturity was determined by the method of Manuelyan et al. (1973). The correlation coefficients between the studied characters were calculated by Brave-Pirson (Lakin, 1990).

The degree of maturity varies from 93 mg to 162 mg at group of the cultivars of flat pods and from 107 mg to 235 mg at the group of cultivars with oval pods. The studied characters vary from 8.70% to 15.96% for the dry matter, 1.27% to 3.47% for total sugars, from 0.48% to 3.41% for the starch and from 0.77% to 1.91% for the cellulose.

In the flat pods variety type the great correlation between the starch content and the cellulose content was established (table 1). The dry matter content correlates with the content of starch and cellulose. Being principal components of the dry matter with high correlation with it, the starch and the cellulose strongly correlate each other.

Table 1. Correlation coefficients among studied characters of flat bean pods

	Maturity	Dry matter	Total sugars	Starch	Cellulose	
Maturity	♦	*0.470*	*0.054*	*0.399*	*0.545**	b)
Dry matter	0.148 / 0.646*	♦	*0.427*	*0.787***	*0.773***	
Total sugars	0.055 /-0.075	0.477 / 0.323	♦	*0.036*	*-0.041*	
a) Starch	0.101 / 0.575	0.816*/	0.200 /-0.387	♦	*0.860***	
Cellulose	0.135 / 0.675*	0.656 /	0.155 / 0.128	0.901** /	♦	

a) 2000 / 2001, b) *total from both years*

In the oval pods the correlations of the dry matter content with the starch and cellulose content are the greatest (table 2). The correlation coefficients are with high value and they are statistically proven during the individual years. In contrast to the flat pods the relation between the starch content and the cellulose content is rather slighter. Probably, the explanation can be connected to the different pod structure of both variety types, different number and size of the grains where the starch is manly synthesized.

The method of Manuelyan et al. (1973) for determination of the degree of maturity of oval green bean pods is based on the established by the authors significant correlation between the weight of the mean grain and the dry matter content. In the experiment performed by us the correlation coefficient for these two characters is not significant during the second experimental year and the value of the total correlation coefficient is not high enough. Nevertheless, there is a trend, in which as a result of the increase of the dry matter content there is an increase of the weight of the mean grain. In contrast to the oval bean pods, in flat ones a great dispersing of data is observed. This doesn't permit to prognosticate the maturity in dependence of the change in the mean grain.

Table 2. Correlation coefficients among studied characters of oval bean pods

	Maturity	Dry matter	Total sugars	Starch	Cellulose
Maturity	♦	*0.582**	*0.229*	*0.364*	*0.549**
Dry matter	0.778**/ 0.447	♦	*0.248*	*0.719***	*0.869***
Total sugars	-0.036 / 0.783	-0.203 / 0.414	♦	*-0.060*	*0.106*
Starch	0.412 / 0.302	0.820**/ 0.806**	-0.224 / 0.101	♦	*0.556**
Cellulose	0.745**/ 0.449	0.786**/ 0.963**	-0.535 / 0.375	0.513 / 0.859*	♦

a) b)

a) 2000 / 2001, b) *total from both years*

For successful application of the correlations in breeding process it is important to establish to what extent they are influenced by climatic, agrochemical, technological and other factors. One correlation is really significant only in cases when it has constant character of appearance. The high correlation of the dry matter content with the content of starch and cellulose (table 3) allow us to calculate the regression equations. In the case of the dry matter content and content of starch we have y = 0.34x - 2.12, where 'y' means content of starch and 'x' means content of dry matter (figure 1).

Table 3. Total correlation coefficients among studied characters of flat and oval bean pods

	Maturity	Dry matter	Total sugars	Starch	Cellulose
Maturity	♦				
Dry matter	0.402*	♦			
Total sugars	0.148	0.384*	♦		
Starch	0.300	0.860**	0.115	♦	
Cellulose	0.501**	0.739**	0.047	0.685**	♦

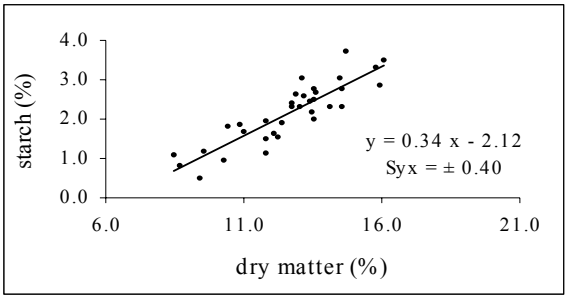

Figure 1. Regression of the dry matter and starch content of green bean pods

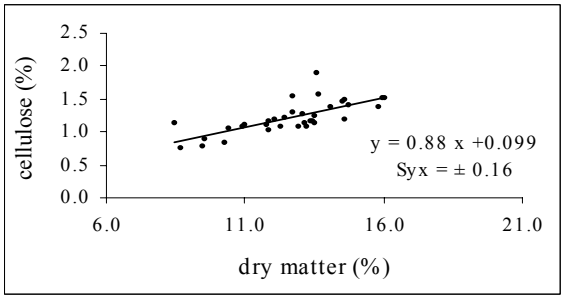

Figure 2. Regression of the dry matter and cellulose content of green bean pods

In the case of the dry matter content and content of cellulose we have y = 0.88x + 0.099, where 'y' means content of cellulose and 'x' means content of dry matter (figure 2).

Both equations allow calculating the starch content and the cellulose content by the weight dry matter content. They are especially valuable in making of primary screening, which is connected with an analysis of great number of samples. The standard errors of evaluation are within limits, which guarantee an accuracy satisfying the breeding work.

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PROCESSING OF VALUE-ADDED SUGAR BEANS

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Introduction

Sugar-cooked beans are snack items in countries other than the U.S., particularly in Asia. The sugar-beans can be eaten as is, or mixed with other foods, such as salads. The snack has potential in the U.S., given the recent consumer interest in "healthy" foods, and the increasing ethnic population. A typical batch process for sugar beans is used in the present work. The objective of this study was to investigate the weight gain and the textural changes in the sugar-cooked beans throughout the process.

Materials and Method

Whole dry Michigan cranberry beans (*Phaseolus vulgaris)* were used. The process flow was 1) soak beans for 12 hr in water at an initial temperature of 77°C containing less than 0.1% (w/w) sodium bicarbonate and sodium polyphosphate; 2) blanch beans in 98°C [containing above additives] for different heating times [9, 10, 11, and 12 min.]; 3) Sugar-cook in 50% sugar/water solution at 70°C for 45 minutes. Beans were prepared in duplicate treatments and evaluated for physical quality characteristics.

Weight gain and bean texture during and after soak, after blanch, and after the sugar cook were measured using a Kramer Shear Press at 23°C. Maximum force at a shear press speed of 4 was measured. Texture measurements were expressed in units of Newtons per reference weight of 20.3 g of beans. Five samples were measured for each run. Hydration ratio = drained weight/initial dry weight. Initial dry weight was 100 g.

Results and Discussion

Bean Soaking

Greater than 95% of the weight gain was achieved within the first four hours of a 12-hour soaking procedure (Figure 1). The bean softening followed a similar trend. Within 4 hours of soaking, the maximum force during shearing of the beans had been reduced to 90% of the total reduction at 12 hours (Figure 1). Therefore, it may not be necessary to soak these beans for the full 12 hours to obtain nearly

Figure 1. Relative weight gain and texture during soaking of beans

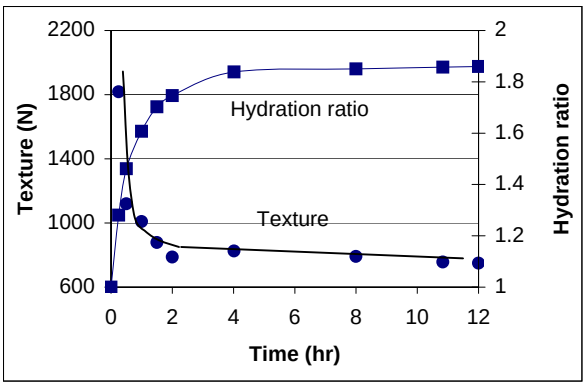
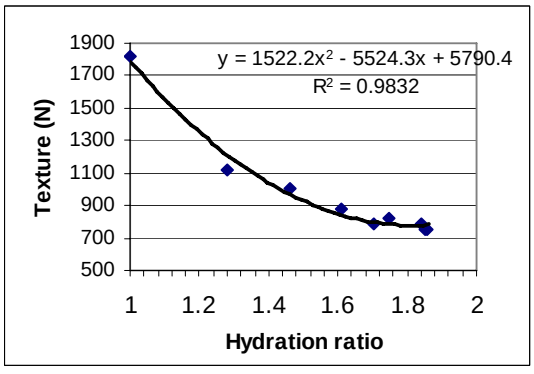


Figure 2. Correlation of drained weight and texture



identical texture results. An inverse relationship between texture and hydration ratio was demonstrated (Fig. 2), similar to results for Anasazi beans (Occena et al 1992).

Thermal Treatments

The greatest component (90%) of the total weight gain for the sugar-cooked beans occurred during soaking (Fig. 3). Weight gain increased slightly as blanch time increased. Further increases in total weight occurred during the sugar-cook phase of the bean preparation procedure (Fig. 3).

A comparison of Figures 3 and 4 shows that an increase in bean weight during blanching softens the bean, while increases in weight during the sugar cook lead to hardening. The relatively lower temperature and longer heating time established during the tertiary sugar-cook cycle may influence bean firmness due to osmotic pressure differential and/or molecular cross-linking of the cotyledon constituents. Thus, the initial force required to shear the beans is increased.

Figure 3. Relative weight gain at end of soak and at end of various cook times

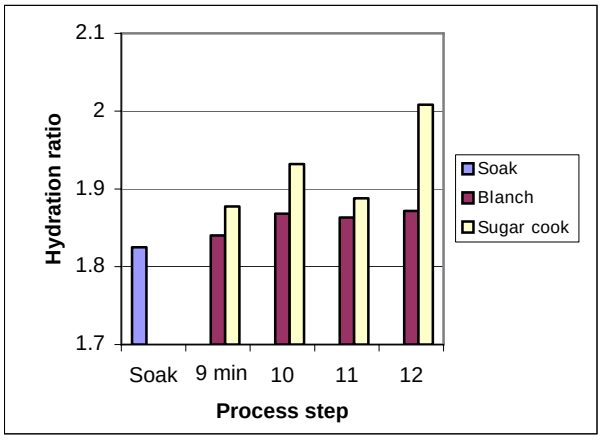
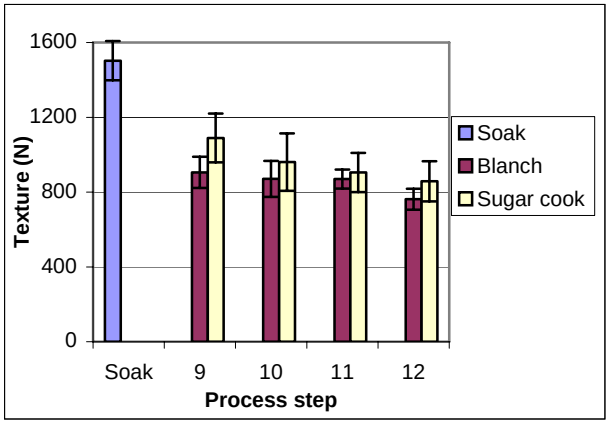


Figure 4. Bean texture during processing



The processes utilized were effective for producing a palatable bean snack product. These data will be useful for scale-up and optimization of the process.

Acknowledgement

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CASES OF PAST CYTOPLASMIC INTROGRESSION AND INTROGRESSION OF NUCLEAR GENES IN COMMON BEAN (*Phaseolus vulgaris* L.)

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Introduction

Domesticated common bean is in genetic contact with its conspecific wild relative in various locations in the highlands of Latin America. Wild-weed-crop complexes resulting from intraspecific hybridisation have been reported from several sites [1-3] and studies on genebank accessions have provided additional instances of probable introgression between wild and domesticated beans and between different races of domesticated bean [4-6]. Gene flow from wild to domesticated beans may generate variability in landrace populations [2]; gene flow from domesticated to wild beans, or between domesticated beans, is relevant to concerns about possible escape of transgenes.

These reports of introgression all involve markers coded by nuclear genes (morphology, phaseolin type, RAPD bands). However, studies of organellar DNA may demonstrate cytoplasmic introgression, undetectable by study of nuclear markers alone [7]. This phenomenon is known as cytoplasmic capture or chloroplast capture and occurs when a hybrid and its derivatives backcross repeatedly, as seed parent, to the pollen parent. Eventually the nuclear genome of the pollen parent is more or less restored, but now occurs in the cytoplasm of the original seed parent. In a survey of variation in chloroplast haplotypes in wild and domesticated common beans from the CIAT genebank, we found examples of probable introgression, including chloroplast introgression, additional to those that have been previously reported.

Materials and Methods

Chloroplast haplotypes were determined as described previously [8] for 127 accessions of wild beans and 160 accessions of domesticated beans from the CIAT genebank. Information on phaseolin type and racial classification (domesticated accessions only, following Beebe *et al.* and Singh *et al.* [4, 9]) was available for most of these accessions.

Results and discussion

Sixteen chloroplast haplotypes (A to P) were found among wild beans: eight in Mesoamerica (B, H to K, N to P), five in South America (C to F, and M) and three in both regions (A, G and L). Four haplotypes characterise domesticated beans, with different haplotypes being associated with different races (Table 1). We found 25 cases (approximately 9 %) in which the expected correlations between morphology and/or phaseolin type and/or chloroplast haplotype broke down. We suggest that these represent cases of introgression. Since common bean is often reported as autogamous, these cases represent an important proportion of the accessions examined. In Table 2, we have classified these according to the probable direction of gene flow.

These results suggest that introgression may proceed in both directions: from wild to crop and from crop to wild, the former one being more frequent. Gene flow from crop to wild may be reduced when characteristics of the crop that affect survival in the wild habitat make a proportion of the F2 and further generations be quickly eliminated, but still may occur in common bean. Cytoplasmic introgression may be as frequent as introgression of nuclear genes, but they are not necessarily correlated. It means, cytoplasmic introgression may occur without significant introgression of nuclear genes, and the opposite situation may also occur. These results are reported for genebank accessions, therefore field research should be conducted to estimate the frequencies of these types of introgression and their impact on natural populations and local landraces.

Table 1**.** Chloroplast haplotypes and phaseolin types associated with different races of domesticated beans

Mesoamerican Races	Chloroplast haplotype	Phaseolin type
Mesoamerica	K	S
Durango	K	S
Jalisco	L	S
Guatemala	I	S
Andean Races (Nueva Granada, Peru, Chile)	C	T, C, H

Table 2. Introgression in accessions of common bean.

Type of introgression	No. of cases	Evidence	Accession nos.	Provenance
Nuclear	11			
Gene flow via pollen from wild to domesticated beans	8	Morphology and chloroplast haplotype of domesticated bean but phaseolin type of local wild bean	G1791, G1797, G4342, G7237, G8167, G24648, G24674, G24738	Mexico (Jalisco, Michoacán), Colombia (Boyacá, Cundinamarca, Huila)
Gene flow via pollen from domesticated to wild beans	1	Morphology and chloroplast haplotype of local wild bean but phaseolin type of domesticated bean	G24758	Colombia (Boyacá)
Gene flow via pollen between domesticated races	2	Morphology of one race but phaseolin of a different race	G11751, G21222	Colombia (Nariño), Peru (Junín)
Cytoplasmic	14			
Chloroplast introgression from wild to domesticated beans	2	Morphology and phaseolin type of domesticated bean but chloroplast haplotype of local wild bean	G799 (1 of 5 plants), G11010 (1 of 5 plants)	Mexico (Durango), Guatemala (Sacatepéquez)
Chloroplast introgression from domesticated to wild beans	3	Aberrant morphology and/or polymorphism for phaseolin types of local wild/domesticated beans, chloroplast haplotype of local landrace	G21197, G24798, G50388	Guatemala (Quetzaltenango), Colombia (Cundinamarca), Argentina (Jujuy)
Chloroplast introgression between domesticated races	9	Morphology of one race but chloroplast haplotype of another race	G1042, G1809, G2568, G3161, G3886, G11010 (1 of 5 plants), G23993, G21222 (1 of 5 plants)	Mexico (Durango, Jalisco, Sinaloa), Guatemala (Santa Rosa), Colombia (Antioquia, Huila, Nariño, Tolima), Ecuador (Loja)

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LEVEL OF INTROGRESSION IN INTER-SPECIFIC (*Phaseolus vulgaris* x *P. acutifolius*) CONGRUITY-BACKCROSS LINES

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International Center for Tropical Agriculture. CIAT

Introduction

Congruity backcrossing (CBC) was shown by Mejía-Jiménez et al. (1994) to be a useful technique for increasing the success of introgressing tepary bean (*Phaseolus acutifolius*) genes into a common bean (*P. vulgaris*) background. This technique uses multiple back-crosses done alternately between both species which allows the recovery of mature back-cross F1 hybrid plants. Congruity backcrossing was postulated by the authors to reduce inter-specific hybridization barriers such as hybrid sterility, genotype incompatibility, embryo abortion often found in crosses between *P. vulgaris* and *P. acutifolius*. In their original study, Mejía-Jiménez et al. (1994) used seed protein (phaseolin) and morphological markers to verify introgression among the congruity backcross lines generated by a cross between the Tepary accession G4001and the common bean variety, ICA Pijao. In this study, our primary objective was to quantify the level of introgression in the same lines using amplified fragment length polymorphism (AFLP) marker analysis.

Materials and Methods

Plant Material: A total of 61 genotypes were used. The first part of the experiment included four lines that were derived from a single backcross of the inter-specific F1 hybrid to the common bean parent (CBC_1F_5), and 35 lines that were derived from five alternating congruity backcrosses (CBC_5F_{10}). Among the latter, 18 lines were drought tolerant and 17 were drought susceptible. The parents of the introgression lines, G40001 and ICA Pijao, were used as controls to confirm the identity and source of the DNA polymorphisms. In a second part of the experiment the level of introgression in lines derived from recurrent backcrossing and selection was compared with the level of introgression found in the lines developed by congruity backcrossing. These included 14 common bean advanced lines (from the XAN and VAX series) and varieties ('Tara' and 'Jules') derived from tepary beans. Many of these genotypes were originally developed for resistance to common bacterial blight, a trait that was identified in several tepary bean accessions, such as G40020, that were also included in this study. Three other tepary beans, G40035, G40036, G40006, were selected to represent other accessions of *P. acutifolius* and to confirm the identity of markers introgressed from tepary parents into the common bean advanced lines and varieties.

AFLP and genetic similarity analysis: Total genomic DNA was extracted from 2 g of fresh leaf tissue. Amplicon-template preparation, pre-amplification, and selective amplification followed the protocol of the Gibco BRL small genome AFLP analysis system kit based on *Eco*RI (E) -*Mse*I (M) adapters. An initial parental screening was conducted with 24 primer combinations all with 3 selective nucleotides. The four most polymorphic primer combinations for the two parents, G40001 and ICA Pijao, were chosen and run on the full set of introgression lines. Fragments were sized by comparison to a 50bp ladder molecular weight size standard. All the polymorphic AFLP bands between 100 and 350 bp were scored for presence or absence among the lines. Genetic similarities between genotypes was determined with the Dice coefficient using the software packages SAS and NTSYS 2.02.

Results

From 73% to 95% of bands were polymorphic in the twenty-four AFLP combinations that were tested on the parents and bulks from the congruent backcross set. Four AFLP combinations had high polymorphism rates, clear amplification profiles and well-distributed ranges in PCR product sizes. These four combinations generated 207 bands including monomorphic and polymorphic bands across the 41 congruent backcross individuals and their two parents (Table 1). On average each primer combination produced 51.8 bands, however some primer combinations produced more bands than others. Each band was scored as a possible introgression event if it was present in one of the parents and in several of the introgression lines. Markers showing predominantly the tepary allele in the congruent backcross lines were

not considered. Non-parental bands (bands present in the introgression lines but absent in either parent) occurred on average only 2.25 times per AFLP combination. The remaining genotypes, including the common bean varieties and advanced lines and the tepary accessions, were evaluated for the same bands that were present in the introgression lines and their parents. Analysis of the genotypes with the four different primer combinations produced genetic similarity matrices that were significantly correlated. A multiple correspondance analysis was done for the datasets from each AFLP combination for the complete set of 61 genotypes. The results were similar for each combination, consistently showing three major clusters among the genotypes: 1) The most distinct cluster consisted of the four tepary bean accessions; 2) a second cluster contained all of the common bean varieties or advanced lines without tepary bean parentage (ICA Pijao, DOR476, BAT41). This cluster also contained previously developed genotypes with tepary beans in their pedigrees (Tara, Jules, MAR1, SEL1309 as well as all the VAX and XAN lines), all of the CBC_1F_5 introgression lines, and a group of nine CBC_5F_{10} introgression lines all of which had low introgression rates. 3) The remaining cluster was closely related to the other common beans but consisted solely of CBC_5F_{10} introgression lines with higher levels of introgression than the previous group. Drought tolerant introgression lines were found in both of the common bean clusters. Congruity backcross lines derived from 5 cycles of inter-specific hybridization (CBC_5F_{10}) showed higher introgression on average than the lines derived from a single backcross (CBC_1F_5) (Table 2).

Discussion

Compared to a single recurrent backcross, the process of congruity backcrossing helped increase the level of introgression from *P. acutifolius* into *P. vulgaris*. However, the overall amount of introgression was still below the level expected, indicating that there must be genetic incompatibilities in the inter-specific crosses that are still not resolved even with five cycles of congruity backcrossing. Introgression was seen to be even lower in cultivated varieties and advanced lines previously generated from inter-specific hybridization with tepary beans that did not use congruent backcrossing. Many of the initial common bean genotypes containing tepary genes have now been used in multiple generations of crosses and recurrent selection further diluting the introgression that has occurred. The lower than expected introgression rate has serious implications for the transfer of quantitative traits from tepary bean to common bean. While a simple trait such as common bacterial blight resistance has been transferred successfully from tepary bean to common bean advanced lines and varieties, it may be more difficult to transfer complex traits such as drought tolerance which would rely on the simultaneous introgression of the right combination of tepary genes.

Table 1. Number of bands that were monomorphic, non-parental or polymorphic (introgressed or non-introgressed) in four AFLP combinations tested on the congruent backcross individuals and parents, ICA Pijao (*Phaseolus vulgaris*) and G40001 (*P. acutifolius*).

					Polymorphic			
	combination	total	Mono-morphic	Non-parental	Introgressed	%	Non-introgressed	%
1	E-ACC M-CTA	48	7	1	10	25.0	30	75.0
2	E-ACA M-CAT	62	10	5	23	48.9	24	51.1
3	E-AAG M-CTC	34	9	2	11	47.8	12	52.2
4	E-AAG M-CTT	63	10	3	16	32.0	34	68.0
	Total	207	36	11	60	37.5	100	62.5

Table 2. Level of introgression in congruity backcross lines and their parents.

species/generation	genotype - selection history	% bands introgressed			Expected introgression
		min	max	avg	
P. vulgaris parent	ICA Pijao (parent)	--	--	0.0	Na
P. acutifolius parent	G40001(male parent)	--	--	100.0	Na
inter-specific CBC_1	ICA Pijao x (ICA Pijao x G40001)F1	2.5	4.9	3.6	25.0
inter-specific CBC_5	ICA Pijao x (CBC_4)	1.2	11.0	6.0	32.8

Reference

Mejía-Jiménez A, Muñoz C, Jacobsen HJ, Roca WM, Singh SP (1994) Theor. Appl. Genet. 88: 324-331

GENETIC DIVERSITY OF THE CIAT TEPARY BEAN (*Phaseolus acutifolius* A. Gray) COLLECTION MEASURED WITH AMPLIFIED FRAGMENT LENGTH POLYMORPHISM MARKERS

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Rationale

Tepary bean (*Phaseolus acutifolius* A.Gray) is in the tertiary gene pool of common beans (*P. vulgaris* L.) and as such represents a potential but difficult to use genetic resource for the improvement of common beans. The two species have been crossed, despite high embryo abortion, using congruity or recurrent backcrossing and these interspecific hybridizations have been used to incorporate common bacterial blight resistance into common bean. Notwithstanding their utility for the improvement of other species, tepary beans are a useful and interesting crop in their own right, especially for dryland agricultural systems. They are known to have high drought and salinity tolerance, good nutritional quality and a tradition of cultivation in Mexico, South western United States and Central America, that goes back 5000 years. Many landraces exist but there are no improved varieties. For this it is critical to have a baseline data on the diversity that exists within tepary beans. Previous authors (Scinkel and Gepts, 1989, Plant Breeding 102: 182-195; Garvin and Weeden, 1994; Crop Science 34: 1390-1395) have suggested that tepary beans seem to be less diverse than common bean or lima beans. They are thought to have had a single center of origin and to have been distributed from a few original sites across the present distribution. The objective of this research was to study the patterns of diversity within the species and its placement relative to other *Phaseolus* species that were used as outgroups, using amplified fragment length polymorphism (AFLP) markers. Relative to other molecular markers, AFLPs tend to be evolutionarily conserved markers and serve to reference different species relative to each other. Additional objectives of the study were to determine if *P. acutifolius* and *P. parvifolius* merit being separate species and if molecular markers can distinguish between the botanical varieties var. *acutifolius* and var. *tenuifolius* within the species *P. acutifolius.* AFLP markers have been applied before to study wild species of *Phaseolus* (Tohme et al., 1996; Crop Science 36: 1375-84) and lima bean, *P. lunatus*, accessions and their close relatives (Caicedo et al., 1999; Crop Science 39: 1497-1507) but not to tepary beans.

Materials and Methods

A total of 127 genotypes from the Genetic Resources Unit of CIAT were analyzed in the experiments. The outgroup consisted in 10 genotype from the *Phaseolus* genus including 4 *P. vulgaris* (common bean); 4 *P. lunatus* (lima bean); 1 *P. coccineus* (scarlet runner bean); and 1 *P. glabellus* genotype. For both common and lima beans a wild and a cultivated representative from both the Andean and Mesoamerican genepools was included in the analysis. For the other species only a single representative was analyzed. A total of 117 tepary beans and their close relatives were analyzed, consisting in 49 cultivated *P. acutifolius* var. *acutifolius*; 44 wild *P. acutifolius* var. *acutifolius*; 12 *P. acutifolius* var. *tenuifolius;* and 12 *P. parvifolius* accessions. The genotypes were grown in the greenhouse and total genomic DNA was extracted from 2 g of fresh leaf tissue. AFLP fragments were generated with the Gibco BRL AFLP analysis system I kit. In a previous study, we chose the combination of E-AAG and M-CTT primers based on a survey of *Eco*RI (E) -*Mse*I (M) adapters and primers with 3 selective nucleotides each. PCR products were run on 4% silver-stained polyacrylamide gels for 1, 1.5 and 2 hours to resolve as many fragments as possible. Bands were sized by comparison to a 50bp ladder molecular weight size standard. All the polymorphic AFLP bands between 100 and 400 bp were scored for presence or absence among the lines and used to calculate the similarity matrix. Genetic similarities between genotypes was determined with the Dice coefficient using the software packages SAS (SAS Institute, 1989) and NTSYS 2.02.

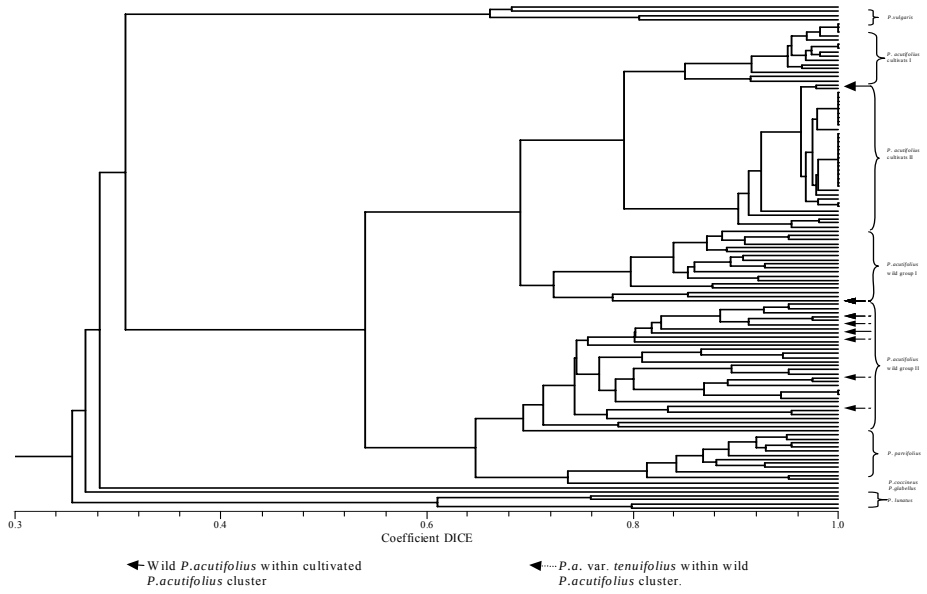
Results and Discussion

The AFLP combination used in this study had a good polymorphism rate, clear amplification profile and well-distributed range in PCR product sizes. The AFLP combination produced a total of 167 bands. Of these, 99.5% of the bands were polymorphic across all species although there was substantial monomorphism within the cultivated *P. acutifolius*. Both monomorphic and polymorphic band were used to determine the genetic similarity between genotypes. Figure 1 shows the dendrogram created for the AFLP bands. The structure of the dendrogram agrees with known taxonomic relationships for the six species represented in the study. *P. lunatus* was the most distant group, followed by *P. glabellus* and *P. coccineus*. *P. vulgaris* was the closest to the *P. acutifolius - parvifolius* clade. The level of similarity was around 35% between the five species/groups. Within both *P. vulgaris* and *P. lunatus* the distinction between Andean and Mesomerican genepools was clear. The level of similarity between genepools was higher in *P. vulgaris* (68%) than in *P. lunatus* (62%). Within the *P. acutifolius – parvifolius* clade, all the accessions shared up to 54% similarity. Five groups could be distinguished within this clade: 1) cultivated *P. acutifolius* from Central and North America 2) cultivated *P. acutifolius* from North America (mainly Sonora and Sinaloa), 3) wild *P. acutifolius* var.

acutifolius 4) wild *P. acutifolius* var *acutifolius* and *tenuifolius*; and 45) *P. parvifolius*. These five groups could be organized hierarchically into two supergroups, consisting of groups 1, 2 and 3 together and groups 4 and 5 together. The first grouping contained all the cultivated *P. acutifolius*, while the second grouping contained all the *P. acutifolius* var. *tenuifolius* and *P. parvifolius* accessions. The wild *P. acutifolius* accessions were distributed among the two groups, with some more allied to the cultivated accesssions of the same species and others allied to the *P. parvifolius* group. Within the first group, the two cultivated groups (1 and 2) were related at 80% similarity and these were related to the wild accessions (group 3) within that group at 68% similarity. Within the second group, the P. parvifolius and P. acutifolius (both var. acutifolius and tenuifolius) were related at 64% similarity. The groups were distinguishable at 54% similarity.

The high genetic similarity detected with AFLP markers for all the cultivated tepary beans, seems to indicate that the crop may have arisen from a single domestication event that led to a genetic bottleneck which limits diversity within the cultivars. From this study, there is very little evidence for introgression from wild relatives into the cultivated genepool after the initial domestication event. Tepary beans are known to have a very low crossing rate that limits the creation of new diversity within the crop. The lack of diversity within the cultivated tepary bean is a serious limitation for improvement of the crop although it belies some of the variability found for disease and insect resistance within the cultivars. Biotic resistances are often fast evolving characteristics so could be expected to have been generated by mutation even without a lot of initial diversity or inter-crossing. However, that lack of diversity in other characteristics such as plant morphology and adaptation range has serious implications for improving the species agronomically and using the species in inter-specific hybridization. The AFLP data presented here also clarifies the relationships within the *P. acutifolius – parvifolius* clade which has been controversial and suggest that *P. acutifolius* and *P. parvifolius* probably do not deserve to be different species, but could qualify as possible subspecies or varieties within the species. The high amounts of diversity found in the wild *P. acutifolius* and *P. parvifolius* accessions are an interesting resource for breeding tepary bean cultivars in the future.

Figure 1. Dendrogram showing the associations among 114 accessions of cultivated and wild tepary beans (*Phaseolus acutifolius* and *P. parvifolius)* and 10 other related species, based on UPGMA clustering for the AFLP banding pattern from primer combination E-AAG, M-CTT.



POSSIBLE ORIGINS OF COMMON BEAN (*Phaseolus vulgaris* L.) CULTIVATED IN SPAIN IN RELATION TO THE WILD GENETIC POOLS OF THE AMERICAS

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Introduction

Common beans, as well as many other crops, were brought to the Old World soon after the discovery of the Americas. It is highly probable that Spain has been the entry point for beans in Europe (Gepts & Bliss 1988). Authors from the 18th and 19th centuries mentioned the great variation found in Spain for this crop, that has been conserved up to now (Moreno et al., 1983). Historical and linguistic sources provide little evidence on the spread of beans in Spain (Lora González & Hernández Bermejo, 1990). Molecular data have been used widely to suggest evolutionary patterns of origin and dispersal of plants in secondary centers of domestication, although by no means are the only argument (Gepts, 1993). Here we compare Spanish cultivars and wild genetic pools distributed in the Americas using molecular markers (phaseolin and RAPDs), trying to infer the origin of Spanish cultivars from the Mesoamerican, Colombian and Andean domestication centers.

Materials and Methods

A total of 88 genotypes were selected (Table 1). Fifty-four Spanish cultivars were used: 21 from CIAT (CIAT, 2001) and 33 from CRF-INIA of Spain (CRF-INIA, 2001). Thirty wild accessions from CIAT (Tohme et al., 1996), representative of the major geographical and ecological regions in the area of domestication centers of common beans, were also used. Four accessions were chosen as controls: two cultivated *P. vulgaris* from the Andes and Mesoamerica (CIAT G Numbers G4494 and G5773, respectively), one *P. coccineus* (G35243) and one *Vigna unguiculata* (G24133) from the Old World that served as outgroup. RAPD analysis was carried out according to Williams et al. (1990), modified by Martín & Hernández Bermejo (2000). Twenty decamer primers ("Kit O" from Operon Technologies Inc., Alameda, CA) were initially tested with six different genotypes. Four of these RAPD primers were selected as most informative and consistent to analyze all samples. Molecular sizes of the amplification fragments were estimated by reference to a 100-bp ladder (Pharmacia). RAPD data were used to obtain the relationships among accessions with the Dice's similarity index (Dice, 1945) to construct the UPGMA dendrogram, using the SAHN-clustering and TREE programs of NTSYS-pc v. 1.8 (Rohlf, 1994). Phaseolin patterns were analyzed by SDS-PAGE as in Gepts et al. (1986).

Results and Discussion

The four selected primers generated 136 reliable bands. The size range of these fragments varied between 230 and 1500 bp. The cluster analysis obtained using the RAPD data formed, near to the 0.80 similarity level, a main cluster (Groups Ia, Ib and Ic in Table 1) and other smaller groups of genotypes (Groups II, III and IV). Group Ia contains 57 accessions (42 cultivated Spanish samples, 14 wild accessions and the Andean control), that represent 78 % of the Spanish cultivars and 47 % of the wild accessions. Group Ib contains only wild populations (center of Peru and Western center of Bolivia) and Group Ic only includes one cultivar from Spain. Group II has 11 samples: 10 Spanish cultivars and the Mesoamerican control. Groups III and IV include mostly wild populations. *Phaseolus coccineus* appears between groups II and III, located at very close similarity level; the Mexican accession (G23470) and *Vigna unguiculata* appear in the cluster analysis very distant of the above mentioned groups. Diversity in phaseolin patterns was also compared among the 54 cultivars from Spain, and four types were found. The "C" phaseolin was present at the highest frequency (57 %), followed by "T" type (26 %), "S" type (15%) and "H" type (2 %). The clustering obtained using RAPD data, and taking account the phaseolin data, suggests that most Spanish beans (Groups Ia and Ic) share the Andean South wild gene pool (center and South of Peru, Bolivia and Argentina, included in the groups Ia and Ib). In the Andean zone, South of Peru, Northern Argentina or Eastern Bolivia (Group Ia) are the most likely candidates as the primary domestication sites of the Spanish domesticated common bean. Furthermore, a few Spanish cultivars (Group II) could be related with the Mesoamerican gene pool. These results are concordant with those obtained by Espejo-Ibáñez et al., (1994) using isoenzymes, and those obtained by Gepts and Bliss (1988) using phaseolin evidence.

Table 1. List of the plant material selected for this study and groups (I to IV) defined using RAPD markers

Acces. (1)	B. S. (2)	Origin		P. T.	Group	Acces. (1)	B.S. (2)	Origin		P. T.	Group
		Country	Province					Countr	Province		
G1820	C	Spain	Albacete	T	Ia	G19895	W	Argenti	Tucumán	I1*	Ia
G1016	C	Spain	Burgos	C	Ia	G19901	W	Argenti	Tucumán	T*	Ia
G1017	C	Spain	Burgos	T	Ia	G19890	W	Argenti	Salta	T*	Ia
G1820	C	Spain	Cádiz	T	Ia	G19893	W	Argenti	Salta	T*	Ia
G1820	C	Spain	Cuenca	C	Ia	G24318	W	Argenti	Salta	T*	Ia
G1525	C	Spain	Granada	C	Ia	G19889	W	Argenti	Jujuy	T*	Ia
G1526	C	Spain	Granada	T	Ia	G21197	W	Argenti	Jujuy	I3*	Ia
G1015	C	Spain	Huesca	C	Ia	G35243	P. c.	Portugal			
G1818	C	Spain	La Coruña	T	Ia	G24133	V. u.	China			
G1019	C	Spain	León	C	Ia	G4494	Contr	Colomb		T	Ia
G1819	C	Spain	León	T	Ia	G5773	Contr	Colomb		S	II
G1019	C	Spain	León	C	Ia	BG-	C	Spain	Barcelona	T	II
G1468	C	Spain	La Rioja	C	Ia	BG-	C	Spain	Teruel	S	II
G1017	C	Spain	Navarra	T	Ia	BG-	C	Spain	Cantabria	S	II
G1019	C	Spain	Orense	C	Ia	BG-	C	Spain	Orense	C	Ia
G1016	C	Spain	Palencia	C	Ia	BG-	C	Spain	Pontevedr	T	II
G1819	C	Spain	Palencia	C	Ia	BG-	C	Spain	Asturias	S	II
G1443	C	Spain	Pontevedra	C	Ia	BG-	C	Spain	Asturias	C	Ia
G1440	C	Spain	Salamanca	C	Ia	BG-	C	Spain	Asturias	C	Ia
G1578	C	Spain	Zamora	H	Ia	BG-	C	Spain	Asturias	C	Ia
G1441	C	Spain	Zamora	T	Ia	BG-	C	Spain	Asturias	T	Ia
G2347	W	México	Oaxaca	S*		BG	C	Spain	Asturias	C	Ia
G1990	W	Guatemala	Sacatepeque	S*	III	BG-	C	Spain	Ávila	T	Ia
G2341	W	Costa Rica	San José	M1*	III	BG-	C	Spain	Soria	C	Ia
G2111	W	Colombia	Cundinamar	CH*	III	BG-	C	Spain	Burgos	T	Ic
G2469	W	Colombia	Boyacá	S*	III	BG-	C	Spain	Burgos	C	Ia
G2475	W	Colombia	Boyacá	T*	III	BG-	C	Spain	Zamora	S	II
G2358	W	Ecuador	Azuay	I*	III	BG-	C	Spain	Zamora	C	Ia
G2372	W	Ecuador	Chimborazo	I*	IV	BG-	C	Spain	León	C	Ia
G2372	W	Ecuador	Loja	I*	IV	BG-	C	Spain	Ávila	C	Ia
G2124	W	Peru	Cajamarca	I*	IV	BG-4496	C	Spain	Zamora	C	Ia
G2358	W	Peru	Cajamarca	I*	IV	BG-10548	C	Spain	Pontevedr	S	II
G2358	W	Peru	Piura	I*	IV	BG-11037	C	Spain	Navarra	S	III
G1285	W	Peru	Huanuco	T*	Ib	BG-11058	C	Spain	La Rioja	C	Ia
G2341	W	Peru	Junín	T*	Ib	BG-11731	C	Spain	Cuenca	S	Ia
G2342	W	Peru	Junín	T*	Ib	BG-11732	C	Spain	Cuenca	S	II
G2342	W	Peru	Apurimac	H*	Ia	BG-11736	C	Spain	Cuenca	C	Ia
G2342	W	Peru	Apurimac	H*	Ia	BG-22827	C	Spain	Cantabria	C	Ia
G2345	W	Peru	Cuzco	Pa*	Ia	BG-22832	C	Spain	Cantabria	C	Ia
G2345	W	Peru	Cuzco	T*	Ia	BG-22836	C	Spain	Cantabria	C	Ia
G2344	W	Bolivia	Tarija	T*	Ia	BG-26166	C	Spain	Orense	C	Ia
G2344	W	Bolivia	Tarija	Ta*	Ia	BG-26172	C	Spain	Orense	T	II
G2344	W	Bolivia	Cochabamb	T*	Ib	BG-27961	C	Spain	León	C	Ia
G1989	W	Argentina	Tucumán	C*	Ia	BG-27962	C	Spain	Asturias	C	II

[1] Acc.= accession number in the germplasm bank of the CIAT; BG…= accession number in the germplasm bank of CRF-INIA.. [2] B.S.= Biological Status of the analyzed materials: Cultivated; W= Wild; *P. c.= P. coccineus; V. u.= Vigna unguiculata.* [3] P.T. = Phaseolin types; those marked (*) as per Tohme et al. (1996).

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BROADENING THE USE OF DRY BEAN SEEDS

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Introduction

The surface area for cultivating dry beans in Spain has become much smaller over the last years and although the average consumption per person has increased, the overall figure for consumption is still low. This increase in unitary consumption is directly related to the coming into fashion of the "Mediterranean diet". Even so, rejection among the younger population can be observed; probably, among other reasons, due to the flavour, unattractive appearance and problems with flatulence.

It is a particularly unattractive crop for Spanish producers as the production costs in Spain, and other industrialised countries, are high and sale prices low, and therefore the industry has to import the product from other countries. The varieties that have been a "Guarantee of quality and origin" do, however, sell at much higher prices and are more widely accepted.

It is well known that leguminous plants and cereals complement each other regarding several essential amino acids. The consumption of both products has always been recommended when considering a healthy balanced diet. On the other hand, products for human consumption coming from organic agriculture are being well accepted, and increasing their offer.

Bearing in mind aspects of healthy eating, environmental sustainability and other economic factors (giving the end product a higher added value), we have set up a R & D project.

Objectives

The objectives are:

- To increase the nutritional value and dietary convenience.
- To broaden the present market offer.
- To reduce imports in this sector.
- To increase the added value of cereals and leguminous crops.
- To ensure good preservation through the whole commercial chain.
- To make consumption easier.

Methods and Materials

The following stages of the general plan have been developed: Evaluation and selection of different genotypes under ecological cultivation, according to their vegetative and productive characteristics and the physical / chemical quality of the seed.

Fifty-one dry bean seed genotypes were evaluated from different varietal types (Sanjuán et al., 1998); were included USWK-6 and USWA-70 kindly supplied by Dr. Miklas. The assay took place under two different cultivation methods in the Valencian Community (Spain): a) ecological cultivation, and b) traditional cultivation. The design was made up of random blocks with two repeat series and plots of 25-30 plants, in each cultivation method. Measurements were made on a plot level, and at individual level taking into account 3-5 plants per cultivation method, block, and plot. Both vegetative and productive factors were evaluated.

At present the measurements regarding chemical composition are being finished. The development of a coated snack made from a mixture of bean flours and wheat (Llorca et al., 2001).

Results

Significant differences between cultivation means for vegetative and productive factors were observed and also between genotypes in each environment. Ecological cultivation gave rise to: more vegetation overall and higher production, greater presence of the *Acanthoscelides obtectus* bruchid, and *Coccinella septempunctata* (beneficial insect), better overall health.

Regarding the development of a snack, positive results have been obtained from: a) the study of the most suitable mixture of bean and wheat flours for the coating of the snack; b) the technological procedure for the preparation of the aforementioned coating.

At present a chemical and micro-structural study of the snack is being carried out on the bean flour, produced from ecological agriculture, and wheat.

Acknowledgements

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COMPARATIVE ANALYSIS OF GENETIC STRUCTURE AND DIVERSITY IN WILD LIMA BEAN POPULATIONS FROM THE CENTRAL VALLEY OF COSTA RICA, USING MICROSATELLITE AND ISOZYME MARKERS.

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Introduction

A considerable amount of information has been accumulated on the genetic diversity and structure of the wild populations of Lima bean (*Phaseolus lunatus* L.) in the Central Valley of Costa Rica. Data on isozyme markers showed low interpopulation gene flow, a balance between among- and within- population variability, a predominantly autogamous breeding system and consequently a lack of heterozygosity in the sampled populations (Maquet *et al*., 1996; Zoro, 1999; Maquet *et al.*, 2001). Field estimation of mating system parameters and analysis of factors influencing these parameters in the valley (such as pollen and seed dispersal, vegetative growth) confirmed also the mixed mating but predominantly selfing breeding system of wild Lima beans (Hardy *et al.*, 1997; Baudoin *et al.*, 1998).

Nevertheless, isozyme markers did not allow to analyze a high number of loci and revealed only a low level of allelic diversity in our case, which constitute limiting factors to study a key element in genetic structure of populations : gene flow at both short and long distance. Therefore, a study was initiated using higher polymorphic loci (i.e. microsatellites) and considering the maternal genotypes (leaves) rather than progenies (seeds). For this purpose, geographical coordinates of many wild populations in the Central Valley were encoded to obtain a distant matrix. In three representative regions of the valley (Escazú, Heredia and San Ramón), individuals of different populations were sampled in 2000 and 2001 from a central population and along a grid (each four meters in both directions) with the aim to measure gene flow using microsatellite markers.

Material and Methods

The first step was to develop a protocol of DNA extraction from leaves dried with silicagel and collected from populations in the Central Valley. The second step was to choose primers allowing DNA amplification and expressing polymorphism. For this purpose, we tested 73 primers isolated from *P. vulgaris* L. and provided by the Unit of Biotechnology at CIAT (Cali, Colombia). The third step was to determine the primer pairs likely to reveal polymorphism among individuals of wild populations directly collected along the grid established in the three regions of the Central Valley. The last step was to compare the parameters of population genetics obtained from the microsatellite and isozyme markers.

Results and Discussion

Using different protocols of DNA extraction, we retained with some adjustments the DNA extraction kit provided by Promega due to its lowest time consumption and efficiency for extracting DNA from dried old leaves. PCR reactions were carried out in 20µL mixtures containing 20 ng of genomic DNA template, 1 x reaction buffer, 0.1µM of each primer, 0.25 mM of each dNTPs, 1.5 U Taq DNA polymerase. After a thermocycle program, 5µL of PCR products were separated on 6% polyacrylamide gels in denaturing conditions for about 1h15 min at 1800 V and visualized using a silver staining procedure.

Out of 73 primer pairs isolated from *P. vulgaris*, 57 (78%) amplified in *P. lunatus*, with a good polymorphism obtained from 12 primer pairs : AG1, BM98, BM114, BM141, BM142, BM149, BM156, BM159, BM160, BM161, GAT554 and GAT591. Among the latter, four primers AG1, BM160, BM161 and GAT554 were applied in a preliminary trial to amplify 191 individuals belonging to 10 populations distributed in Heredia. Sequencing gels were analyzed with the software GelCompar ver. 4.2. and the population genetics parameters were determined for the 191 individuals using the softwares FSTAT ver. 2.9.3.2. (Goudet, 1995) and Popgene ver. 3.2. (Yeh, Boylen, 1997). Table 1 show the first results obtained from Nei diversity indices (1973) and F-statistics (Weir, Cockerham, 1984).

Isozyme data were obtained from seeds collected during several years in 138 wild populations distributed in the whole valley and from the 13 following enzyme systems : ADH, DIA, END, EST, G6PDH, GDH, GPI, LAP, MDH, PGDH, PGM, SKDH and SOD. Number of individuals per population varies according to the period of collecting missions and the population size. Table 1 shows similar results between the two markers for F_{IT}, F_{IS} and F_{ST}. However, as expected by the studies of Ciofi *et al.* (1998), H_o, H_s, H_T and D_{ST} values are lower for isozymes than for microsatellites.

The same trend is observed in *Nm*, as expected by the study of Slatkin, Barton (1989).

Table 1. Genetic diversity indices and F-statistics in 10 wild Lima bean populations in the Central Valley of Costa Rica

Parameter marker	N_m [1]	H_o [2]	H_s [3]	H_T [4]	D_{ST} [5]	G_{ST} [6]	F_{IT} [7]	F_{IS} [8]	F_{ST} [9]
Microsatellites	0.17	0.031	0.215	0.489	0.273	0.560	0.924	0.841	0.519
Isozymes	0.04	0.012	0.060	0.186	0.126	0.680	0.929	0.831	0.578

[1]Nb of migrants according to Wright method, based on F_{ST}; [2] observed genetic diversity; [3] intrapopulation genetic diversity; [4] total genetic diversity; [5] interpopulation genetic diversity; [6] among population differenciation coefficient; [7] total consanguinity coefficient; [8] intrapopulation consanguinity coefficient; [9] fixation coefficient

Such preliminary results suggest a better estimation of gene flow and parameters of genetic diversity and structure with the use of microsatellites. Because the latter are more polymorphic and constitute co-dominant markers, a more precise measurement of gene flow using exclusion paternity analysis will be expected from all the leave samples taken in the three regions of the Central Valley.
We thank CIAT for providing the primers isolated from P. vulgaris.

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LEGUME MICROSATELLITES TESTED IN COMMON BEAN

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Introduction

Common bean (*Phaseolus vulgaris*) belongs to the Phaseoleae subtribe along with several other legume crops originally from the tropics or subtropics, like cowpea (*Vigna unguiculata*), mung bean (*Vigna mungo*), soybean (*Glycine max*) and pidgeon pea (*Cajanus cajan*). The Phaseoleae tribe contains the largest number of genera and most economically important crops of any tribe in the Legume family. The genetic similarity between Phaseoleae species has been confirmed by karyotyping and comparative mapping studies and most of the species in this tribe share the same or similar basic number of chromosomes (either n=10 or 11), although they vary greatly in the size of their genomes sometimes due to polyploidization events, as is the case for soybean. The legumes that originated in temperate regions, such as peas, lentils, chickpeas, alfalfa and clover, belong to other tribes and typically have basic chromosome sets of 7 or 8. Peanuts are also from a different branch of legumes than either the tropical or temperate legumes mentioned above. The close relationship between certain legume species led us to the hypothesis that molecular markers from one species could be used in another. In recent years, a large number of PCR-based microsatellite markers have been developed for soybean (Cregan et al., 1999; Peakall et al., 1998) and to a lesser degree the other tropical legumes, common beans, cowpeas, peanuts (Yu et al., 2000; C. Fatokun, pers. comm.; Hopkins et al., 1999). Our objective in this study was to try to adapt the microsatellites available for other Phaseoleae legume crops (especially soybean and cowpea) to common bean. If successful, the transfer of microsatellites between legume species, would reduce the cost and time needed to develop markers specific for each crop individually.

Materials and Methods

The microsatellites tested included 423 from *Glycine max* (408 with ATTn motif, 3 with GAn motif and 12 from coding sequences) and 118 from *Vigna* (mixture of GA, CA, AT and compound motif genomic microsatellites and two cDNA based markers). These markers were tested against a panel of nine legumes, that included the soybean, Mesomerican and Andean common bean genotypes (Williams, DOR364 and G19833, respectively) that have been the sources of microsatellite libraries made for these crops so as to provide a control in the size of the allele that was detected. For the other legume species (cowpea, lima bean, tepary bean, pidgeon pea and chacha fruit) we used genotypes that were representative of varieties grown in the Andean region or that were in the CIAT germplasm collection. Primer amplification was tested with a range of conditions. The soybean microsatellites were tested initially with lax amplification conditions using 45 to 47C for annealing temperature and 2.0 to 2.5 mM final concentrations of MgCl2. The cowpea microsatellites were amplified with 52 C annealing temperature and 2.5 MgCl2. Both sets of markers were analyzed on agarose (2.0%) gels with ethidium bromide staining. The cowpea microsatellites and the other microsatellites with single amplification products were analyzed on polyacrylamide (6%) gels with silver staining.

Results

Soybean and common bean appeared to be especially divergent in regard to their microsatellite loci. The soybean genomic ATTn microsatellites generally did not amplify well in common bean. The cDNA based microsatellites were also poorly conserved. At low annealing temperature and lax conditions required to amplify PCR products with these primers in common bean, these markers amplified multiple bands from common bean, tepary bean, lima bean, cowpea or mung bean DNA that were in general completely different in size compared to the soybean allele. The likelihood that these represent homoelogous microsatellite loci was deemed low and these microsatellites were not investigated further. Table 2 shows the nine most transferable microsatellites from soybean and the molecular weight of the amplified products. The total rate of transferability to the six species was between 2 and 0.5%. Cowpea genomic microsatellites were slightly more useful than the soybean genomic microsatellies for amplification in *Phaseolus* species. The transferability rates were between 7.6 and 11.0 %. The gene-derived microsatellites were more conserved than the microsatellites from non-coding sequences, and four out of six primer pairs from Table 1 amplified well across the subtribe. For example one cowpea microsatellite, VM21, amplified well in a range of legumes and represented a gene from *Vigna radiata* for ACC oxidase that contained an ATn repeat in the 3' untranslated region.

Discussion

Soybean and common bean appeared to be especially divergent in regard to their microsatellite loci and the soybean genomic ATTn microsatellites generally did not amplify well in common bean. Most of these microsatellites amplified multiple bands from common bean, tepary bean, lima bean, cowpea or mung bean DNA that were completely different in size compared to the soybean allele. Soybean primers may be less useful because of the evolutionary distance separating this species. This is in marked contract to studies with animals where microsatellites have been successfully transferred among related species such as birds, tortoises, primates (eg. gorillas/apes), ungulates (eg.

horses/cattle) and rodents (eg. rats/mice). Ultimately it is the genetic distance between species and genera that determines the ability of SSR primers to amplify in different genomes and the ability to transfer microsatellites between species must be determined empirically. And it seems that in plants, unlike animals, microsatellite loci are not well conserved over large genetic distance between species. Transferability of microsatellites between cowpeas and common beans appeared to be more promising than between soybeans and common bean suggesting that the close phylogenetic relationship between these species allowed us to exchange some of the microsatellites. We also noted that gene-derived microsatellites may be more conserved than the microsatellites from non-coding sequences and therefore, gene sequences in *Vigna* species which have microsatellites in them may be good candidates for comparative markers that will amplify in common bean and other *Phaseolus* species. In summary, we found that the pattern of amplification of legume microsatellites from soybean, cowpea and common bean, agreed well with the tribe, subtribe and genus designations of the legumes being tested.

Table 1. Size (in nucleotides) of amplification products of soybean and cowpea microsatellites on a panel of economically-important legumes from the Phaseoleae tribe[1].

		Family	***Fabacea***							
		Tribe	***Phaseoleae***							
		Sub-tribe	***Phaseolinae***						***Glycinin.***	***Cajanin.***
		Genus	***Phaseolus***				***Vigna***		***Glycine***	***Cajanus***
		Species	**P.v (M)**	**P.v (A)**	**P.a**	**P.l**	**V.u**	**V.r**	**G.m**	**C.c**
		Variety	**DOR364**	**G19833**	**G40001**	**Peru**	**Molina**	**Mungo**	**Williams**	**IS-10**
Source of Microsatellite	**Marker Type**	**Primer**								
Soybean	cDNA[2]	SoyPRP1	240	240	-	-	240	240	*120-240*	240
	cDNA	SoySc514	260	260	-	-	260	260	*120*	-
	Genomic	Satt 511	360	360	-	540	-	270	*250*	470
	Genomic	Satt 401	160	160	-	-	500	670	*170*	-
	Genomic	Satt 206	140	140	-	220	-	-	*220*	-
	Genomic	Satt 237	320	320	320	320	320	320	*250-320*	320
	Genomic	Satt 305	-	-	-	290	-	-	*200*	-
	Genomic	Satt 411	-	-	-	-	-	290	*100*	-
	Genomic	Satt 275	140	140	140	-	-	-	*160*	-
	Transferability (%)		**1.89**	**1.89**	**0.47**	**1.18**	**1.18**	**1.66**	**100.00**	**0.71**
Cowpea	Genomic	VM26	-	-	90	160	*160*	140	140	140
	Genomic	VM63	300	300	300	300	*300*	190	190	190
	cDNA	VM21	208	202	208	208	*240*	240	260-202	-
	Genomic	VM91	330	330-510	330	330	*330*	150	330	330
	Genomic	VM98	160	160	160	160	*160*	160-90	160	160
	Genomic	VM114	270	270	-	-	*270*	270	270	-
	Genomic	VM118	280	280	280	280	*280*	280	280	300
	Transferability (%)		**11.02**	**11.02**	**10.17**	**9.32**	**7.63**	**100.00**	**11.02**	**5.93**

1/ P.v. (M) : *Phaseolus vulgaris* – Mesoamerican; P.v. (A): *Phaseolus vulgaris* – Andean; P.a: *Phaseolus acutifolius*; P. l: *Phaseolus lunatus*; V.u: *Vigna unguiculata*; V.r: *Vigna radiata*; G.m: *Glycine max* and C.c: *Cajanus cajan*. 2/ source of sequence information used to design microsatellite marker. Note: Number in italics indicate allele size in reference species.

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In vitro CULTURE OF IMMATURE EMBRYOS OF Phaseolus polyanthus Greenm. AND Phaseolus vulgaris L.

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Introduction

Intraspecific hybrids in the common bean *Phaseolus vulgaris* L. have allowed to develop very high seed yielding cultivars, but without a significant improvement of the resistance to several diseases and pests responsible for half of the losses of yields in the tropics (Singh 1999). Interspecific hybridization between *P.vulgaris* and species belonging to the secondary gene pool, such as *P. polyanthus* Greenm., would allow to introgress genes of resistance to some economically important diseases (Schmit, Baudoin 1992). In order to maintain the desired characteristics, it is essential to use *P. polyanthus* as the female parent. In this case, however, the early abortion of hybrid embryos constitutes a very strong incompatibility barrier (Baudoin *et al*. 1992; Geerts 2001). One of the techniques used for the rescue of embryos is the *in vitro* culture (Sharma *et al*. 1996). Geerts *et al*. (2001) have generated and acclimatized plantlets of *P. vulgaris* from two days old pods cultivated *in vitro*. This study compares the growth and development parameters of immature embryos of *P. vulgaris* and *P. polyanthus* with the aim to create interspecific crosses between the two species.

Material and Methods,

The tested genotypes are NI 637 for *P. vulgaris* and NI 1015 and G 35348 for *P. polyanthus*, all cultivated forms. Pod and embryo culture techniques are those described by Geerts *et al.* (2001). These are summarized in Table 1.

Table 1. : *In vitro* culture stages of pods and embryos and for each stage technical conditions.

Technical conditions	*Pod culture*	*Embryo maturation*	Embryo dehydration	*Germination, growth and rooting*	*Acclimatization*
Media	P_{00}[(1)] (580 mosm) P_{01}[(1)] (450 and 350 mosm)	P_{01}[(1)] at 350 mosm	G6[(2)]	G7[(3)]	garden mould [(4)]
Light *Photoperiod*	60 µmol m^{-2} s^{-1} 16h	darkness -	darkness -	60 µmol m^{-2} s^{-1} 16h	580 µmol m^{-2} s^{-1} 11h30
Temp. (D/N)	25/25°C	25/25°C	25/25°C	25/25°C	24/20°C
Cult. duration	7 days	14 days	14 days	14 days [(5)]	
Containers	Petri dish	Petri dish	Petri dish	Petri dish	polyethylene pot

[(1)] P_{00} & P_{01} : Phillips modified medium (Geerts 2001); [(2)] G6 : Hu et Zanettini modified medium (Geerts 2001); [(3)] G7 : Mergeai *et al.* modified medium (Geerts 2001); [(4)] Klasmann 4 special n^r 26 (80 %), moss peat (15 %), Rhine sand (5 %) & 100 mg organic fertilizer (2.9 % total N, 2.9 % P_2O_5, 2.0 % K_20); [(5)] duration average according to the vigour of the plantlet before acclimatization.

The osmotic pressure of culture media evolves according to pod age. It varies in continuous way from 580 to 350 mosm when using liquid medium but by steps, successively 580, 450 and 350 mosm when using solid media. The studied parameters concern the influence of the genotype, the

maturity level of pods before its *in vitro* culture (from 3 to 5 days after pod setting on the mother plant) and pod culture techniques (comparing liquid and solid media).

Results and Discussion

Pod growth rates differ between the two tested species according to the culture technique : NI 637 shows a 32.3 % increase (with number of embryos : n = 48) in solid medium and 48.2 % increase (n = 24) in liquid medium, while NI 1015 and G 35348 show on an average a lower rate, respectively 22.7 % and 19.8 % increase (n = 86 and n = 77) in solid medium and 30.9 % and 27.3 % increase (n = 86 and n = 89) in liquid medium.

After seven days of *in vitro* pod culture, ovules, and then embryos, are extracted and placed in petri dishes containing *in vitro* solid media. For ovules, one notes a regular growth of half of these in NI 637 (*P. vulgaris*) and only of 21.5% and 23.5%, respectively in NI 1015 and G 35348, the two *P. polyanthus* genotypes. During ovule growth, many ovules died due to several factors : the disinfection technique of the plant material (phytotoxicity, necrosis), the *in vitro* conditions or a poor pollination and/or fertilization of mother plants maintained in growth chamber. The rate of embryo extraction is double in *P. vulgaris* compared to *P. polyanthus*. This may be related with a delay of embryos evolution in *P. polyanthus*. Indeed, in *P. vulgaris*, the extracted embryos had often reached heart-shaped or cotyledonar stage, while in *P. polyanthus*, embryos were hardly developed. On the other hand, no difference in rate of embryo extraction is observed between the two *P. polyanthus* genotypes NI 1015 and G 35348.

Germination of extracted embryos is higher in *P. vulgaris* (68.7%) than in *P. polyanthus* (28.4% in NI 1015 and 20.7% in G 35348). Pod age at the time of *in vitro* culture does not appear to influence the germinating capacity of the embryo. No significant difference has been observed between the three durations pods stay on the mother plant (3,4 and 5 days after pod setting). The ratio between the number of plantlets under acclimatization conditions and the number of germinated embryos is higher in *P. polyanthus* (76.2% for NI 1015 and 73.7% for G 35348) than in *P. vulgaris* (51,1%). Nevertheless, six weeks after the onset of acclimatization, the percent of growing plantlets out of the number of extracted embryos is higher in *P. vulgaris* (> 30%) than in *P. polyanthus* (≤ 5%).

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IN VITRO REGENERATION OF *Phaseolus vulgaris* L.

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Introduction

Common bean is among of the most cultivated species in family Leguminosae. Classical breeding is the basic approach for production of the widespread cultivars. Some problems based on the less genetic variations, low surviving ability of the interspecific hybrids, specific inheritances of some valueble characteristics as yield, disease and pests' resistance, harvesting characteristics, etc. are time and labour consuming or difficult to be resolve by conventional techniques. Plant biotechnology offers different strategies to overcome these difficulties. With some exemptions, species belonging to Leguminosae are difficult to regenerate *in vitro*. Grain legumes have less regeneration potential compared to the forage ones. Regeneration ability depends on the genotype, physiological state of the explant, tissue and cell specialization of the culture and the cultivation conditions (Jacobsen, 1992, Mohamed et al., 1996, Zhang-Zhang, 1997). Recently, several systems for bean regeneration were published (Mohamed et al., 1996, Zambre et al., 1998). However, the regeneration protocols are with low repeatability and due for specific individual genotypes. Aim of this investigation was:

i) to develop a system for *in vitro* regeneration in three different Bulgarian cultivars of common bean;

ii) to determine the factors influencing the process of bean regeneration *in vitro.*

Material and Methods

Three different genotypes were investigated for their *in vitro* ability to regenerate plants. Seeds from Bulgarian cultivars - Plovdiv 11M, Plovdiv 10 and Dobrudjanski 7 were sterilized by routine procedure (Svetleva et al., 1999). Sterile seeds were cultivated on MS base medium for 7 and 14 days under standard conditions of growth room - 16/8 hours photoperiod, 2500 Lx light intensity at 24° C temperature and 70% humidity. Leave petioles excised from *in vitro* plants were used as primary explants. Petiole explants were cultivated on MS medium supplemented with different concentrations of TDZ (2-4 μM), NAA (0.3 - 0.6 μM) and Paclobutrazol (2 μM), applied alone or in combinations. Ten explants per petri dish in 5 replicates were designed for all variants. Shoot initiation were carried out on dark, at 26° C temperature for two passages (two fresh media changes), each one per four weeks. Initiated shoots were elongated on MSE media - MS base enriched with 1 or 5 mg/l BA and 0.001 mg/l IAA. Shoots were cultivated in jars (volume 190 cm^3) in average of 5 initiated explants per container for four weeks in the standard conditions of growth room. Elongated shoots were detached from primary explants and transferred to a conversion media - MSG - MS with 1 mg/l BA and 0.2 mg/l GA_3, where they rooted also.

Results and Discussion

Data have shown no significant difference in the capacity for somatic organogenesis of the investigated bean cultivars (Table 1). All cultivars - Dobrudjanski 7, Plovdiv 11M and Plovdiv 10 were able to induced shoot organogenesis after callus formation. Best results according mean numbers of the explants regenerated shoots was observed when Plovdiv 11M was used. An average of 26% of the explants initiated shoots was found. Highest numbers of shoots per one explant were initiated in the same cultivar. Explants arising from seven days old plants in all genotypes were estimated to be competent for *in vitro* regeneration and form shoots after dedifferentiation. Explants from older plants are non-regenerable at the investigated conditions. Age of the explant is an important factor for *in vitro* regeneration (Sharp et al., 1982; Mohamed 1996). A specific physiological state corresponds to the regeneration competence of the cells (Veltcheva et al., in press). Organogenesis is limited around the embryonic tissues - pedicells from flowering buds (Mohamed et al., 1996), cotyledons (Mohamed et al., 1992; Yancheva et al., 1999) embryonic axis (Mohamed et al., 1992) etc. It could be assumed that the cells of young petioles are still competent for regeneration. After this period the regeneration ability of the cells is strongly inhibited. Different cytokinins,

used in already published protocols for bean regeneration - CPPU (Mohamed et al., 1996), BA (Malik and Sacsena, 1992; Yancheva et al., 1999), kinetine (Genga and Alavena, 1991), 2iP (McClean and Grafton, 1989) etc., were investigated for their ability to induce regeneration (data not shown). Our preliminary results indicated that TDZ benefits the process of organogenesis. Four weeks after cultivation of the explants on MSI mediums callus formation took place. Type of the callus differs according the type and the concentration of applied growth regulators. Regenerable callus was estimated to be brown - green, with granulated structures. Highest total number of shoots has been induced when 2 μM TDZ in combination with 0.3 μM NAA (MSI2) were applied to MS base media (Table 1). Paclobutrazol has stimulated callus proliferation. Maximum proliferation for one passage has been observed on AVI3 media, supplemented with TDZ/IAA and paclobutrazol media with Plovdiv 10 cultivar. Difference between fresh weight in 1st and 2nd passages was calculated to be 0.143 g. However, shoot induction on paclobutrazol containing medium has been inhibited. Most of the initiated shoots on TDZ/IAA media ether proliferated callus cells on their surface or get necroses and degenerate after transfer to new media with reduced or lacking growth regulators. Shoot elongation has been suppressed. Cultivation of the initiated shoots on media supplemented with antioxidant - glutation (10 mg/l) and growth regulators - BA and IAA stimulated the process of shoot elongation. Optimal concentration for growth regulators was estimated to be BA - 22,2 μM and IAA - 0.057 μM. Shoot elongation took place in 37% of initiated shoots of bean cultivar - Plovdiv 11M. Plant regeneration was observed on media with BA - 4,44 μM and GA_3 - 0.58 μM. Cultivation of the shoots and plantlets on BA enriched media, decreased root initiation of regenerants on MS base media. Root initiation was achieved either after two times transfer on fresh MS base media or on media supplemented with 0.057 μM IAA. Described system is highly repeatable and efforts will be towered to a optimization of the efficiency of regeneration.

Table 1. Influence of induction medium on in vitro regeneration of three different Bulgarian common bean cultivars.

Cultivars	Total number of shoots			Shoots per explant			Regenerants per explant		
	AVI1	AVI2	AVI3	AVI1	AVI2	AVI3	AVI1	AVI2	AVI3
Dobrudjanski 7	7	35	0	0	4	0		4	
Plovdiv 11M	8	40	2	1.2	7	1.0		3	
Plovdiv 10	7	38	0	0	5	0		2	
	Callus Growth - Fresh Weight [g]								
	1st passage			2nd passage			3rd passage		
	AVI1	AVI2	AVI3	AVI1	AVI2	AVI3	AVI1	AVI2	AVI3
Dobrudjanski 7	0.079	0.073	0.141	0.099	0.095	0.174	0.147	0.115	0.332
Plovdiv 11M	0.037	0.023	0.365	0.091	0.084	0.447	0.184	0.126	0.508
Plovdiv 10	0.077	0.089	0.334	0.144	0.110	0.477	0.161	0.125	0.573

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NAMING AND RELEASE OF "GRAND MESA"

The Colorado Agricultural Experiment Station announces the release of 'Grand Mesa' disease-resistant pinto bean (*Phaseolus vulgaris* L.) variety with high yield potential. Grand Mesa was developed by personnel at Colorado State University and tested in the Cooperative Dry Bean Nursery, Western Regional Bean Trial, Midwest Regional Performance Nursery, and Colorado Crop Testing Program as CO 75511.

Grand Mesa is an F_3 derived line from the single cross 34596-1/RNK-178 made in 1994. The cross between upright pinto breeding line 34596-1 and the experimental line RNK-178 was made to combine upright architecture and resistance to the prevalent strains of rust in a pinto cultivar. The inbred line 34596-1 was derived from the cross CO 56249/83b235. CO 56249 is a pinto line that has good agronomic and seed characteristics, but is susceptible to the predominant races of bean rust found in Colorado. 83b235 is a high-yielding experimental pinto line from the University of Idaho, developed by James Myers and R.J. Kolar. RNK 178 is an experimental line developed by Rogers NK (Syngenta) with high yield potential and rust resistance. In 1997, one $F_{3:5}$ line was selected and bulked for testing and used to produce 24 plant rows for seed increase at Fruita, Colorado. Approximately 18 of the 24 plant rows were bulked based on uniformity for plant type and seed characteristics to produce the initial breeder seed.

Grand Mesa has upright architecture in most environments (Type IIb), however in some environments it expresses semi-vine architecture (Type III). It also possesses resistance to the prevalent races of rust in the High Plains, bean common mosaic virus resistance, and white mold tolerance. Rust resistance is conditioned by the Ur-3 allele from the parent, RNK-178. Resistance to bean common mosaic appears to be conditioned by the recessive allele $bc2^2$ from the line 34596-1, however that has not been confirmed in duplicate tests. White mold tolerance ratings is based on field performance at three locations in 2000. Yield levels averaged 320 and 91 pounds per acre less than 'Montrose' and 'Buster', respectively, across 13 location-years in 1999 and 2000.

Foundation seed of Grand Mesa was released to seed producers in April 2001. Application for PVP under Title V will be sought. A "Technology Fee" paid to the Certification agency in the state of production will be assessed on all Registered and Certified seed produced. Small amounts of seed are available from Mark Brick, Department of Soil and Crop Sciences, Colorado State University, Fort Collins, CO 80523, mbrick@lamar.colostate.edu.

Mark A. Brick, Howard Schwartz, J. Barry Ogg, Jerry J. Johnson, and Fred Judson.
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RELEASE OF 'ORCA' BLACK AND WHITE ANASAZI[@] - TYPE DRY BEAN

A.N. Hang, M.J. Silbernagel and P.N. Miklas

The Agricultural Research Center of Washington State University, Idaho Agricultural Experiment Station and Agricultural Research Service, and U.S. Department of Agriculture announce the release of 'Orca', a black and white Anasazi-type bean (*Phaseolus vulgaris* L.) cultivar. Orca is a black and white mottled seeded dry bean, with upright growth habit, mid to late season maturity with resistance to bean common mosaic virus (BCMV). This is the first Anasazi-type bean that will reach harvest maturity within 100 to 110 days in North America. Scientists participating in the development of this variety were A.N. Hang Washington State University-Prosser, M.J. Silbernagel (retired USDA-ARS) and P.N. Miklas, USDA-ARS, Prosser.

Orca is an F_6-derived F_{10} population from the cross A 55/Anasazi. A-55 is a black-seeded, upright II-A plant habit type developed by S.P. Singh (CIAT Columbia), with dominant *I* gene resistance to BCMV and high tolerance to curly top virus (CTV). It is also tolerant to root rot complex (*Fusarium, Rhizoctonia,* and *Pythium* spp.) found in the bean production areas of the U.S. Pacific Northwest. The Native American landrace Anasazi-type dry bean (red and white mottled) of the U.S. Southwest has a late-maturing, vigorous, recumbent plant habit II-B and is very susceptible to BCMV and CTV. This landrace is uniquely well adapted to the arid high-altitude regions of the U.S. Southwest. Planted in the spring, it emerges on residual winter moisture, and develops deep roots and restricted top growth until the August monsoonal rains, after which plants put on a rapid spurt of top growth and then flower and mature rapidly in the dry fall that follows. They are more photosensitive than the dry bean cultivars normally grown in bean-producing areas of North America. In the northern latitude, landrace Anasazi dry bean will not bloom until late in the growing season. This lateness in the northern latitudes often results in the crop being frozen before harvest. Plant growth habit of Orca is upright and the line is lodging resistant; they have unprotected dominant *I* gene resistance to BCMV and complete resistance to CTV (presumed to be due to dominant epistatic genes). In replicated yield trials at Othello, WA, Orca bloomed at 60 days and matured 102 days after planting. Average yield of Orca was 4110 kg ha^{-1} and is comparable to UI- 906 or UI-911 with the same growing season. Orca planted in Vancouver Washington bloomed later than UI-911 but matured a day earlier. Yield of Orca was lower than UI-911 but seed was much bigger and very attractive. Seeds of Orca are black-and-white mottled, seed is plump, medium sized and bigger than those of its parent A-55 or any other commercial black bean cultivars with a 100-seed weight of 30 g (vs. 25 g for A-55). After cooking the dark part of the seed appears dark maroon, similar to its Anasazi parent.

Orca has been released as a WSU variety and may be sold for seed by name only under the certified class. Breeder and foundation seed will be maintained by Washington State Crop Improvement Association, Foundation Seed Service - WSU Seed House, Pullman, WA 99164-6420. Phone (509) 335-4365, Fax (509) 335-7007, or email Greg Vollmer <wscia@wsu.edu>. A research fee will be assessed on each unit of foundation seed sold. Plant variety protection will not be applied.

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