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Report of The

BEAN IMPROVEMENT COOPERATIVE

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Coordinating Committee

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THE 47th ANNUAL REPORT OF THE BEAN IMPROVEMENT COOPERATIVE

The Bean Improvement Cooperative enjoyed a stimulating meeting at the 2003 Biennial Meeting in Sacramento, California. The meeting had 130 registered participants and featured 32 oral presentations and 37 poster presentations. The quality of both the oral and poster presentations was excellent. The focus of the National Dry Bean Symposium on bean germplasm was to recognize the contributions of Dr. George Freytag and the publication of the *Phaseolus* monograph ' Taxonomy, Distribution and Ecology of the Genus *Phaseolus* (Leguminosae–Papilionoideae) in North America, Mexico and Central America' authored by G. F. Freytag, and D. G. Debouck. The meeting began with the Frazier-Zaumeyer Distinguished Lecture, entitled: 'Phenotypic and genotypic selection: integrating the use of molecular information.' The lecture was presented by Dr. Frederick A. Bliss, Professor Emeritus University of California, Davis and Research Director Seminis Seeds Company, Woodland CA.

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

The outstanding student oral presentation was entitled: '*Identification and mapping bean root rot resistance in a population of Mesoamerican x Andean origin*' presented by F. M. Navarro, University of Wisconsin – Jim Nienhuis, advisor.

The second place oral presentation was entitled: ' *Identification of germplasm and resistance to the soybean aphid-transmitted virus complex*' presented by M.E. Sass, University of Wisconsin. – Jim Nienhuis, advisor.

The outstanding poster presentation was entitled: '*Phenotypic and genotypic variation in Uromyces appendiculatus from regions in commercial production and centers of common bean domestication'* presented by M. Acevedo, University of Nebraska – Jim Steadmam, advisor.

The second place poster presentation was entitled: '*Elucidation of the genotype of a virgarcus seedcoat pattern mutant of Red Hawk Dark Red Kidney Bean*' presented by E. Ernest, Michigan State University – Jim Kelly, advisor.

The meeting received excellent and generous support from the following organizations: Seminis Seed Company; Department of Agronomy and Range Science UC Davis; Harris Moran Seed Company; National Dry Bean Council; Basin Seed; Department of Plant Pathology UC Davis; Department of Homeland Security Washington DC; Michigan Bean Commission; Saskatchewan Pulse Growers; and SeedGro. 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. Paul Gepts and I wish to thank the sponsors and the participants for making the meeting a success. Details of the next BIC meeting in Delaware in 2005 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. Roger F. Sandsted** from Cornell University and **Dr. Eelco Drijfhout** from Wageningen University, the Netherlands. Roger Sandsted was awarded the BIC Meritorious Service Award in 1983 and he is best known for the development and release of the Aurora small white and Midnight black bean varieties. Eelco Drijfhout was awarded the BIC Meritorious Service Award in 1982 and is widely recognized for his scientific treatise 'Genetic interaction between *Phaseolus vulgaris* and bean common mosaic virus with implications for strain identification and breeding for resistance' which provided a clear understanding of the pathogenic variability of bean common mosaic virus and the elucidation and interaction of major dominant and recessive genes conditioning resistance to bean common mosaic virus. The BIC recognizes both individuals for their significant achievements to bean research.

Dr. James D. Kelly, BIC President

REPORT OF THE BIC GENETICS COMMITTEE

The Genetic Committee met in Sacramento CA on October 27, 2003 at 7:30pm. The meeting began with a motion to accept the genetic stocks submitted (two groups; Mar. 1, 2003 and Sept. 18, 2003 cover letters) by Mark J. Bassett to Molly Welsh at Pullman, WA into the Genetic Stock Collection. The motion stated that the stocks #92 through 102 and 108 through 111 be accepted without condition, but stocks #103 through 108 (all carrying the unpublished gene *Prp*^{i}*-2* for intensified anthocyanin expression in a syndrome of plant organs) and stock #112 with genotype *t z*^{zel} *bip*^{ana} were given conditional acceptance, pending publication of the supporting data. The motion was supported. James Kelly presented evidence that characterized and gave new gene symbols to additional alleles at *Co-1* and *Co-4* for anthracnose resistance. A motion to accept the new gene symbols was made and carried. A formal letter addressed to A. L. Alzate-Marin and her research group in Brazil was, subsequently, sent to notify them of the acceptance of gene symbols for the new anthracnose resistance genes. Phil Miklas made two presentations to the committee. In the first, he reviewed the evidence for a gene (*Ctv-1*, or alternatively *Bct*) for resistance to curly top virus in common bean. In the second, he described the present system of host differential varieties for halo blight (HB) and proposed a new binary or triplex system for designating HB races and HB resistance gene symbols. James Myers rotated off the Genetics Committee and Matthew Blair was appointed in his place.

BIC COMMITTEE MEMBERSHIP - 1957 to 2004

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
2001 Antonius, Beaver, **Kelly**, Kotch, Miklas, Myers, Park, Riley, de Ron, Schwartz (ex officio), Vandenberg
2003 Beaver, **Kelly**, Kmiecik, Kurowski, Miklas, Myers, Park, Riley, de Ron, Schwartz (ex officio), Vandenberg

Awards Committee:

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

2003 BIC AWARD RECIPIENTS

2003 FRAZIER - ZAUMEYER DISTINGUISHED LECTURESHIP AWARD

FREDRICK A. BLISS

Prof. Fredrick A. Bliss was born into a farming family in Red Cloud, Nebraska, on Dec. 5, 1938. He earned a B.S. degree with Distinction in Agronomy from the University of Nebraska in 1960. He went on to obtain a PhD degree with W. Gabelman at the University of Wisconsin-Madison in Horticulture-Genetics in 1965, followed by a postdoctoral fellowship at the University of Minnesota. He was appointed Assistant Professor in the Department of Horticulture in 1966 but the spent the first years abroad. First he was a lecturer at the University of Ife in Nigeria as a member of a USAID/UW team from 1966-1968. He became then a visiting scientist at the University of Goettingen (West Germany) in 1968 before re-joining the faculty at the University of Wisconsin in 1969.

There were two significant changes in Dr. Bliss's career following his Wisconsin era. In 1988, he accepted the Will W. Lester Endowed Chair in the Department of Pomology at the University of California, Davis. He led that department as chair from 1991-1994. In 1998, he joined the Seminis Seed Co., where is currently employed in addition to being a Professor Emeritus at UC Davis.

Throughout his varied career, Fred - as he is known to all who have worked with him - has had a significant impact not only in plant genetics and breeding but also on his students, postdocs and collaborators. Among his many accomplishments are his work on seed proteins and nitrogen fixation in beans. He was able to show that genetically complex traits can be introduced into adapted, elite bean lines, using the inbred backcross breeding method. For seed proteins, he conducted both basic and applied studies that allowed him to identify specific seed protein components and use these as selection tools to manipulate both the quality and quantity of proteins in bean seeds.

For nitrogen fixation, he paid attention to individual traits (nodule number) or organs (root types) to again subdivide a complex trait into more manageable sub-traits. With this approach, he was a precursor by more than a decade of the current QTL-candidate gene approach. It also allowed the discovery of the insecticidal role of the arcelin seed protein.

A major preoccupation was to broaden the narrow germplasm basis of the crops he worked with. One of the advantages of the inbred backcross methods was to allow the use of exotic, unadapted germplasm. While the focus was initially on landraces from the centers of origin, it has now shifted to wild beans as well. Thus, Fred was also a pioneer in the active use of a broad array of crop germplasm.

Mentoring has been one of the hallmarks of Fred's career. He has advised 6 postdocs, 9 visiting scientists, 6 Master of Science degree students and 27 Ph.D. students, from the U.S. and foreign countries. He has always shown the utmost concern for the professional and personal well-being of his students and provided sound, impartial advice. He received the Outstanding Graduate Educator Award from the American Society for Horticultural Science in 1988.

Among the many other awards Fred has received are the Meritorious Service Award from the Bean Improvement Cooperative for "outstanding scientific accomplishments relating to bean (*Phaseolus*) improvement" and his election as fellow of the American Society for Horticultural Science (1985), the Crop Science Society of America (1986), and the American Association for the Advancement of Science (1990). He received the ASSINSEL - Grand Prize from the International Association of Plant Breeders for the Protection of Plant Varieties in 1986 for "outstanding work in the field of plant genetics and plant breeding."

2003 MERITORIOUS SERVICE AWARD RECIPIENTS

S. BEEBE, P. GEPTS & M. A. PASTOR-CORRALES

STEVEN BEEBE

Dr. Steven Beebe was born in Burlington, Iowa on September 27, 1952. He obtained a B.S. degree in horticulture from Iowa State University in 1974. He earned both his M.S. and Ph.D. degrees in plant breeding and genetics under the supervision of Dr. Fred Bliss at the University of Wisconsin. The topic of his Ph.D. dissertation was breeding for resistance to soil-borne pathogens of common bean and part of the research was conducted in CIAT (International Center for Tropical Agriculture), Palmira, Colombia.

After graduation, Dr. Beebe was stationed in Jutiapa, Guatemala from 1981 to 1985 where he worked as a bean breeder for CIAT. His responsibilities included increasing bean production in the region and the strengthening of bean research programs in Central America. Dr. Beebe participated in the research in Guatemala that led to the development and release of the first cultivars with resistance to bean golden yellow mosaic virus (BGYM). In 1985, he moved to the CIAT headquarters in Cali, Colombia where he assumed the responsibility for germplasm and varietal development for Coastal Mexico, the Caribbean, Central America and Southern Brazil. Dr. Beebe focused on the genetic improvement of beans for resistance to BGYM, bacterial and fungal diseases, tolerance to acid soils and enhanced biological nitrogen fixation. From 1992 to 1998, Dr. Beebe worked as a germplasm specialist responsible for the agronomic, morphological and molecular characterization of the Phaseolus bean germplasm.

He served as the Project Manager of the Bean Improvement Project at CIAT from 1996 to 1998. During this period, he was CIAT's representative on the Technical Committee of the Bean/Cowpea CRSP. Dr. Beebe continues to work on the genetic improvement of beans in the CIAT bean and biotechnology research projects.

During the past 20 years, he has been an active participant in the PROFRIJOL regional bean network and a frequent contributor at the annual meeting of the Programa Cooperativo Centroamericano para el Mejoramiento de Cultivos y Animales. Dr. Beebe has also provided informal training at CIAT in plant breeding, and has served as a mentor for numerous bean researchers from Central America and the Caribbean. He is author and co-author of more than 30 refereed journal articles and two book chapters. These publications describe research dealing with biodiversity, crop evolution and breeding for resistance to a wide range of biotic and abiotic factors that limit bean production in the tropics. He has developed bean cultivars that are used in at least ten countries. In addition, improved bean germplasm lines developed and released by Dr. Beebe have been widely used as parents by bean breeding programs throughout Latin America.

PAUL GEPTS

Paul Gepts is a Professor of Agronomy at the University of California, Davis. He was born in Brussels, Belgium in 1953. He earned his "Candidature en Sciences Agronomiques" in 1973. In 1976, he earned a "Diplome d' Ingenieur Agronome" from "Faculté des Sciences Agronomiques", Gembloux, Belgium, where he worked on chemical control of cereal diseases. Dr. Gepts began his research career in *Phaseolus* beans at Gembloux in 1977. He worked on interspecific crosses between common and scarlet runner beans, and continued his research during his stay at Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia from 1978 to 1981. He then moved to the University of Wisconsin at Madison, where he earned his Ph.D. degree in Plant Breeding and Genetics in 1984 under the direction of Dr. Fred Bliss.

From 1985 to 1986, he was a post-doctoral research geneticist at the University of California, Riverside, where he worked on genetic variability of nuclear and chloroplast DNA sequences in pearl millet. His strong desire to conduct fundamental research in *Phaseolus* beans was re-invigorated when he joined the faculty at the University of California, Davis in 1986.

Dr. Gepts' most outstanding and pioneering research used phaseolin seed protein, and unequivocally revealed, once and for all, that wild beans are the immediate ancestor of common bean cultivars, common bean had non-centric domestication in both Middle America and Andean South America, there are two major gene pools in wild populations and in cultivars, and a large reduction in genetic diversity occurred in cultivars during domestication. This was truly a milestone achievement in common bean evolutionary research. In addition, he traced the worldwide dispersal of cultivars from their centers of origin and domestication in the Americas. More recently, Dr. Gepts' collaborative research using multivariate statistical analyses of molecular, morphological, and agronomic traits identified races of common bean. Also worthy of special mention are his research on genetics of heat tolerance and major domestication traits, identification of the ancestral and most primitive phaseolin seed protein and molecular markers for various traits, and development of the core linkage map of the bean genome.

Dr. Gepts has made 114 national and international presentations in scholarly meetings and conferences, published 108 research articles and chapters in books and symposium proceedings, and edited a book on *Phaseolus* germplasm resources. He has trained 8 M.S. and 16 Ph.D. students from around the world. Dr. Gepts is an active member of the BIC and several other professional societies and committees. He served as a Chair and has been a member of the Genetics Committee of the BIC and *Phaseolus* Crop Advisory Committee of the National Plant Germplasm System of the USDA-ARS. He has received half a dozen honors and awards including the BIC Distinguished Achievement Award in 1991.

MARCIAL A. 'TALO' PASTOR-CORRALES

Marcial A. Pastor-Corrales (Talo) was born May 2, 1948 in Arrozal, Peru. He received his B.S. in Botany in 1972 and his M.S. in Botany and Mycology in 1974 from Eastern Illinois University University. He then went to Texas A & M where he obtained a second M.S. in 1977 and a Ph.D. in 1980 in Plant Pathology. He then went to CIAT as a Post-Doctoral Fellow in the Bean Program from 1980-1981. He assumed the Senior Staff Plant Pathologist position in the Bean Program from 1981 to 1997 at Cali, Colombia. Talo then jointed Novartis Crop Protection, Northeast Research Station, in Hudson, New York from 1997-2000. From April 2000 to the present, Talo has served as the Research Plant Pathologist with the USDA-ARS at Beltsville, Maryland.

Dr. Pastor-Corrales has 20 years of experience in basic and applied research in bean disease management, especially selecting for genetic resistance; seventeen years as the principal Plant Pathologist of the Bean Program at CIAT in Cali, Colombia, and the last three years as a USDA-ARS Research Plant Pathologist at Beltsville, MD. Dr. Pastor-Corrales was an essential member of the CIAT Bean Program that developed the disease resistant bean lines that dramatically increased bean production in Latin America, and later impacted bean production in Africa. He discovered common bean accession G2333, arguably the most important source of bean anthracnose resistance in the world, and elucidated its inheritance of resistance. Furthermore, he identified many other important and widely used sources of anthracnose resistance. This is significant due to the high variability exhibited by populations of the anthracnose pathogen, *Colletotrichum lindemuthianum*. Dr. Pastor-Corrales and colleagues identified new sources of resistance to the soilborne pathogens that cause Fusarium wilt, charcoal rot, Rhizoctonia root rot, and root knot nematode. Because diseases caused by soilborne pathogens are often difficult to work with, few sources of resistance existed. Many of these sources of resistance have been used to develop commercial bean cultivars with resistance to diseases and have contributed to a broader genetic base of the bean crop.

Talo was the PI of a team that developed methodology for effective selection of bean breeding lines with resistance to many diseases in Latin America and Africa. Laboratory, greenhouse and field techniques were used for evaluating the reaction of bean germplasm to fungal and bacterial pathogens. This research permitted scientists in national programs to evaluate sources of disease resistance. It also led to development of bean cultivars with the highest levels of resistance to common bacterial blight in the world. He researched the extensive and previously unsuspected virulence and genetic diversity of the bean angular leaf spot pathogen *Phaeoisariopsis griseola*. He also demonstrated that this was an important yield-reducing disease.

Dr. Pastor-Corrales discovered that the diversity of the anthracnose and angular leaf spot pathogens was nearly identical to the diversity of their bean host, and proposed that these two pathogens had co-evolved with their bean host. He introduced the use of Andean and Middle American beans to characterize the virulence diversity of these two pathogens. He and others also used molecular tools to demonstrate that these two pathogens had co-evolved with their bean host. This research contributed greatly to the idea that combining disease resistance genes from Andean and Middle American beans will improve management of anthracnose and angular leaf spot.

Dr. Pastor-Corrales co-edited the second edition of the book "Bean Production Problems in the Tropics", and assumed the role of principal editor for the Spanish version of this popular book. He was a prominent member of a CIAT multidisciplinary team that developed high yielding bean lines with resistance to the most widespread and damaging diseases of common bean in Latin America – bean golden mosaic, anthracnose, angular leaf spot, common mosaic, and common bacterial blight. He is now leading a multiregional effort to produce multiple disease resistance in high-yielding North American pinto, great northern, navy and red bean advanced germplasm.

2005 BIENNIAL BIC/NDBC MEETING

The 2005 BIC meeting will take place in Newark, Delaware on the campus of the University of Delaware on dates to be determined in mid-November, 2005. 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 Winterthur and Hagley Museums. Winterthur is the 180 room home of the H.F. duPont featuring his early American decorative arts collection, his garden, and his farm. Hagley features the original black powder mills of the DuPont Company. Further information will be forthcoming through the BIC web site (www.css.msu.edu/bic), the 2004 annual report, and individual mailings to members. For further information contact Ed Kee at kee@udel.edu.

ASA FELLOWS

The BIC wishes to recognize the achievements of three BIC members, Dr. Mark Brick, Dr. Paul Gepts and Dr. Shree Singh who were recognized as ASA fellows at the Annual meeting of the American Society of Agronomy in Denver in 2003. Congratulations!!

BEAN GENE LIST

The BIC is deeply indebted to Dr. Mark Bassett for his very substantial effort in updating the List of Genes – *Phaseolus vulgaris* L. last published in the BIC in 1996. The Gene List for 2004 has been extensively updated by Mark, but due to space limitations he could not include complete information on the linked SCAR markers for the different genes. Links to where information is available on these markers are provided.

IN MEMORY OF ROGER F. SANDSTED

On Wednesday, March 12, 2003, Professor Roger F Sandsted, 84 of 22 Dutcher Road, Freeville, New York, passed from this world in the same manner he lived his life; in quiet dignity and in gentle poise. Roger was born August 5th, 1918 in Holdrege, Nebraska to the late William and Otelia Sandsted. The family lived on a farm where Roger participated in many of the farming operations.

He was graduated from the Holdrege High School in 1936 and went to work on the family farm before entering the armed forces just prior to World War II. He joined the Air Corps and became a pilot of a B29, "Superfortress". He flew 30 missions over Japan, while stationed on Tinian Island in the South Pacific. He was discharged from the army in October 1945.

After the war Roger finished his college studies at The University of Nebraska, College of Agriculture, receiving a B.S. in 1948. He continued his education at the University of Minnesota in the Horticulture Department, acquiring a PhD in 1954.

Roger's first job was at the University of Idaho in the Agriculture Department, where he lived in Parma, Idaho. He then went to the Cornell University as an Assistant Professor of Vegetable Crops in 1957. He was elevated to Associate Professor in 1963 and to Professor in 1977. He also held the title of Department Extension Leader from 1976 to 1983.

As a research and extension Horticulturist with primary responsibility for legume vegetables Roger made numerous contributions to the bean industry. He conducted yearly variety and observation trials on snap and dry beans. His keen observations led to the selection and development of the small white bean "Aurora", which was released in 1973, and the black bean "Midnight", which was released in 1980. "Midnight" attracted national attention due to its improved growing characteristics. Another notable accomplishment as a result of his selection and breeding efforts is the red kidney bean "Ruddy". Roger also conducted numerous other university cultural experiments with an emphasis on dry beans.

Roger made valuable contributions to the bean industry and was awarded the Meritorious Service Award of the Bean Improvement Cooperative in 1983. The results of his research have been effectively communicated in extension bulletins, newsletter articles, motion pictures, and professional publications noted for their straight-forward language. He was a cornerstone of the New York Bean Industry who made his mark on the national level through devoted research and infectious enthusiasm for beans.

He retired from Cornell in 1983 and was named Professor Emeritus. Roger maintained a strong interest in agriculture establishing many gardens at his home. Roger became a Master Gardener with the Tompkins County Cooperative Extensive, helping home gardeners with problems and answering questions.

Professionally, he was a member of Alpha Zeta, Alpha Gamma Rho, American Society for Horticultural Science, Bean Improvement Cooperative and Epsilon Sigma Phi. Roger became a member of the Town of Dryden Historical Society and served on the Board of Trustees. He was chairman of the Collections Committee and was a valued member for many years. Roger was a member of the Presbyterian Church in Dryden and served as a trustee. For many years, he was a member of the 40th Bomb Group Association, made up of members of the squadron he flew with. He enjoyed many reunions of the group.

Roger was known as a kind and generous man who always found time to help others. His quiet, sincere and gentle manner was a calming influence for many and will be remembered by his family, friends and colleagues. In lieu of flowers, friends may send memorial contributions to "Roger Sandsted Memorial" c/o Monica Roth, T.C.C.E., Master Gardeners Program, 615 Willow Ave. Ithaca, NY 14850.

LIST OF GENES - *Phaseolus vulgaris* L.

The original comprehensive gene list was prepared by S.H. Yarnell (Bot. Rev. 31:247-330, 1965) and published in the BIC 8:4-20, 1965. An updated list was prepared by M.H. Dickson and associates and published in the BIC 25:109-127, 1982. The next update (BIC 32:1-15, 1989) was prepared by M.J. Bassett, involving extensive additions, corrections, revisions, and style changes. Subsequent updates (BIC 36:vi-xxiii, 1993; BIC 39:1-19; and the current one) were prepared by M. J. Bassett. A table of SCAR molecular markers for many genes in the common bean Gene List (below) is available at http://www.usda.prosser.wsu.edu/miklas/Scartable3.pdf.

Acc — *Accompanying* colors, i.e., the formerly "pleiotropic effects of R^{st} on the color of pods, the top edge of the standard, and the hypocotyl" (Prakken 1974).

ace — *acera* (Latin): produces shiny pod (Yen 1957). *Ace* is linked to *V* (Bassett 1997a), which is located (McClean et al. 2002) on chromosome 1 (Freyre et al. 1998; Pedrosa et al. 2003).

Adk — structural gene for *adenylate kinase* enzyme (Weeden 1984).

Am — *amaranth*: with *No* and *Sal* geranium flower color, and scarlet flower with *Beg No Sal* (Lamprecht 1948b, 1961a). Scarlet flower (Fan 1, 43C; Royal Hort. Soc. fans) is expressed by *Sal Am* V^{wf} (or *v*), and *Sal Am v* expressed oxblood red seed coats (vs. mineral brown) due either to a pleiotropic effect of *Am* or a very closely linked dominant gene (Bassett 2003b). *Am* has no expression with *sal*, and *Am* is located 9 cM from *V* (Bassett 2003b) on (McClean et al. 2002) chromosome 1 (Freyre et al. 1998; Pedrosa et al. 2003).

Amv-1 — high level resistance to a strain of *alfalfa mosaic virus* (Wade and Zaumeyer 1940).

Amv-2 — resistance to the same strain of *alfalfa mosaic virus* as for *Amv* (Wade and Zaumeyer 1940).

Ane — *Anebulosus* (Latin): produces nebulosus-mottling on testa (Prakken 1977a); observable only in c^u *J* and *C*/c^u *J* backgrounds. Not allelic with *V* or *R*, but linked to *B* (Lamprecht 1964). This trait is more commonly known as strong (grayish brown) vein pattern of seed coats (Bassett, editor).

aph — *aphyllus* (Latin): plants are sterile and have only two (unifoliate) leaves and 4 to 6 nodes. (Lamprecht 1958).

Arc — *arcus* (Latin): with *Bip* gives virgarcus seed coat pattern, with *bip* gives virgata; *arc* with *Bip* gives *arcus*, with *bip* gives bipunctata; extends seed coat color in partly colored seeds (Lamprecht 1940b). The arcus pattern is also expressed by *t z Bip J Fib*; possible allelism between *Arc* and *Fib* has not been tested (Bassett and McClean 2000; Lamprecht 1940b), whereas *J* and *Fib* are not allelic (Bassett 2001).

arg — *argentum* (Latin): with *Y* produces a "silver" or greenish gray pod (Lamprecht 1947b), formerly *s* (Currence 1930, 1931); *arg* with *y* gives a white pod (Currence 1931; Lamprecht 1947b).

Arl (*Arc*) — structural gene for the seed protein *arcelin* (Osborn et al. 1986).

asp — *asper* (Latin): very dull (non-shiny) seed coat that is slightly rough textured due to the pyramidal shape of the outer epidermal palisade cells (Lamprecht, 1940c). With *P C J G B V*, *asp* seed coats had only 19% of the total anthocyanin content (delphinidin 3-*O*-glucoside, petunidin 3-*O*-glucoside, and malvidin 3-*O*-glucoside) compared with *Asp*; this was achieved by *asp* changing the size and shape of the palisade cells of the seed coat epidermis, making the cells significantly smaller than with *Asp* (Beninger et al. 2000). *Asp* is located (Miklas et al. 2000) on chromosome 4 (Freyre et al. 1998; Pedrosa et al. 2003).

B (*Br, Vir*) — as used by Lamprecht (1932a, 1939, 1951a); the greenish brown factor of Prakken (1970). Similar or equivalent genes, according to Feenstra (1960), are the *C* of Tschermak (1912), the *D* of Shull (1908), the *E* of Kooiman (1920), the *H* of Shaw and Norton (1918), and the *L* of Sirks (1922). Smith (1961) used the gene symbol *Br* for *B*, according to Prakken (1972b). Lamprecht (1932b) used the gene symbol *Vir* for the effects of segregation at *B* in the genotype *P C j g B/b v*, according to Prakken (1970). The interactions of *B* with nearly all combinations of genes for seed coat color were summarized by Prakken (1972b). With *P C J G V Asp*, the *B* gene acts to regulate the production of precursors of anthocyanins in the seed coat color pathway above the level of dihydrokaempferol formation (Beninger et al. 2000). With *P C J G v Asp*, the *B* gene acts to regulate the production of astragalin and kaempferol 3-*O*-glucoside (Beninger et al. 1999). *B* is very tightly linked (Kyle and Dickson 1988) to the virus resistance gene *I* on chromosome 9 (Freyre et al. 1998; Pedrosa et al. 2003; Vallejos et al. 2000).

bc-u — strain-*unspecific* complementary gene, giving resistance to strains of *bean common* mosaic virus (BCMV) only when together with one or more of the strain-specific resistance genes (Drijfhout 1978b).

bc-1^{1} with *bc-u* gives resistance to BCMV strains NL1 and NL8 (Drijfhout 1978b).

bc-1^{2} with *bc-u* gives resistance to BCMV strains NL1, NL2, NL7, and NL8 (Drijfhout 1978b).

bc-2^{1} with *bc-u* gives resistance to BCMV strains NL1, NL4, NL6, and NL7 (Drijfhout 1978b).

bc-2^{2} with *bc-u* gives resistance to BCMV strains NL1, NL2, NL5, NL6, NL7, and NL8 (Drijfhout 1978b).

bc-3 with *bc-u* gives resistance to all strains of BCMV (Drijfhout 1978b).

Bcm confers temperature-sensitive resistance to *blackeye cowpea mosaic* virus. Tightly linked, if not identical, to the *I* gene for resistance to bean common mosaic virus (Kyle and Provvidenti 1987; Provvidenti et al. 1983).

Bct (*Ctv-1*) a gene conditioning resistance to *beet curly top virus* discovered by Schultz and Dean (1947). The *Ctv-1* symbol was proposed by Provvidenti (1987) and updated to *Bct* by Larsen and Miklas (2004). *Bct* is located between the *Phs* and *Asp* loci (Miklas et al. 2000) on chromosome 4 (Freyre et al. 1998; Pedrosa et al. 2003).

Bdm confers resistance to *Bean dwarf mosaic* virus (BDMV) through the blockage of long-distance movement in the phloem (may or may not be associated with a hypersensitive response) (Seo et al. 2004).

Beg with *P v* (Line 214), gives *begonia* red flower color by fully dominant action, but with *P v*^{lae}, expresses partial dominance for *begonia* red flower (Lamprecht 1948b). Allelism of *Beg* with *Sal* was not tested (Bassett 2003b).

bgm confers resistance (prevents a chlorotic response) to bean golden yellow mosaic virus (BGYMV) (Velez et al. 1998).

bgm-2 confers resistance (prevents a chlorotic response) to BGYMV (Velez et al. 1998).

Bip *bipunctata* (Latin): *Bip* and *bip* combine with *Arc* and *arc* to form seed coat patterns based on the hilum; extends seed coat color in partly colored seeds (Lamprecht 1932d, 1940b). Genotype *t z bip* expresses the bipunctata pattern of partly colored seed coats; whereas *t z Bip* expresses virgarcus pattern (Bassett 1996c; Schreiber 1940). *Bip* is linked to *J* and is located (McClean et al. 2002) on chromosome 8 (Freyre et al. 1998; Pedrosa et al. 2003).

bip^{ana} Anasazi pattern of partly colored seed coats is expressed by genotype *t Z bip*^{ana}; whereas *t z bip*^{ana} expresses the Anabip pattern (Bassett et al. 2000).

blu *blue* flower color mutant (Bassett 1992a).

Bpm confers resistance to *bean pod mottle* virus (Thomas and Zaumeyer 1950); symbol proposed by Provvidenti (1987).

Bsm confers resistance to *bean southern mosaic* virus (Zaumeyer and Harter 1943); symbol proposed by Provvidenti (1987).

By-1 confers strain-specific resistance to pea mosaic virus, a strain of *bean yellow* mosaic virus (Schroeder and Provvidenti 1968).

By-2 strain-unspecific gene for temperature sensitive resistance to *bean yellow* mosaic virus (Dickson and Natti 1968).

C with *P z j g b v*, sulfur-white or primrose yellow testa; no color in the hilum ring (Lamprecht 1932a, 1939, 1951a, 1951b; Tjebbes and Kooiman 1922b). According to Feenstra (1960), this *C* is the equivalent of the *B* of Tjebbes (1927), of Kooiman (1920), and of Sirks (1922), and the *Cm* of Prakken (1934). From the early 20th century until the present, the regulation of color and pattern expression (especially in seed coats, but also in other plants organs, e.g., flowers, pods, petioles and stems) at *C* has had dual characterization as both a series of alleles at a locus and a series of very tightly linked genes in one chromosome region (Prakken, 1974). Plant introduction (PI) lines with various seed coat patterns were identified and demonstrated to be allelic (Troy and Hartman 1978). The interactions of *C* and *J* were summarized by Prakken (1972b). *C* is located (McClean et al. 2002) on chromosome 8 (Freyre et al. 1998; Pedrosa et al. 2003).

C/c inconstant (ever-segregating) mottling with color genes (Lamprecht 1932a, 1939; Prakken 1940-1941; Shaw and Norton 1918; Tschermak 1912). According to Prakken (1974), the "complex *C* locus" includes 6 tightly linked loci, including *M, Pr, Acc, C/c*, *R,* and *C*st.

c^{cr} superscript cr, *completely recessive*: the heterozygote *C/c*^{cr} shows the pure dark pattern color *C/C*, without mottling as in *C/c* and *C/c*^{u} (Nakayama 1965).

C^{cir} superscript cir, *circumdatus* (Latin): lateral accumulation of medium sized spots on the testa (Lamprecht 1947a).

C^{ma} (M, R^{ma}) responsible for constant (not heterozygosity dependent) (superscript ma) *marbling* of the seed coat; the colors depend on other genes (Emerson 1909a; Shull 1908; Smith 1939, 1947; Tschermak 1912). Later interpreted to be an allele of R and re-designated R^{ma} (Lamprecht 1947a). M was originally used by Shull (1908) for inconstant mottling. M with Ro and V produces marbling of the pod (Lamprecht 1940a, 1951b). According to Prakken (1974), C, R, and M are 3 distinct but very closely linked loci that are included in the "complex C locus."

C^{r} indistinct, inconstant mottling of the seed coat (Lamprecht 1940a, 1947a; Smith 1939).

C^{res} superscript res, *resperus* (Latin): sprinkled or speckled seed coat (Lamprecht 1940a, 1947a).

C^{rho} superscript rho, *rhomboidus* (Latin): rhomboid spotting of the testa (Lamprecht 1947a; Troy and Hartman 1978).

C^{st} superscript st, *striping* on seed coat and pod (Kooiman 1931; Lamprecht 1939; Sirks 1922; Smith 1939; Tjebbes and Kooiman 1919b; Tschermak 1912); considered by Lamprecht (1947a) to be due to R^{st}. The C^{st} allele in 'La Gaude' has the pleiotropic effect of producing blackish violet zebra-like veins on the standard petal of the flowers (Prakken 1977a).

[C^{st} R Acc] (*Aeq*) with *v*, also "darkens" the tip of the banner petal (Prakken 1972b and 1974), i.e., the otherwise white standard has a red tip; the genes R and Acc are tightly linked within the "complex C locus" (Prakken 1974); the *Terminalverstärkung der Blütenfarbe* character of Lamprecht (1961a) does not require his *Uc, Unc* genes to account for its highly variable penetrance (color intensity).

c^{u} (*inh*, i_e) superscript u, *unchangeable*: produces a creamish testa (Feenstra 1960); the modifier genes G, B, and V do not change the pale background color of P J c^{u} (Prakken 1970). With v^{lse}, c^{u} blocks production of flavonol glycosides; with V, c^{u} blocks production of flavonol glycosides and anthocyanin (Feenstra 1960).

[c^{u} Prp^{i}] (*Prp*, c^{ui}, *Nud*) with T P V produces cartridge buff seed coats, with very tight genetic linkage to a syndrome of anthocyanin (superscript i) *intensification* effects: *purple* flower buds, *intense purple* flowers, *purple* pods, *purple* petioles and stems, and a blush of *purple* on leaf lamina as found in 'Royal Burgundy' (Bassett 1994a; Kooiman 1931); a series of purple pod "alleles" exist at the complex C locus (Bassett 1994a; Okonkwo and Clayberg 1984). The same anthocyanin intensification syndrome has been reported repeatedly (but incompletely), each time with a new gene symbol: *Nud* by Lamprecht (1935e), c^{ui} by Nakayama (1964), and *Prp* by Okonkwo and Clayberg (1984).

[c^{u} prp^{st}] (prp^{st}) with T P V produces cartridge buff seed coats with very tight genetic linkage to green pods with *purple* (superscript st) *stripes* as found in Contender (Bassett 1994a).

[C Prp] (*Prp*, *Ro*) with T P J B V produces black seed coats and purple pods as found in 'Preto 146' (Bassett 1994a).

c^{v} a completely recessive c that does not show heterozygous mottling and has no effect on seed coat color except with V, producing a grayish brown with G B V (Bassett 1995b).

[C R] (R) with P, produces a *red* seed coat (Emerson 1909b; Lamprecht 1935a; Tjebbes and Kooiman 1921) that has been variously described as light vinaceous (Tjebbes and Kooiman 1921), light purple vinaceous (Lamprecht 1947a), and deep oxblood red (Smith 1939), the differences possibly due to modifying genes. The flowers are red (Tjebbes and Kooiman 1922b). It does not affect the color of the hilum ring (Lamprecht 1939). R, R^{cir}, R^{r}, R^{res}, R^{rho}, and r are allelic, according to Lamprecht (1947a); but Prakken (1977b) has shown that C^{st} patterns can exist without the R locus red color. Therefore, the striping, marbling, and other patterns are more correctly designated as properties of the C locus, and the bracket notation, [C R], is used to indicate two genes with nearly unbreakable linkage (Bassett 1991b). The interactions of [C R] with other genes controlling seed coat color were summarized by Prakken (1972b).

[C r] (r) with appropriate modifier genes gives white seed coat (Emerson 1909b; Lamprecht 1940a, 1947a).

Ca *caruncula* (Latin): expresses a stripe pattern, originating at the caruncula and extending away from the hilum (Lamprecht 1932c and 1934a).

Cam confers temperature sensitive resistance to *cowpea aphid-borne mosaic* virus. Tightly linked, if not identical, to the I gene for resistance to bean common mosaic virus (Kyle and Provvidenti 1987; Provvidenti et al. 1983).

Cav *Caruncula verruca* (Latin): causes a wrinkling of the testa radiating from the caruncula (Lamprecht 1955). The heterozygote is less distinct.

cc *chlorotic cup* leaf mutation (Nagata and Bassett 1984).

chl pale green *chlorophyll* deficiency (Nakayama 1959a).

cl *circumlineatus* (Latin): in partly colored seed coats, each of the color centers and even the smallest dots are bordered by (circumlineated) a sharp precipitation-like line (Prakken 1972b).

cml *chlorotic moderately lanceolate* leaf mutant (Bassett 1992c).

Co-1 (*A*) an anthracnose [*Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams.-Scrib.] resistance gene discovered by McRostie (1919) and found in the Andean variety Michigan Dark Red Kidney. *Co-1* is located (Kelly et al. 2003) on chromosome 2 (Freyre et al. 1998; Pedrosa et al. 2003). The gene symbol base *Co* was proposed for all anthracnose resistance genes by Kelly and Young (1996). A recent comprehensive review of the genetics of anthracnose resistance in common bean is available (Kelly and Vallejo 2004).

Co-1^2 an anthracnose resistance gene discovered by Melotto and Kelly (2000) and found in 'Kaboon'.

Co-1^3 an anthracnose resistance gene discovered by Melotto and Kelly (2000) and found in 'Perry Marrow'.

Co-1^4 an anthracnose resistance gene discovered by Alzate-Marin et al. (2003a) and found in 'AND277'.

Co-2 (*Are*) an anthracnose resistance gene discovered by Mastenbroek (1960) and found in the Middle American differential variety Cornell 49242. *Co-2* is located (Adam-Blondon et al. 1994) on chromosome 6 (Freyre et al. 1998; Pedrosa et al. 2003).

Co-3 (*Mexique 1*) an anthracnose resistance gene discovered by Bannerot (1965) and found in the Middle American variety Mexico 222. *Co-3* is located (Rodriguez-Suarez et al. 2004) on chromosome 10 (Freyre et al. 1998; Pedrosa 2003).

Co-3^2 an anthracnose resistance gene found in the Middle American variety Mexico 227 (Fouilloux 1979).

Co-4 (*Mexique 2*) an anthracnose resistance gene discovered by Bannerot in 1969 (Fouilloux 1976, 1979) and found in the Middle American differential variety TO. *Co-4* is located (Kelly et al. 2003) on chromosome 3 (Freyre et al. 1998; Pedrosa et al. 2003).

Co-4^2 an anthracnose resistance gene found in SEL 1308 and G2333 (Young et al. 1998).

Co-4^3 an anthracnose resistance gene found in PI 207262 (Alzate-Marin et al. 2002).

Co-5 (*Mexique 3*) an anthracnose resistance gene discovered by Bannerot in 1969 (Fouilloux 1976, 1979) and found in the Middle American differential variety TU and G2333, SEL 1360 (Young et al. 1998).

Co-6 an anthracnose resistance gene discovered by Schwartz et al. (1982) and found in the Middle American differential variety AB136. *Co-6* is located (Kelly et al. 2003; Mendez de Vigo 2002) on chromosome 4 (Freyre et al. 1998; Pedrosa et al. 2003).

Co-7 an anthracnose resistance gene discovered by Pastor-Corrales et al. (1994) and found in the Middle American differential variety G2333 and selection 1308 from G2333 (Young et al. 1998).

co-8 an anthracnose resistance gene first described in differential variety AB136 (Alzate-Marin et al. 1997).

Co-9 an anthracnose resistance gene first described by Geffroy et al. (1999) in the variety BAT93. The *Co-9* gene is also present in the differential variety PI 207262 (Alzate-Marin et al. 2003c). *Co-9* is located (Geffroy et al. 1999) on chromosome 10 (Freyre et al. 1998; Pedrosa et al. 2003). Preliminary data demonstrate that *Co-9* may be an allele of *Co-3*, and appropriate changes in gene symbols may soon be forthcoming (Rodriguez-Suarez et al. 2004).

Co-10 an anthracnose resistance gene described by Alzate-Marin et al. (2003b) in the variety Ouro Negro. *Co-10* is located (Alzate-Marin et al. 2003b) on chromosome 10 (Freyre et al. 1998; Pedrosa et al. 2003).

cr-1 cr-2 complementary recessive genes for crippled morphology, i.e., stunted plants with small, crinkled leaves (Coyne 1965; Finke et al. 1986).

Crg this *complements resistance gene* is a factor necessary for the expression of *Ur-3*-mediated bean rust resistance and is located (Kalavacharla et al. 2000) on chromosome 3 (Freyre et al. 1998; Pedrosa et al. 2003).

cry *crypto-dwarf*: a dwarfing gene; with *Fin* intermediate height (Nakayama 1957); with *la* produces long internodes resulting in slender type of growth in bush (*fin*) but not in tall (*Fin*) forms (Lamprecht 1947b).

cs *chlorotic stem* mutant (Nagata and Bassett 1984).

Ct for *curved* pod *tip* shape; *ct* for straight pod tip (Al-Muktar and Coyne 1981).

ctv-1 ctv-2 confer resistance to beet *curly top virus* (Schultz and Dean 1947); symbol proposed by Provvidenti (1987).

cyv (*by-3*) confers high level resistance to *clover yellow vein* virus, formerly known as the severe, necrotic, or pod-distorting strain of bean yellow mosaic virus (Provvidenti and Schroeder 1973; Tu 1983); symbol proposed by Provvidenti (1987).

Da straight pod (Lamprecht 1932b).

Db polymeric with *Da* for straight pod (Lamprecht 1932b, 1947b). [Polymeric genes have identical functions (expression) but different loci].

dgs (*gl, le*) *dark green savoy* leaf mutant (Frazier and Davis 1966b; Nagata and Bassett 1984). According to Nagata and Bassett (1984), *dgs* is synonymous with the *wrinkled leaf* mutant of Moh (1968) and the *gl* (*glossy*) of Motto et al. (1979); also synonymous with the *le* (*leathery* leaf) of Van Rheenen et al. (1984).

dia *diamond* leaf mutant (Nagata and Bassett 1984). Leaflets are angular, slightly chlorotic, thick, and reduced in area.

Diap-1 structural gene for *diaphorase* enzyme (Weeden and Liang 1985).

Diap-2 structural gene for *diaphorase* enzyme (Sprecher 1988).

diff *diffundere* (Latin): with *exp* gives completely colored testa except for one end of the seed; *diff* with *Bip Arc* gives maximus phenotype, with *bip Arc* gives major phenotype; extends seed coat color in partly colored seeds (Lamprecht 1940b).

dis *dispares* (Latin): mottled or striped flower of scarlet runner bean (Lamprecht 1951c).

Dl-1 Dl-2 (DL_1 DL_2) complementary genes for *dosage-dependent lethality* and developmental abnormality; *Dl Dl Dl-2 Dl-2* is lethal, *Dl dl Dl-2 Dl-2* and *Dl Dl Dl-2 dl-2* are sublethal, *Dl dl Dl-2 dl-2* is temperature dependent abnormal, and *Dl Dl dl-2 dl-2, dl dl Dl-2 Dl-2, Dl dl dl-2 dl-2, dl dl Dl-2 dl-2*, and *dl dl dl-2 dl-2* are normal; *Dl* inhibits root development and *Dl-2* inhibits shoot development (Shii et al. 1980).

do *dwarf out-crossing* mutant (Nagata and Bassett 1984). Out-crossing rates up to 56% are observed due to delayed pollen dehiscence (Nagata and Bassett 1985).

ds (*te*) *dwarf seed*: produces small seeds and short pods with deep constrictions between the seeds; cross pollination with *Ds* gives normal size seeds and pods on *ds*/*ds* plants, breaking the usual dominance of maternal genotype over embryo genotype for seed size development (Bassett 1982); the xenia effect was first described by Tschermak (1931) and the trait was named *tenuis* (Latin) for "narrow" pod by Lamprecht (1961a).

dt-1^{a} *dt-2*^{a} *daylength temperature*: produce early, day-length neutral flowering with complex temperature interactions (Massaya 1978).

dt-1^{b} *dt-2*^{b} *daylength temperature*: control flowering response to short days with complex temperature interactions; *dt-2*^{b} causes increased production of branches (Massaya 1978).

dw-1 dw-2 duplicate genes causing *dwarf* plant (Nakayama 1957).

Ea Eb polymeric genes for "flat" pod, elliptical in cross-section vs. *ea eb* round pod (Lamprecht 1932b, 1947b; Tschermak 1916).

Est-1 structural gene for most anodal *esterase* enzyme (Weeden and Liang 1985).

Est-2 structural gene for second most anodal *esterase* enzyme (Weeden and Liang 1985).

exp *expandere* (Latin): with *diff* gives solid color to seed coat except for one end of the seed, giving minimus and minor phenotypes (Lamprecht 1940b).

F confers resistance to the *F* strain of anthracnose found in variety Robust (McRostie 1919); 'Robust' is extinct, but it was a parent of variety Michelite, which has not been fully characterized for anthracnose resistence although close to *Co-1* type (Kelly, personal communication).

Fa basic gene for pod membrane (Lamprecht 1932b).

fast *fastigate* shape of seed (Lamprecht 1934a).

Fb Fc supplementary genes for pod membrane (Lamprecht 1932b).

fa fb fc weak pod membrane; pod may be constricted (Lamprecht 1932b); may give 9:7, 15:1, or 63:1 ratios (Lamprecht 1932b, 1947b).

fd *delayed flowering* response under long days (Coyne 1970).

Fe-1 Fe-2 *Ferrum* (Latin): complementary dominant genes controlling resistance to leaf chlorosis due to iron deficiency in plants grown on calcareous soils (Coyne et al. 1982; Zaiter et al. 1987).

Fib *fibula* arcs, with *t*, white arcs (bows) expressed in the corona zone of seed coats, together with expansa partly colored pattern (Bassett 2001; Bassett and McClean 2000).

Fin (*in*) *Finitus* (Latin): indeterminate vs. *fin* determinate plant growth (Lamprecht 1935b; Rudorf 1958); long vs. short internode; later vs. earlier flowering. *Fin* is 1 cM from *Z* (Bassett 1997c) and located (McClean et al. 2002) on chromosome 1 (Freyre et al. 1998; Pedrosa et al. 2003).

Fop-1 confers resistance to the Brazilian race of *Fusarium oxysporum* f. sp. *phaseoli* (Ribeiro and Hagedorn 1979).

Fop-2 confers resistance to the U.S. race of *Fusarium oxysporum* f. sp. *phaseoli* (Ribeiro and Hagedorn 1979).

Fr a *fertility restoring* gene (Mackenzie and Bassett 1987) for the cytoplasmic male sterility source derived from CIAT accession line G08063 (Bassett and Shuh 1982). Restoration is partial in F_1, complete and irreversible in fertile F_2 segregants, i.e., the gene alters the mitochondrial DNA, deleting a fragment of at least 25 kilobases in restored plants (Mackenzie et al. 1988; Mackenzie and Chase 1990).

Fr-2 a *fertility restoring* gene that is derived from CIAT accession line G08063 and that restores fertility without deleting the same mitochondrial DNA fragment affected by *Fr* (Mackenzie 1991).

G (*Flav, Ca, Och*) The yellow-brown factor of Prakken (1970). The equivalent of *C* of Shaw and Norton (1918). Prakken (1970) believed that Lamprecht (1951a) genes *Flav, Ca,* and *Och* are synonyms for *G*. The interactions of *G* with other combinations of seed coat color genes are summarized by Prakken (1972b). *G* is located (McClean et al. 2002) on chromosome 10 (Freyre et al. 1998; Pedrosa et al. 2003).

Ga *gametophyte* factor, which achieves complete selection for pollen carrying *Ga*, i.e., no pollen carrying *ga* achieves fertilization (Bassett et al. 1990).

gas *gamete-sterile*: causes both male and female sterility (Lamprecht 1952b).

glb *glossy bronzing* leaf mutant (Bassett 1992c).

Gpi-c1 structural gene for *glucose phosphate isomerase* enzyme, i.e., the more anodal of the two *cytosolic* isozymes (Weeden 1986).

Gr in the presence of *ih*, produces *green* dry pod color; in the presence of *Ih*, produces tan dry pod color; *gr* in the presence of *ih* or *Ih*, produces tan dry pod color (Honma et al. 1968).

gy *greenish yellow* seed coat, usually with *P* [*C r*] *gy J g b v* (or v^{lae}) *Rk* of the Mayocoba market class, but also expressed with *G b v* or *G B v* (Bassett et al. 2002a). A second gene (tentative symbol *Chr*) is necessary to express greenish yellow color in the corona (with *g b* v^{lae}) and hilum ring with *g b* v^{lae} or *g b v* (Bassett 2003c). *Gy* is either closely linked to *C* or is part of the 'complex *C* locus' (Bassett et al. 2002a) on chromosome 3 (Freyre et al. 1998; Pedrosa et al. 2003).

Hbl (L_{HB-1}) controls expression of *halo blight* tolerance in *leaves* (Hill et al. 1972).

Hbnc (SC_{HB-1}) controls expression of *halo blight* tolerance resulting in *nonsystemic chlorosis* of leaves (Hill et al. 1972).

Hbp (PD_{HB-1}) controls expression of *halo blight* tolerance in *pods* (Hill et al. 1972).

hmb controls expression of sensitivity to the *herbicide metobromuron*, where *Hmb* expresses metobromuron insensitivity (Park and Hamill 1993).

Hss *hypersensitivity soybean*: confers a rapid lethal necrotic response to soybean mosaic virus (SMV) that is not temperature sensitive (Kyle and Provvidenti 1993).

Hsw *hypersensitivity watermelon*: confers temperature sensitive resistance (lethal hypersensitivity) to watermelon mosaic virus 2. Very tightly linked, if not identical, to the *I* gene for bean common mosaic virus (Kyle and Provvidenti 1987).

Ht-1 Ht-2 (*L-1 L-2*) genes of equal value for height of plant (Norton 1915). They also increase length of seed (Frets 1951).

I confers temperature sensitive resistance to bean common mosaic virus. Tightly linked, if not identical, to *Bcm*, *Cam*, *Hsw*, and *Hss* (Ali 1950; Kyle et al. 1986; Kyle and Provvidenti 1993). The *I* gene (or the complex *I* region) conditions resistance and/or lethal necrosis to a set of nine potyviruses, BCMV, WMV, BlCMV, CAbMV, AzMV, ThPV, SMV, PWV-K, and ZYMV (Fisher and Kyle 1994). *I* has a nearly terminal position (Vallejos et al. 2000) on chromosome 9 (Freyre et al. 1998; Pedrosa et al. 2003).

Ia Ib parchmented vs. *ia* tender pod (Lamprecht 1947b). Flat or deep (elliptical cross-section) vs. round pod (Lamprecht 1932b, 1947b, 1961a).

ian-1 ian-2 (*ia*) *indehiscent anther* where the heterozygote produces partial indehiscence (Wyatt 1984); currently, two unlinked mimic genes can produce indehiscent anther (Wyatt, personal communication).

lbd *leaf-bleaching dwarf* mutant (Bassett 1992c).

ico *internodia contracta* (Latin): internodes 4-7 cm long instead of the normal 8-11 cm (Lamprecht 1961b).

Igr (*Ih*) *inhibits* the action of *Gr*, conferring tan dry pod color in the presence of *Gr* or *gr* (Honma et al. 1968).

ilo *inflorescentia longa* (Latin): 5-7 long internodes in the inflorescence instead of the usual 2-3 (Lamprecht 1961b).

ip (i_1) *inhibits* the action of *P* with respect to the color of the hypocotyl (Nakayama 1958).

iter iteratus-ramifera (Latin): with *ram* produces triple branched inflorescence (Lamprecht 1935b, 1935d).

iv (i_2) *inhibits* the action of *V* with respect to the color of the hypocotyl; is lethal with v^{lae} (Nakayama 1958).

iw — *immature white* seed coat in the presence of *p* (Baggett and Kean 1984).

J (*Sh*) — With *P*, gives light yellow-brown or pale ochraceous buff testa (Lamprecht 1933), Rohseidengelb testa (Lamprecht 1939), raw silk testa (Lamprecht 1932a, 1951a) and the same color to the hilum ring (Lamprecht 1951a; Prakken 1934). The equivalent of the *Sh* of Prakken (1934) (Lamprecht 1960; Prakken 1970). Similar to *Asp* (Lamprecht 1940c) only in seed coat shininess (Bassett 1996b). It causes seed coats to glisten and to darken with age (Lamprecht 1939). *J* is linked to *Bip* and is located (McClean et al. 2002) on chromosome 8 (Freyre et al. 1998; Pedrosa et al. 2003).

j (*mar*) — Expresses "immature" seed coat colors, viz., paler and highly variable (seed to seed) along the ventral (darker relative to dorsal) to dorsal surface transition, for whatever combination of other seed coat color genes are present (Bassett 1996b; Prakken 1972b). *j* produces dull (mat) seed coat (Prakken 1940-41), nearly white corona with *Z*, and nearly white corona and hilum ring with *z* (Bassett 1996b; Bassett et al. 1996b). Same as *mar* of Lamprecht (1933) for a broad band of color about the hilum. With *j*, no leuco-anthocyanidins are synthesized and production of anthocyanins and flavonol glycosides is low (Feenstra 1960).

j^{ers} (*ers-2*) — The j^{ers} allele (from 'Early Wax') differs from *j* expression: *T Z* j^{ers} fails to express the margo pattern of *T Z j*, *T z* j^{ers} fails to express the margo *z* pattern of *T z j*, and *t Z* j^{ers} fails to express marginata of *t Z j*; but *t z* j^{ers} and *t z j* express white seed coats (Bassett 1997d; Bassett et al. 2002b). *T/t z/z j/*j^{ers} in a *P C J G B V* background expresses reverse margo pattern (Bassett et al. 2002b).

Ke — *potassium* utilization *efficiency* (Shea et al. 1967).

la — *Lamm*: with *cry* gives long internode; *la* with *Fin* is dwarf; *la cry fin* is slender (Lamprecht 1947b).

Lan — *lanceolate* leaf mutant; *Lan*/*Lan* is usually a zygotic lethal, and survivors are dwarfs that do not flower; *Lan*/*lan* segregates 2:1 (lanceolate to normal) in selfed progeny (Bassett 1981).

Ld — *leaf distortion* resembling phenoxy herbicide injury, with interveinal clearing, slight chlorosis, necrotic scarring of the midrib, altered leaf shape, and extra leaflets (Rabakoarihanta and Baggett 1983).

Lds (*Ds*) — *Ld suppressor* (Rabakoarihanta and Baggett 1983).

Lec — structural gene for the seed protein *lectin* or phytohemagglutinin (Osborn et al. 1986).

Li (*L*) — *long* vs. *li* short *internodes* (Lamprecht 1947b; Norton 1915).

lo — plants have a short inflorescence (Lamprecht 1958).

lr-1 lr-2 — the double recessive genotype produces *leaf rolling* of trifoliolate leaves through the third or fourth nodes, ending in stem and apical necrosis and death of the plant (Provvidenti and Schroeder 1969).

Me — structural gene for *malic enzyme* (Weeden 1984).

Mel (*Me*) — confers nematode resistance to *Meloidogyne incognita* (some isolates of race 1), *M. javanica*, and *M. arenaria* (Omwega et al. 1990).

Mel-2 (*Me-2*) — confers nematode resistance to *Meloidogyne incognita* race 1 (isolates to which *Mel* is susceptible), race 2 and race 3, but is susceptible to *M. javanica* and *M. arenaria* (Omwega and Roberts 1992).

mel-3 (*me-3*) — confers temperature sensitive nematode resistance (resistant at 26 C but susceptible at 28 C) to the same species, races, and isolates as with *Mel-2* (Omwega and Roberts 1992).

Mf — *mancha na flor* (Portuguese): brownish-violet blotch on the base of the standard flower petal (Vieira and Shands 1969).

mi, *mia* — micropylar stripe pattern (Lamprecht 1932c and 1934a); both 3:1 and 15:1 segregation were observed.

Mic (*Mip*) — *micropyle inpunctata* (Latin): small dots near the micropyle (Lamprecht 1940c).

miv — *minor intervallis* (Latin): end of seed flattened and a short distance between funicles (Lamprecht 1952a).

Mrf — *Mosaico rugoso del frijol* (Portuguese): confers immunity to bean rugose mosaic virus (Machado and Pinchinat 1975).

Mrf^2 — *Mosaico rugoso del frijol* (Portuguese): confers the localized lesion type of resistance to bean regose mosaic virus; the order of dominance in the allelic series is *Mrf*>Mrf^2>*mrf* (Machado and Pinchinat 1975).

mrf — *mosaico rugoso del frijol* (Portuguese): confers susceptibility (systemic infection) to bean rugose mosaic virus (Machado and Pinchinat 1975).

ms-1 — an induced mutant for genic *male sterility*, where no pollen is produced but female fertility is unimpaired (Bassett and Silbernagel 1992).

Mue — structural gene for *methylumbelliferyl esterase* (Garrido et al. 1991).

mu — *mutator* locus that produces mutations of *us* to *Us*, thus giving normal green leaf sectors in yellow leaves due to *us mu*, where the ratio of normal to variegated plants is 15:1 (Coyne 1966).

Nag — structural gene for *N-acetyl glucoseaminidase* enzyme (Weeden 1986).

Nd-1 Nd-2 (*D-1 D-2*) additively control the variation in *node* number on the main stem of determinate beans and additively control the number of days to flowering (Evans et al. 1975).

nie an induced mutation for *ineffective nodulation* by *Rhizobium* (Park and Buttery 1994).

nnd (*sym-1*) an induced mutation for *non-nodulation* by *Rhizobium*, i.e., lacking capacity for *symbiosis* (Pedalino et al. 1992).

nnd-2 an induced mutation for *non-nodulation* by *Rhizobium* (Park and Buttery 1994).

No with *P v*, expresses Light *Nopal* Red (light salmon with brownish tinge) flower color and much darker reddish color of flower buds by pleiotropic action; with *P V*, expresses Pure *Nopal* Red flower; *No* action is fully dominant; *No* is linked (31 cM) to *Fin* (Lamprecht 1948b, 1961a). Allelism of *No* with *Sal* was not tested (Bassett 2003b).

nts (*nod*) *nitrogen tolerant supernodulation*: an induced mutation that permits abundant nodulation in the presence of high nitrogen (Park and Buttery 1989).

ol *overlapping leaflets* mutant (Bassett 1992c).

P basic color gene (Emerson 1909a; North and Squibbs 1952; Prakken 1934; Schreiber 1934; Shaw and Norton 1918; Shull 1908; Skoog 1952). *P* without color genes is colorless as is *p* (Lamprecht 1939; Smith 1939). According to Feenstra (1960), *P* is the equivalent of the *A* of Tschermak (1912), of Kooiman (1920), and of Sirks (1922). *P* has a nearly terminal location (Erdmann et al. 2002; Vallejos et al. 1992) on chromosome 4 (Freyre et al. 1998; Pedrosa et al. 2003).

p white seed coat and flower (Emerson 1909a).

p^{gri} (*Gri,* v^{pal}) superscript gri, *griseoalbus* (Latin): p^{gri} with *C J B V* produces grayish white (blubber white) seed coat without a hilum ring, giving the dominance order $P>p^{gri}>p$ (Bassett 1994b; Lamprecht 1936); p^{gri} with *C J B V* produces flowers with very pale lavender wing petals and two dots of violet on the upper edge (center) of an otherwise near white standard petal (Bassett 1992b); formerly a second basic color factor like *P* (Lamprecht 1936). Lamprecht (1936) speculated that the flower color observed with p^{gri} segregation must be due to an undiscovered new allele (tentatively v^{pal}) at *V*. p^{stp}superscript stp, *stippled* seed coat and white flowers with a narrow, violet banner tip and pale violet periphery (2-3 mm) on the wing petals (Bassett 1996a, 2003a).

p^{hbw} stippled seed coat (different from p^{stp}) and violet flowers with the lower (superscript hbw) *half* of the *banner* petal *white* (Bassett 1996a, 2003a).

p^{mic} self-colored seed coat except for a white (superscript mic) *micropyle* stripe and violet flowers without pattern (Bassett 1998, 2003a).

pa *pale* green leaves (Smith 1934).

pc *persistant* green pod *color* (Dean 1968).

pg (pa_1) *pale-green* foliage mutant (Wyatt 1981).

Pha structural gene for the seed protein *phaseolin* (Osborn et al. 1986).

Pmv confers incomplete dominance for resistance to *peanut mottle virus* (Provvidenti and Chirco 1987).

ppd (*neu*) *photoperiod-insensitive* gene found in 'Redkloud' with a syndrome of effects (Wallace et al. 1993); an allele-specific associated primer is now available for *ppd* (Gu et al. 1995); probably the same locus as Neu^+ for short day vs. *neu* for day *neutral* flowering response to length of day of Rudorf (1958).

Pr *Preventing* the "flowing out" of red color (Prakken 1972b, 1974); *pr* with pattern alleles at *C* and *R* allow the red color in the dark pattern color zones to "flow out" into the light pattern color areas, producing various light red hues such that the contrast between the dark and light pattern colors is very small; tightly linked to the *C* locus.

Prp^i*-2* a gene controlling (superscript i) *intensified* anthocyanin (*purple*) expression syndrome (not linked to *C*) in flower buds, corolla, pods, stems and leaf lamina (Bassett 2004a).

prc (*pc*) *progressive chlorosis* mutant (Nagata and Bassett 1984); redesignated *prc* (Awuma and Bassett 1988).

Prx structural gene for *peroxidase* enzyme, i.e., the most cathodal of the peroxidase isozymes (Weeden 1986).

Pse-1 (*R1*) a halo blight resistance gene described by Walker and Patel (1964) and reported as the *R1* gene by Teverson (1991) and Taylor et al. (1996); present in the halo blight differential variety Red Mexican UI-3. *Pse-1* is linked with rust and anthracnose resistance genes and located (Fourie et al. 2004) on chromosome 10 (Freyre et al. 1998; Pedrosa et al. 2003).

Pse-2 (*R2*) a halo blight resistance gene described by Teverson (1991) and Taylor et al. (1996) as present (as *R2*) in the halo blight differential variety A43 (ZAA12).

Pse-3 (*R3*) a halo blight resistance gene described by Teverson (1991) and Taylor et al. (1996) as present (as *R3*) in the halo blight differential variety Tendergreen. *Pse-3* is completely linked with the *I* gene locus (Fourie et al. 2004; Teverson 1991) on chromosome 4 (Freyre et al. 1998; Pedrosa et al. 2003).

Pse-4 (*R4*) a halo blight resistance gene discovered by Teverson (1991) and Taylor et al. (1996) to be present (as *R4*) in the halo blight differential variety Red Mexican UI-3. *Pse-4* is linked (14.7 cM) to the *Pse-1* locus (Fourie et al. 2004) on chromosome 10 (Freyre et al. 1998; Pedrosa et al. 2003).

Pse-5 (*R5*) a halo blight resistance gene described by Teverson (1991) and Taylor et al. (1996) as present (as *R5*) in the halo blight differential variety A43 (ZAA12).

punc *punctatus* (Latin): causes dotting of the testa (Lamprecht 1940c).

ram *ramifera* (Latin): branched inflorescence (Lamprecht 1935b).

Rbcs (*rbcS*) *small* subunit of the *rubisco* enzyme (Weeden 1984).

rf-1 *reclining foliage* due to downward slanting petioles (Bassett 1976). *Rf-1* is linked (11 cM) to *V* (Bassett 1997a), and *V* is located (McClean et al. 2002) on chromosome 1 (Freyre et al. 1998; Pedrosa et al. 2003).

rf-2 *reclining foliage* mutant due to downward slanting petioles (Bassett and Awuma 1989).

rf-3 *reclining foliage* mutant due to downward slanting petioles (Bassett and Awuma 1989).

rfi (*i*) *reclining foliage inhibitor*: recessive epistatic factor to *rf-1* and *rf-3* (Bassett 1976; Bassett and Awuma 1989).

Rfs (*m*) *reclining foliage suppressor*: dominant suppressor of *rf-1* (Bassett 1976).

Rk *red kidney*: the *Rk* allele does not express testaceous (pink) color of light red kidney beans (Gloyer 1928; Smith 1939) or garnet brown color of dark red kidney beans (Smith and Madsen 1948); interactions of *rk* and rk^{d} with *C*, *D* (now *Z*, Bassett et al. 1999b), *J*, *B*, and *V* (using Prakken's symbols) were investigated (Smith 1961). According to Prakken (1972b), *Rk* is linked (28 cM) to *B*, which is located (Kyle and Dickson 1988; Vallejos et al. 2000) on chromosome 9 (Freyre et al. 1998; Pedrosa et al. 2003).

rk *red kidney*: with *m* or *c* (now c^{u}), *rk* expresses testaceous (pink) seed coat color; with *M* (red/buff marbled pattern), *rk* modifies cartridge buff expression to testaceous (Smith 1939, 1947); *rk* is dominant over rk^{d} (Smith and Madsen 1948); *rk* has no expression with *j* (Lamprecht 1961c; Smith 1961).

rk^{d} (*lin*) *red kidney* (superscript d) *dark*: with *r* (now c^{u}) and *J*, rk^{d} expresses garnet brown testa (Smith and Madsen 1948); rk^{d} has no expression with *j* (Smith 1961). With *P v* (or v^{lae}) and either *T*/- or *t*/*t*/, rk^{d} always gives red veins in the wing petals, whether clear or faint (Prakken 1972a, b); in some genetic backgrounds the red veins are "incompletely recessive", i.e., Rk/rk^{cd} gives very faint red veins (Prakken 1972b). The red color of red kidney beans (all recessive alleles) is expressed by proanthocyanidins although three yellow flavonol glycosides are also present in the seed coats (Beninger and Hosfield 1999).

rk^{drv} *red kidney* (superscript drv) *dark red vein*: with *P v*, a spontaneous mutant of the rk^{d} gene expressing red wing petal veins that are "expanded" (larger in diameter and diffuse) compared to those of rk^{d}, creating the illusion of pale pink flowers when viewed at one meter or more (Bassett 2004b).

rk^{cd} *red kidney* (superscript cd) *convertible dark*: *C* rk^{cd} expresses garnet brown seed coats, whereas c^{u} rk^{cd} expresses pink (testaceous) seed coats; thus, expression at rk^{cd} (from 'NW 63') is a function of interaction with *C* (Bassett and Miklas 2003).

rk^{p} *red kidney* (superscript p) *pink*: rk^{p} (from 'Sutter Pink') expresses consistently very weak pink color under humid growing conditions, unlike *rk* from 'Redkloud' (Bassett and Miklas 2003).

rn-1 rn-2 (*r r*N) together confer resistance to *root-knot nematode*, where 2-4 dominant alleles give susceptible reaction and 1 dominant allele gives intermediate resistance in a 11:4:1 ratio (Barrons 1940).

rnd *round* leaf mutant with lateral leaflet tips rounded (Nagata and Bassett 1984).

Sal with *P, Sal* expresses *salmon* red flower color and a reddish tinge to the testa; scarlet red flower is expressed with *Sal Am Beg N*o (Lamprecht 1948b). *Salmon* red flower color (Fan 1, 52C or D; Royal Hort. Soc. fans) is expressed by *Sal am* V^{wf} (or *v*), and scarlet flower (Fan 1, 43C; Royal Hort. Soc. fans) is expressed by *Sal Am* V^{wf} (or *v*) (Bassett 2003b). *Sal Am v* expressed oxblood red seed coats (vs. mineral brown tinged with red) due either to a pleiotropic effect of *Am* or a very closely linked dominant gene (Bassett 2003b), and *Am* has no expression with *sal* (Bassett 2003b).

sb *spindly branch* mutant; the stems are thinner and more highly branched than normal (Awuma and Bassett 1988).

sb^{ms} *spindly branch* (superscript ms) *male sterile* mutant; allelic with *sb*; anthers are atrophied and produce no viable pollen, but there is no loss of female fertility (Bassett 1991a)

sb-2 *spindly branch* mutant; the stems are thinner and more highly branched than normal (Bassett 1990).

sb-3 *spindly branch* mutant; the stems are thinner and more highly branched than normal (Bassett 1990).

sil *silver* colored leaves and severe plant stunting under high intensity light in the field; no stunting under glasshouse culture (Frazier and Davis 1966a; Nagata and Bassett 1984).

Skdh structural gene for *shikimate dehydrogenase* enzyme (Weeden 1984).

sl *stipelless lanceolate* leaf mutant (Nagata and Bassett 1984) gives a lanceolate leaf form with loss of stipels from the terminal leaflet.

Smv confers incompletely dominant resistance to *soybean mosaic virus* (Provvidenti et al. 1982).

St *stringless* pod; *st* gives a complete string (Prakken 1934); has modifiers.

Sur *Sursum versus* (Latin): causes leaves and petioles to point downward (Lamprecht 1937) with pulvinule rotated 180E. See X^{su}.

sw-1 sw-2 the double recessive genotype produces *seedling wilt* (Provvidenti and Schroeder 1969), i.e., epinasty of primary leaves, necrosis of terminal bud, and death of the plant in primary leaf stage.

T self-colored seed coat and colored flowers (Emerson 1909a; Lamprecht 1934b; Shaw and Norton 1918). *T* is located (McClean et al. 2002) on chromosome 11 (Freyre et al. 1998; Pedrosa et al. 2003).

t (*z-1*) a seed coat pattern gene required for all partly colored seed coat patterns; has pleiotropic expression for white flowers (Schreiber 1934; Shaw and Norton 1918) and green cotyledons and hypocotyls (Prakken, 1972b). Early reports of interactions of *t* with *Z* and *z* (Lamprecht 1934b; Sax 1923; Shaw and Norton 1918) were later extended to *t* interactions with *Z*, *J*, and *Bip* (Bassett 1994c, 1996b and c, 1997c and d; Bassett et al. 2000, 2002b; Lamprecht 1940b; Schreiber 1940).

t^{cf} superscript cf, *colored flower*: a seed coat gene (from PI 597984) for partly colored patterns without pleiotropic expression for white flowers; necessary for expression of the two-points pattern (Bassett et al. 1999a).

Th-1 Th-2 genes of equal value for seed *thickness* (Frets 1951).

Tm confers immunity to *tobacco mosaic* virus (Thompson et al. 1952).

To cell wall fiber (Prakken 1934).

top *topiary* plant architecture; a spontaneous mutant with determinate habit (terminal bud is reproductive); dark green leaves on shortened rachis, petiolules, and petioles that cause overlapping leaflets held close to the stem (Guner and Myers 2000).

Tor (*T*) *torquere* (Latin): twining habit vs. *tor* non-twining (Norton 1915; Lamprecht 1947b); confers phytochrome-controlled climbing habit in indeterminate bush bean types (Kretchner et al. 1961; Kretchmer and Wallace 1978).

Tr *testa rupture* (Dickson 1969); an incompletely dominant gene with 25-30% penetrance.

tri *tricotyledonae* (Latin): produces three cotyledons (Lamprecht 1961b) with 40-50% penetrance.

trv confers resistance to *tobacco ringspot virus* (Tu 1983); symbol proposed by Provvidenti (1987).

Ts *temperature-dependant string* formation (Drijfhout 1978a); *St ts* is without string, *St Ts* expresses incomplete string, and *st Ts* and *st ts* have complete string.

tw *twisted* pod character produces pod rotation that is highly variable, from slight to more than 360 degrees in snap bean germplasm (Baggett and Kean 1995).

uni *unifoliata* (Latin): unifoliate leaves; complete sterility (Lamprecht 1935c); this material is lost, and no allelism tests were made with other unifoliate mutants before *uni-1* was lost.

Uni-2 a dominant mutation for *unifoliate* true leaves (Garrido et al. 1991).

uni^{nde} induced mutation with *unifoliate* leaves with (superscript nde) *node dependent expression*; partial fertility and shows reversion to normal leaflet number at higher nodes (Myers and Bassett 1993).

uni^{nie} *unifoliate* leaves with (superscript nie) *node independent expression* (natural mutant); completely female sterile but male-fertile and shows consistently strong expression of the unifoliate trait at higher nodes (Myers and Bassett 1993).

Ur-1 a rust [*Uromyces appendiculatus* (Pers.) Unger var. *appendiculatus*] resistance gene discovered by Ballantyne (1978) and found in the Middle American source ‘B1627'. Kelly et al. (1996) proposed using the *Ur* symbol as a base for all rust resistance genes.

Ur-2 a rust resistance gene discovered by Ballantyne (1978) and found in the Middle American source ‘B2090'.

Ur-2^2 a rust resistance allele at the *Ur-2* locus discovered by Ballantyne (1978) and found in the Middle American source 'B2055'. *Ur-3* a rust resistance gene discovered by Ballantyne (1978) (see also Ballantyne and McIntosh 1977) and found in the Middle American sources 'Aurora', 'Mex 235', 'Nep-2', and '51051', albeit with slightly different reaction profiles across a differential set of races for each source (Miklas et al, 2002). *Ur-3* is linked to the *Co-2* gene and has a nearly terminal position (Miklas et al. 2002) on chromosome 6 (Freyre et al. 1998; Kelly et al. 2003; Pedrosa et al. 2003). *Ur-4* (*Up-2, Ur-C*)a rust resistance gene originally discovered by Ballantyne (1978) as *Ur-C* and rediscovered by Christ and Groth (1982) as *Up-2*. *Ur-4* is an Andean gene found in 'Early Gallatin' and is located (Miklas et al. 2002) on chromosome 1 (Freyre et al. 1998; Kelly et al. 2003; Pedrosa et al. 2003).

Ur-5 (B-190) a block (cluster) of eight tightly linked rust resistance genes (*Ur-5A* through *Ur-5H*) found by Stavely (1984) and present in the rust differential variety Mexico 309. *Ur-5* is located (Miklas et al. 2002) in the vicinity of other resistance genes (Kelly et al. 2003) on chromosome 10 (Freyre et al. 1998; Pedrosa et al. 2003).

Ur-6 (*Ur*$_a$, *Ur-G*) a rust resistance gene originally discovered by Ballantyne (1978) as *Ur-G* and rediscovered by Grafton et al. (1985) as *Ur*$_a$. *Ur-6* is an Andean gene present in 'Olathe' and the rust differential variety Golden Gate Wax. *Ur-6* is independent of *Ur-3* and located (Miklas et al. 2002) on chromosome 6 (Freyre et al. 1998; Pedrosa et al. 2003).

Ur-7 (*R*$_{B11}$) a rust resistance gene discovered by Augustin et al. (1972) and found in the Middle American varieties GN 1140 and Pinto US-5. *Ur-7* is independent of *Ur-3* and *Ur-6* and located (Park et al. 2003) on chromosome 6 (Freyre et al. 1998; Pedrosa et al. 2003).

Ur-8 (*Up-1*) a rust resistance gene discovered by Christ and Groth (1982) and found in the Andean variety U.S. #3.

Ur-9 (*Ur*$_p$) a rust resistance gene discovered by Finke el al. (1986) and found in the Andean variety Pompadour Checa. *Ur-9* is located (Miklas et al. 2002) near the *Co-1* locus (Kelly et al. 2003) on chromosome 2 (Freyre et al. 1998; Pedrosa et al. 2003).

Ur-10 (*URPR1*) a rust resistance gene discovered by Webster and Ainsworth (1988) and found in snap bean varieties Cape and Resisto.

Ur-11 (*Ur-3*^{2}) originally a rust resistance allele at the *Ur-3* locus discovered by Stavely (1990), but later found to be tightly linked with *Ur-3* (Stavely 1998). *Ur-11* is located (Miklas et al. 2002) on chromosome 6 (Freyre et al. 1998; Pedrosa et al. 2003).

Ur-12 a gene conditioning adult plant resistance (APR) to bean rust discovered by Jung et al. (1998) that is initially expressed at the fourth trifoliolate leaf stage or later. *Ur-12* is found in the Andean variety Pompadour Checa and is tentatively located at a terminal position (Jung et al. 1998; Miklas et al. 2002) on chromosome 4 (Freyre et al. 1998; Pedrosa et al. 2003).

Ur-13 a rust resistance gene discovered by Liebenberg and Pretorius (2004) and found in the cranberry Andean variety Kranskop; however, the gene appears to be of Middle American origin and is carried by variety Redlands Pioneer (Liebenberg and Pretorius 2004). *Ur-13* is located (Liebenberg 2003) on chromosome 3 (Freyre et al. 1998; Pedrosa et al. 2003).

us *unstable* gene that mutates to *Us* in presence of *mu* to produce green leaf sectors in a yellow leaf background due to *us mu*, resulting in variegation (Coyne 1966).

V (*Bl*) with *P* produces pale glaucescens testa without a hilum ring (Lamprecht 1939). The color ranges from pale violet to black depending upon other color genes present (Lamprecht 1932a; Prakken 1934, 1972b). According to Prakken (1972a) the *Bl* of Smith (1939) is the same as *V*. *Bl* with the basic color factors produces purple-violet seed coat (Smith 1939; Tjebbes and Kooiman 1921, 1922a), changes oxblood red to purple (Smith 1939), and is responsible for bluish tints to plant colors (Tjebbes and Kooiman 1921). *bl* with appropriate genes produces red seed coat (Tjebbes and Kooiman 1922a). According to Feenstra (1960), *V* is the equivalent of the *B* of Shull (1908) and of Tschermak (1912), the *F* of Kooiman (1931), the *G* of Shaw and Norton (1918), and the *Z* of Sirks (1922). *V* is located (McClean et al. 2002) on chromosome 1 (Freyre et al. 1998; Pedrosa et al. 2003).

V^{wf} a gene with the seed coat color properties of *V* but with the pleiotropic effect of (superscript wf) *white flower* color; a gene derived from *P. coccineus* (Lamprecht line M0137, now PI 527845), permitting black seed coats and scarlet or vermilion flowers in nature (Bassett 1997b).

v^{lae} (*Cor*) superscript lae, *laelia* (Latin): with *T P* gives *laelia* (pink) flowers and rose stem (Lamprecht 1935e); with *P C J G B* produces mineral brown seed coats with the black corona character; expresses dark corona (purple to black) with numerous other genotypes (Bassett 1995a). The *Cor* locus of Lamprecht (1934a, 1936) is a synonym for *v*^{lae}.

v white flowers, and with *P C J G B*, produces mineral brown seed coat (Lamprecht 1935e).

var	*variegated*: environment-sensitive gene, in combination with *mu* and *us* produces yellow lethal plants in a ratio of 63 normal:1 variegated (Coyne 1966).
vi (*vir*$_f$)	*virescent* foliage mutant (Grafton et al. 1983).
wb	with *T P V*, gives flowers with a *white banner* petal and wings of pale violet; the gene is from the *P. coccineus* PI 273666 (Bassett 1993a).
Wmv	confers resistance to *watermelon mosaic virus* 2 (Kyle and Provvidenti 1987; Provvidenti 1974).
X^{su}	*ex parte* (superscript su) *sursum versus* (Latin): causes the leaves and petals to point downward (Lamprecht 1961b); effect is similar to *Sur*, but pulvinule is rotated only 90E.
y	with *Arg*, produces *yellow* wax pod; with *arg*, the pod is white; *Y* with *Arg* produces green pod; *Y* with *arg* gives a greenish gray (silvery) pod (Currence 1931; Lamprecht 1947b).
Z (*D*) (*ers*)	*zonal* partly colored seed coat patterns are expressed with *t z* (Tschermak 1912, as interpreted by Lamprecht 1934b). With *t*, the *Z* locus interacts with *Bip* to express a wide range of partly colored seed coat patterns (Lamprecht 1934b, 1940b). The *L* of Schreiber (1940) was found to be allelic with *J* (Bassett et al. 2002b); hence, all the partly colored patterns controlled by interactions (with *t*) of *Z* and *L* (Schreiber 1940) are really interactions of *Z* with *J*. Similarly, the *mar* gene of Lamprecht (1933) was found to be allelic with *j* (Bassett 1996b); hence, the interaction of *t* with *j* expresses marginata pattern (Bassett 1994c), which is the equivalent of the *t Z L* of Schreiber (1940) for marginata. Similarly, the new allele l^{ers} (Bassett 1997d) is now recognized to be j^{ers} (Bassett et al. 2002b). The *D* gene for hilum ring color was found to be allelic with *Z* (Bassett et al. 1999b). Thus, hilum ring color is controlled by the interaction of *J* and *Z* (Prakken 1970), where colorless hilum ring is expressed by *z j*. Thus, *Z* and *J* have dual roles, 1) color expression of the hilum ring and 2) major roles in the expression of partly colored seed coats. A review of partly colored seed coat patterns with illustrations and genotypes is available (Bassett and McClean 2000). *Z* is located (McClean et al. 2002) on chromosome 5 (Freyre et al. 1998; Pedrosa et al. 2003).
z^{sel}	superscript sel, *sellatus* (Latin): with *t*, z^{sel}/z^{sel} expresses *sellatus* pattern and z^{sel}/z expresses piebald pattern (Bassett 1997c; Lamprecht 1934b; Tschermak 1912).
z	with *t Bip*, expresses virgarcus pattern; with *t bip* expresses bipunctata pattern (Bassett 1996c). For other interactions see Bassett and McClean (2000).
Znd	gene found in the variety Matterhorm for resistance to soil deficiency of Zn (Singh and Westermann 2002).

APPENDIX
Obsolete symbols removed from list

A	basic color factor, producing yellow-brown (Kooiman 1931; Sirks 1922; Tjebbes and Kooiman 1922b; Tschermak 1912). It is the equivalent of *P*, which has priority.
A	indeterminate versus determinate, *a*, plant habit (Emerson 1916; Norton 1915). Symbol superseded by *Fin* (Lamprecht 1935b).
A, B, C	schematic genes contributing to the length and number of internodes (Emerson 1916). Also used as schematic genes contributing to hybrid vigor (Malinowski 1924).
A, B, C, D	schematic genes each contributing 1 cg to a minimum seed weight (Sirks 1925).
Aeq	*Aequicoloratus* (Latin): with *P T E Uc Unc* and R^{st} or R^{ma} darkens the banner petal (Lamprecht 1935e, 1948a); with *Sal* the effect is similar to *V* (Lamprecht 1948b).
an	appears to have the functions of *P* (Hilpert 1949).
av, sv, iv	confer resistance to bean common mosaic virus (Ali 1950; Petersen 1958).
B	originally a "blackener", producing anthocyanin with the basic color gene *P = A* (Shull 1908; Sirks 1922; Tschermak 1912). According to Feenstra (1960) this gene is the equivalent of the *G* of Shaw and Norton (1918), the *F* of Kooiman (1920), the *Z* of Sirks (1922), and the *V* of Lamprecht (1932a) and Prakken (1934). It is the equivalent of Feenstra's *C* (1960).
B I	hypothetical genes for testa vein color and orientation (Sarafi 1974). Data not sufficient to establish new genes (Bassett, editor).
Br	According to Prakken (1972a), the *Br* of Smith (1947, 1961) is the same as *B*. *Br* with *P Rk* produces brown seed coat (Smith 1947), *br* with *P Rk* green seed coat, *br* with *P rk* pink seed coat (Smith 1947).

C R hypothetical genes for seed coat color where *C* gives cream, *R* gives red, *C R* produces milky phenotypes, and *r c* produces pink (Sarafi 1974). The real genotypes probably involve the *Rk* locus and its modifiers (Bassett, editor).

Ca with color genes, *caruncula* stripe (Lamprecht 1932c). Prakken (1970) believed this gene is a synonym for *G*.

Can According to Prakken (1972a), *D* is the equivalent of *Can* or *Ins* of Lamprecht (1939). *Can* with color genes gives a whitish (Speckweiss) testa (Lamprecht 1939) or blubber white (Lamprecht 1951a), with a yellowish brown hilum ring (Lamprecht 1939).

def *defectus* (Latin): gene *def* is a synonym for *gy* (Bassett, editor). The hypothesis of Prakken (1972b) was that the interaction of *G*/*g* with *def* produced zonal variability of greenish yellow expression on seed coats. whereas the seed coat color expression of *gy* was falsely attributed to *G b v* and *g b v*. The hypothesis of Bassett et al. (2002) is that the interaction of (*C J*) *G* or *g* (*b v*) with *gy* expresses greenish yellow seed coat with variable expressivity. Thus, Prakken (1972b) attributed the instability of *gy* expression to a separate and non-existent gene *def* and attributed the greenish yellow color of *gy* to *C J g b v*, whereas the latter genotype has only shamois expression.

E intensifier with color genes (Tjebbes and Kooiman 1922b).

e *E* required for complete coloring of seed coat (Emerson 1909b); the action of *e* is hypostatic on *t*, producing much reduced partial coloring of seed coat and required for the soldier series of seed coat patterns (Emerson 1909b; Lamprecht 1934b; Leakey 1988; Sax and McPhee 1923; Smith 1939). The only published data (Sax and McPhee 1923) supporting the existence of this gene is too preliminary and inadequate to establish the gene.

Epi Hyp interspecific genes for *epigeal* and *hypogeal* cotyledons in *P. vulgaris* and *P. coccineus*, respectively (Lamprecht 1945, 1957). Lamprecht's model with *Epi* and *Hyp* giving 9 distinct phenotypes for cotyledon attachment position has been superseded by a quantitative model (Wall and York 1957).

ers, *ers-2* *erasure*: genes restricting partly colored seed coat patterns, now known to be synonyms for *z* and j^{ers}, respectively (Bassett 1997d; Bassett and Blom 1991; Bassett et al. 2002b).

Ext Int interspecific genes for *external* and *internal* stigma positions in *P. coccineus* and *P. vulgaris*, respectively (Lamprecht 1945). Lamprecht's Mendelian model with the *Ext* and *Int* loci giving 9 distinct phenotypes for stigma form has been superseded by a quantitative model (Manshardt and Bassett 1984).

F was used as a color gene by Shaw and Norton (1918) with basic genes and their *C* for yellow to produce coffee-brown. It was also used similarly by Kooiman (1931) with *C* for yellow or orange-brown plus *E*, producing coffee brown, to give black (*A B C E F*). The combinations *A B F, A C F*, and *A D F* had pale lilac flowers (Tjebbes and Kooiman 1922b) perhaps the equivalent of v^{lae}. The gene is no longer recognized.

Fcr, *Fcr-2* formerly (Bassett 1993b), complementary genes for *flower color restoration* with *t*; but t^{cf} is now known to express flower color normally (no white flower effect) while expressing (with *Z*, *Bip*, and *J*) partly colored seed coat patterns (Bassett et al. 1999a).

Flav has a light yellow influence (Lamprecht 1951a) on seed coat color; previously considered to be recessive (Lamprecht 1939). Prakken (1970) believed this gene is a synonym for *G*.

H described by Shaw and Norton (1918) as producing light brown or olive. Considered by Feenstra (1960) as the equivalent of the *D* of Shull (1908), the *C* of Tschermak (1912), the *E* of Kooiman (1931), the *L* of Sirks (1922), the *B* of Lamprecht (1939), the *B* of Prakken (1934), the *B* of Feenstra (1960), and the *Bl* of Smith (1939).

ie similar to the action of *ip*; also inhibits the action of *B* and *G* (Nakayama 1959b); considered by Lamprecht (1961c) to be equivalent of *c*.

inh *inhibeo* (Latin): inhibits the action of *V* on seed coat colors (Lamprecht 1940c).

Ins According to Prakken (1972a), *D* is the equivalent of *Can* or *Ins* of Lamprecht (1939). *Ins* with appropriate factors gives light buff (Lamprecht 1939) or raw silk (Lamprecht 1951a) testa; has a hilum ring.

L *Löschungsfaktor* (German): inhibits (or *limits*) the partial coloring of the testa; with *t*, producing an entirely white testa (Schreiber 1934). *L* and *l* combine with *Z* and *z* to produce several color patterns (Schreiber 1940). *L* is a synonym for *J* (Bassett et al. 2002b); Schreiber's (1940) *L* is exactly equivalent to *j*.

lin *lineatus* (Latin): produces red veins in wing petals (Lamprecht 1935e). According to Prakken (1972a), red veins in wing petals are a pleiotropic effect of the testa color gene rk^{d}.

M^{st} causes striping of the seed coat (Smith 1947); redesignated R^{st} (Lamprecht 1947a).

mar	*margo* (Latin): broad colored zone around hilum ring (Lamprecht 1933).
Ms In-ms	*Ms* confers *male sterility* and *In-ms inhibits* action of *Ms*, restoring pollen fertility; *in-ms Ms* is lethal (Mutschler and Bliss 1980). Without translocation heterozygosity to account for the semisterile class, the validity of the model is questionable (Ashraf and Bassett 1986).
Nud	*Nudus* (Latin): with *P,* gives purple, waxy stem and crimson flowers (Lamprecht 1935e). *Nud* is a synonym for [c^{u} Prp^{i}] (Bassett 1994a; Bassett,editor).
Och	with *P C j*, gives *ochre* yellow tints such as ochraceous, Hell Lohfarben, light tawny brown, tawny olive to clay (Lamprecht 1933, 1939); has colored hilum ring (Lamprecht 1939); epistatic to *Vir* (Lamprecht 1939). Prakken (1970) believed this gene is a synonym for *G*.
P	(schematic) increases vigor with *A B C* (Malinowski 1924).
Pur	obsolete symbol for *V* (Lam-Sanchez and Vieira 1964; Okonkwo and Clayberg 1984), originally *Pur Ro* has a deep *purple* pod (Lamprecht 1951b).
R	(schematic) increases vigor with *A B C* (Malinowski 1924).
Ro	*Rosa* (German): the *Ro* of Lamprecht (1951b) and Lam-Sanchez and Vieira (1964) is synonymous with the *Prp* of Bassett (1994a) and Okonkwo and Clayberg (1984). With *Pur* (*V*), gives dark purple pod; with *pur* (*v*), gives *rose* pod color (Lamprecht 1951b). Lam-Sanchez and Vieira (1964) report *Ro V* gives dark purple pod and *Ro v* gives red pod; Okonkwo and Clayberg (1984) report *Ro* as a second locus, along with *Prp*, giving purple pods.
S	(schematic) increases vigor with *A B C* (Malinowski 1924).
Uc Unc (I_1 I_2)	*uni coloris* (Latin): with appropriate genes, darken the banner petal (Lamprecht 1948a); either *Uc-uc* and *Unc-unc* (Lamprecht 1948a) or I_1-i_1 and I_2-i_2 (Nakayama 1958) for the presence or not of anthocyanin in hypocotyl and stem. According to Prakken (1972b), both of these gene pairs are synonyms for genes in the "complex *C* locus", e.g., *Unc* is the equivalent of *Str*.
v^{pal}	with *P,* gives clear light red flowers (Lamprecht 1936); later shown to be a pleiotropic effect of p^{gri} (Bassett 1992b, 1994b).
Vir	with *P Gri C virescens* or greenish shades on the testa (Lamprecht 1933); among these are Russgrun or olive black. Prakken (1970) believed that *Vir* is a synonym for *B*.
Ws	confers resistance to *Whetzelinia* (now *Sclerotinia*) *sclerotiorum*. Gene is no longer in use (Abawi et al. 1978).
X x	early designation for inconstant mottling of the seed coat (Emerson 1909a); now *C c* (Lamprecht 1940a).
Z	constant mottling of the seed coat (Tjebbes and Kooiman 1919a); now C^{ma} or R^{ma}.
Z-1	self-colored seed coat (Tschermak 1912); the equivalent of *T*.
Z-2	pigment extender (Tschermak 1912); the equivalent of *Z*.

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Coordination of Genes and Gene Symbol Nomenclature - BIC Genetics Committee

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

We strongly recommend that any researcher conducting studies of potentially new, qualitatively inherited traits of common bean submit his manuscript to the committee prior to publication (concurrent submission can be made to the genetics committee and the journal). The committee will evaluate the data to determine 1) if sufficient evidence exists to establish the inheritance hypothesis, 2) whether any issue of potential allelism of the trait has been met, and 3) whether the proposed gene symbol has been previously assigned to another gene. The evidence must include 1) data from one generation to formulate an hypothesis and 2) data from subsequent generations to test that hypothesis. The population sizes used must be sufficiently large to distinguish (with statistical significance) among potential segregation hypotheses.

During 1999, for example, several gene symbols (*bip*^{ana}, *Co-1*, *Co-1*^{2}, *Co-1*^{3}, and *Top*) and their supporting data were submitted to the committee for approval, which was granted in all cases.

Questions or comments should be addressed to the chairman of the committee: **Dr. Mark J. Bassett, Horticultural Sciences Department, P.O. Box 110690, University of Florida, Gainesville, FL 32611: ph. (352) 392-1928, ext. 326; fax. (352) 392-5653; and e-mail mjb@gnv.ifas.ufl.edu.**

Phenotypic and Genotypic Selection: Integrating the Use of Molecular Information

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Phenotypic selection is one of the most important tools for the breeder. It is intuitive, often sensory, and may be either subjective or objective. Being as old as human-kind, it is one of the most important factors contributing to crop domestication. The period from the mid-1940's through late-1970's was perhaps the "Golden Age" of biometrical genetic analysis of quantitative expression. The distinctions between qualitative and quantitative genes were being debated, genetic analyses were based almost totally on phenotypic observations and progeny tests, and there were few genetic linkage maps.

Response to phenotypic selection depends on relative magnitudes of components that make up phenotypic variance, which is described by the well-know equation:
P = G + E + (GxE) + Exp. Error. Despite its virtues and almost universal use, selection response and rate of gain may be modest for traits with low heritability, especially under field selection where non-genetic variance often is substantial and difficult to control.

After completing the Ph.D. dissertation in 1965, in which I used phenotypic observations and progeny tests to elucidate the basis of cytoplasmic-genic male sterility in table beets (Bliss and Gabelman, 1965), I became interested in the contribution of individual genes to quantitative expression and how to optimize phenotypic selection for quantitative traits in self-pollinated crops (Bliss and Gates, 1968).

I began to adapt the concept of Wehrhahn and Allard (1965) for breeding purposes and proposed what I have called "The Inbred Backcross Line Method of Breeding" for creating populations of lines containing genes introgressed from promising donors in a background of well-adapted germplasm (Bliss, 1981). This method combines backcrossing followed by inbreeding using single seed descent (SSD), then family and individual plant selection. This method is effective for traits showing any level of heritability, gene number can vary from few to many, and gene expression can show additivity, dominance or epistasis. Working with students at the University of Wisconsin-Madison, this approach has been used for improving root traits and biological nitrogen fixation (BNF) (Bliss, 1993) and enhanced seed protein (e.g., Sullivan and Bliss, 1983) in beans as well as traits in several other crops, e.g., cucumbers. Currently this method is being used in bean breeding for introgression of yield genes from wild *P. vulgaris* into commercial common bean types (e.g., P.A.A. Pereira, EMBRAPA/CNPAF, Brazil, personal communication; J.D. Kelly, Michigan State Univ., personal communication) as well as other researchers in an array of different crops.

Selection for increased BNF in common bean is challenging because it is a complex physiological trait. There is limited knowledge about the genetic parameters contributing to phenotypic expression and heritability is low, with large non-genetic influences. Selection for root traits requires destruction of plants prior to reproductive maturity although the primary objective remains increased seed yield due in part to more BNF and a concomitant decrease in need for fertilizer N. The IBL approach is effective because creation of families allows selection on a family basis where part of a family unit can be uprooted to measure BNF traits and the remainder of the family allowed to mature for measuring seed yield, protein (percentage N) content and other important traits. The challenges were overcome by creating populations of IBL's and practicing phenotypic selection among families(Miranda and Bliss, 1991; St. Clair and Bliss, 1991). New cultivars with increased nitrogen-fixing ability are available to provide an alternative to using N fertilizers (e.g., Henson et al., 1993).

Improvement of seed protein quantity and quality of common bean has been a major objective of studies conducted with several graduate students and post-doctoral scientists. Our goal of increasing quality of bean seed protein was inspired by work of Mertz et al. (1964) studying effects of the opaque-2 mutant in maize on levels of lysine in the grain. We had hoped to increase sulfur-containing amino acids which are usually limiting in grain legumes using a similar approach. That goal has been only partially realized (Gepts and Bliss, 1984), but while searching for genetic variability for seed protein expression, we made several other important discoveries.

Initially, we collaborated with Dr. Tim Hall and his associates at U.W.-Madison to understand the genetic control of expression of the principle seed protein constituent, phaseolin (Hall et al., 1979). Understanding the protein sub-unit structure and underlying multi-gene family led to an improved knowledge of evolution and domestication of common bean along the arc of diversity of wild beans in the Americas. This knowledge and ability to clearly delineate diversity in various gene pools is contributing to enhanced use of *Phaseolus* populations for breeding and selection. The ongoing studies of various groups, especially Paul Gepts' at U.C. Davis and Daniel Debouk and others at CIAT, have made extensive use of these seed protein markers for genotypic selection.

The susceptibility of maize having T-type male-sterile cytoplasm to the Southern corn leaf blight pathogen brought attention in the 1970's, to genetic vulnerability of important crop plants because of a narrow gene base among cultivars. Based initially on pedigree analyses, this was definitively shown to be the case in both snap and dry beans using genetic markers, especially those related to seed proteins of common bean (e.g., Brown et al., 1982).

Seed protein quantity was increased through development of populations of IBL's and effective selection not only for seed protein percentage but also seed size (mass) equal to or greater than that of the commercially-acceptable recurrent parent and/or a comparable standard check cultivar. Usually seed protein percentage is negatively correlated with seed yield and yield components (Kelly and Bliss, 1975). Therefore, selection for increased protein percentage often results in lines with lower yield as a consequence of smaller seed mass, in part because percentage protein is a ratio of the amount of protein to non-protein. An increase in percentage is as likely to result from less non-protein as from more protein, i.e., a smaller seed has higher percentage protein when the mass of protein remains unchanged. To overcome this, we selected for increased percentage protein only in families with seeds as large or larger than a comparable standard to achieve increased percentage protein without a corresponding yield decrease. This was accomplished using phenotypic selection; however, QTL analyses using molecular genetic markers allow genotypic identification and use of marker-assisted selection (MAS) for even greater efficiency.

While looking for mutants that alter phaseolin protein quantity, we found variants for other proteins as well. Changing amounts of lectin protein also gave concomitant opposite effects on phaseolin. Additionally, we found large effects for a unique protein that Jeanne Romero described and named arcelin (Romero-Andreas et al., 1986). The amounts and types of arcelin were related to varying levels of bruchid insect resistance that scientists at CIAT had identified only in wild *P. vulgaris* populations from Mexico; the same populations in which we had found arcelin protein being expressed (Osborn et al., 1988). The genotypes were easily identifiable using gel electrophoresis.

Bruchid resistance can be determined phenotypically by measuring the amount of insect damage on seeds of different lines. Most conclusions are that this is a quantitative trait, because of the continuous distributions of phenotypes in segregating populations. Typically, there are sizeable contributions of diverse genetic factors and non-genetic factors interacting to produce

quantitative expression. However, our studies of seed proteins required developing techniques to measure individual gene expression through application of electrophoretic methods to separate and measure individual proteins. Combining slab gel (PAGE) electrophoresis and rocket immunoelectrophoresis (RIE), we were able to study multi-gene families that controlled expression of arcelin and phaseolin proteins and to minimize effects of extraneous variation.

We identified four allelic forms of arcelin that showed different levels of antibiosis against one or both species of bruchid weevils. Using protein electrophoretic patterns allowed us to select specifically for genotypes (indirect genotypic selection) producing different levels and specificity of bruchid resistance. This precision resulting from genotypic selection provides a means of developing commercial cultivars that are either pure lines with defined levels of resistance or multi-line cultivars with diversity for specificity and level of resistance to provide more durable resistance that will be difficult for the insects to overcome (Harmsen, 1989). It has been nearly two decades since the genetic basis of bruchid resistance was established and now resistant lines are being released in East Africa, Brazil and Central America. The description of arcelin-containing lines was published recently and seed is available for further study and use in breeding (Osborn et al., 2003).

I have attempted to describe not only what I consider some research highlights of my work on common bean but also to show the power of both phenotypic and genotypic selection. We have long known that genotypic selection is more precise, however it is often difficult to practice. With studies at the molecular level advancing at a rapid pace, new opportunities available to breeders are limited only by their creativity and their ability to integrate these powerful methods into an efficient breeding program with clear goals and objectives and adequate support.

Despite emergence of powerful new molecular techniques, we cannot ignore plant phenotype. It is the parameter that gives value and meaning to our products and as such we cannot avoid using phenotypic evaluations and selection. Genotypic selection improves precision and efficiency of selection and is a logical complement. Molecular information is a key element for developing genotypic selection strategies, and genetic linkage and physical maps of common bean are critical resources to be developed.

I have mentioned the contributions of only some of the talented and dedicated students, post-doctoral and visiting scientists, and colleagues with whom I was privileged to collaborate over the years. Omissions result from the necessary brevity of this paper, not because of lesser importance of their ideas, contributions, diligence and insight. It has been my good fortune to be advised, taught and mentored by such outstanding people as C.O. Gardner, D.P. McGill, D.P. Coyne and H.O. Werner (Univ. of Nebraska), W.H. Gabelman, S.J. Peloquin, A.B. Chapman, O.B. Combs and J. Torrie (University of Wisconsin) and C.E. Gates and R.E. Comstock (Univ. of Minnesota). People in the international “Bean Community” are a terrific group to whom I will ever be indebted for your collegiality, good will and good science.

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Phylogeographic Migrations of *Phaseolus* Beans in the New World, and Consequences for Taxonomy, Conservation and Breeding

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Botanically beans belong to the legume tribe Phaseoleae within the Papilionoideae-Leguminosae (Freytag & Debouck 2002). Bean species are all from the Americas, and their presence in other parts of the world is due to historic movements by humans after 1492. Although Amerindians may have used several species in pre-Columbian times, only five species have been fully domesticated from still existing wild forms, giving rise to thousands genotypes all depending on humans for their survival (Debouck & Smartt 1995).

In early years of formal botany, authors did not realize the extent of diversity existing in tropical legumes, and early descriptions were often inaccurate, resulting in over 400 *Phaseolus* species described (Debouck 1999). Agronomists had to wait the 1st Legume Conference of 1978 in Kew (Maréchal et al. 1978) to have a precise definition of the genus *Phaseolus*, now widely accepted (e.g. Lackey 1983, Delgado 1985). While the understanding of the genus improved, scientists (e.g. Piper 1926, Delgado 1985) continued to be aware of the existence of natural groups, on the basis of morphology or ecology and distribution or both. Later on, these natural groups were confirmed on the basis of evidences from interspecific crosses (Debouck 1999), palynology, and molecular marker studies (e.g. Delgado et al. 1999, Gaitán et al. 2000). The later were particularly useful, as they used different kinds of DNA sequences (nuclear ITS, cpDNA) and were free of environmental effects; increasingly some will allow time inferences. Currently, 15 sections, 74 species and 103 taxa are recognized within *Phaseolus sensu stricto* (Freytag & Debouck 2002). These species and infraspecific variants are coming from speciation and radiation, not from convergence of phylogenetically distant legumes (Delgado et al. 1993).

The 15 sections, apart from being clusters of species sharing several morphological characters, also represent phylogeographic lineages, that is, ways and patterns of speciation as the phyla were colonizing new ecological niches through space and time in the Neotropics. Most sections have had their primary diversification in Mexico and Central America, with expansions towards the United States and the Andes, thousands of years before humans arrived into the New World. Many sections have few species (e.g. Bracteati, Brevilegumeni, Rugosi), while a few others (e.g. Minkelersia, Paniculati, Pedicellati) have many. A couple of taxa, namely *P. chiapasanus, P. glabellus* and *P. microcarpus*, currently stand alone, with some discrepancies between the morphological and the molecular evidences (Freytag & Debouck 2002). The numbers of taxa in sections are more than gambling numbers, but perhaps the indication of additional species yet to be discovered, and/ or the result of contrasting pulses of speciation. Ranges of distribution are better known, with endemic (e.g. *amblyosepalus, plagiocylix*) and widely distributed species (e.g. *leptostachyus, microcarpus*), the later often displaying an impressive morphological variation. Apart from recent (mostly negative) impacts by humans, these distributions should not be seen as static but shaped by evolutionary forces.

For instance, the Lima bean *P. lunatus* belongs to the section Paniculati (the largest section in the genus with 16 species, offering ample possibilities for wide crossing) (Freytag & Debouck 2002). It is an older species as compared to common bean, and has its tertiary gene pool in Central America and the SE and E USA (to certain extent, the Coriacei can also be linked to the

Paniculati, extending this 3rd gene pool further; see Freytag & Debouck 2002), while its secondary gene pool is in the Andes, from Ecuador to Argentina. It has two families of wild forms, one being restricted to SW Ecuador and NW Peru, and the other widely distributed in the tropical lowlands, from Sinaloa to Chaco (Debouck 2000). It is tempting to hypothesize the formation of the Paniculati into Mesoamerica (Fofana et al. 1999), while the Lima bean lineage migrated to the Andes for the initiation of another speciation process yet incomplete (the *augusti-pachyrrhizoides* complex: Caicedo et al. 1999).

The common bean *P. vulgaris* belongs to the section Phaseoli together with the year-bean, *P. dumosus* and two other wild species (Freytag & Debouck 2002). These are primarily distributed in the mountain forests of Central America from Jalisco to Chiriquí. Wild common bean is the only species of the section to have crossed at least twice the Isthmus of Panamá to colonize montane forests of the Andean region, and once backwards to Central America (Chacón 2001). The arrival of *P. dumosus* in the Andes seems as feral, historic or late pre-Columbian. In the Andean region, there are two groups of wild common beans, one being distributed on the eastern slope of the Andes from Lara to San Luís, and one being distributed on the western slope of the Andes from Chimborazo to Cajamarca. These groups represent different colonization events in the Andes, and the group in SW Ecuador and NW Peru is possibly an ancient lineage of common bean (Kami et al. 1995, Chacón 2001). Much earlier did separate *P. coccineus* from the remaining bulk of the Phaseoli (Gepts et al. 2000), possibly in what is today Central America.

These phylogeographic migrations, often combined with local extinctions, have had many consequences, namely: the structuring of genetic diversity into genepools, itself being reflected in co-evolving symbionts and pathogens, and the progressive building of reproductive barriers, and from there speciation. Breeders and conservationists enjoy them everyday!

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Molecular Phylogeny of the Genus *Phaseolus* L. (Fabaceae)

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Introduction

This study was designed to determine the closest relatives of *Phaseolus* using nucleotide sequences from the cpDNA *trnK* and nrDNA ITS/5.8S regions, as well as morphological data. Such data were utilized because they have been shown to be phylogenetically informative in legumes (e.g., Delgado-Salinas et al. 1999; Lavin et al. 2003; Riley-Hulting et al. 2004; Thulin et al., in press). The goals of this study thus include a systematic and phylogenetic analysis of the genus *Phaseolus*, which addresses the relationships of the species, the identity of species groups within the genus, as well as the relationships of the genus to other neotropical genera. We also explored the various ways these phylogenies can be used to answer questions about the age of diversification of *Phaseolus* species, and the evolution of their characters. The development of a well-supported and informative phylogeny should also facilitate breeding programs by more accurately determining membership in primary and higher order gene pools.

Results and Discussion

Using DNA sequence data from the chloroplast gene *trnK* (which includes *matk*) and the internal transcribed spacer region (ITS) of the nuclear ribosomal RNA genes, a molecular phylogeny of *Phaseolus* was constructed via parsimony and Bayesian likelihood methods. The combined analysis of two genetic loci and morphological characters corroborated the previously detected monophyly of the genus, but more definitively placed it as sister to the rest of the New World Phaseolinae genera, which includes *Macroptilium*, *Mysanthus*, *Oryxis*, *Oxyrhynchus*, *Ramirezella*, *Strophostyles*, and the neotropical species of *Vigna* excluding the species of subgenus Lasiospron. In essence, *Phaseolus* represents an isolated basally branching lineage within the New World radiation of Phaseolinae.

This study combining two genetic loci and morphological data also more accurately determined the phylogenetic relationships among *Phaseolus* species than did the study of Delgado-Salinas et al. (1999), which relied strictly on ITS sequences and morphological data for phylogenetic inference. Some *Phaseolus* species clades were supported in both analyses and these groups also corresponded to the subgeneric classification of *Phaseolus* proposed by Freytag and Debouck (2002). Clades resolved in the combined phylogeny, such as one comprising *P. microcarpus*, *P. glabellus* and *P. oaxacanus*, were surprising because they seeming conflicted with the ITS tree (Delgado-Salinas et al. 1999). However, this putative conflict was really the result of poor clade support for certain of the ITS clades resolved in Delgado-Salinas (1999). Other instances of conflict between the combined analysis including cpDNA *trnK* and that with just the nrDNA ITS region included the conflicting positions of *P. vulgaris*, *P. coccineus*, and *P. dumosus*, which may explain the hybrid origins of the last, as discussed in Delgado-Salinas et al. (1999, p. 448), where the evolution of *Phaseolus* was hypothesized to be complex and potentially involve some reticulate evolution.

The combined phylogenetic analysis also revealed the taxonomic limitations of certain morphological character states. Many of the morphological differences among taxa have arisen multiple times, and appear to reflect adaptation to particular habitats rather than shared phylogenetic history. For example, the manner of germination, root type, and stigma position have carried much taxonomic weight in the past, but are now realized to be much more prone to convergent evolution that previously thought. The new classifications of the species of *Phaseolus* will have to abandon or down weight these traditionally important characters.

In addition, the age of the diversification of the modern species of *Phaseolus* was estimated at pre-Pleistocene, or 4.5 Ma as estimated with the penalized likelihood method (Sanderson, 2002). This method also revealed a relatively fast rate of evolution for the trnK locus in *Phaseolus*. In particular, the matK coding region has an estimated substitution rate that ranges 4.7-6.9 x 10^{-10} substitution per site per year, which is about the fastest rate estimated for all legume subgroups (Lavin et al., in prep.). This strongly suggests that the lack of molecular divergence among the South American species of *Phaseolus* is a result of these species migrating out of an originally Mesoamerican distribution, where present-day genetic diversity is high, and into their present southern hemisphere geographical range.

Finally, there is disagreement among certain of the phylogenetic relationships resolved with our combined molecular analyses and those depicted in the classification recently proposed by Freytag and Debouck (2002). This is mostly due, however, to Freytag and Debouck's classification that does not adhere to the phylogenetic tenets of classification, such as using the criterion of monophyly to formally recognize taxonomic groups.

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STUDIES IN *PHASEOLUS* GERMPLASM DIVERSITY: A REVIEW OF WORK AT CIAT

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Both wild and cultivated *Phaseolus* germplasm were characterized over several years using RAPD and/or AFLP to determine the genetic structure of the species and the association of genetic structure with phenotypic traits. In most cases, multivariate analysis was carried out using Multiple Correspondence Analysis (MCA), which adjusts the weight of each datum inversely to its frequency in the population. For present purposes this implies that rare DNA polymorphisms that occur together get especially high value in the analysis, a phenomenon that is interpreted evolutionarily as reflecting descent from a common ancestor.

A core collection of wild *P. vulgaris* was created, based on geographic distribution and seed protein classification of phaseolin, lectins and alpha-amylase inhibitors (Tohme et al, 1996). A total of 114 accessions were analyzed by AFLP. Reported gene pools of Andean, Mesoamerican and northern Andean (Ecuador and northern Peru) origins were recognized, and additional diversity was found in Colombia, suggesting a fourth wild bean gene pool. This latter pool has been incorporated into a breeding program together with other wild accessions, and one Colombian accession appears to have contributed yield genes that are expressed in the temperate region of the United States (J. Kelly, pers. comm., 2002). Wilds from Guatemala separated only slightly from Mexican wilds, but relatively discreet groups were observed in the Andean pool. It is suggested that the rugged Andean terrain serves to isolate genetic groups more effectively than the topography of Middle America.

Diversity in cultivated bean as revealed by RAPD analysis mirrored the structure of the wild bean gene pools and was largely consistent with results reported by other authors. Two major gene pools, one Middle American and one Andean, were revealed (Gepts et al, 1986). Within the Middle American gene pool, races Durango, Jalisco and Mesoamerica were distinguishable, and groups formed by DNA analysis were consistent with morphological traits associated with these races (Singh et al, 1991). However, a fourth race was distinguished among climbing beans in the south of Mexico and in Guatemala, which separated from race Jalisco climbers in the MCA (Beebe et al, 2000). This race was designated as race Guatemala and was characterized by having several sources of resistance to angular leaf spot. G2333, a widely studied source of anthracnose resistance, also pertains to this race. The distinction of races Jalisco and Guatemala may reflect the a geographical separation created by the isthmus of Tehuantepec in southern Mexico, with a maximum altitude of about 1000 m above sea level

Furthermore, some internal structure was distinguished within races Mesoamerica and Durango (Beebe et al, 2000). Race Mesoamerica separated into two closely related subgroups, one designated M1 that consisted of type 2 habit genotypes (largely black seeded) and one with wide diversity in seed color and type 3 growth habits. Race Durango presented two subgroups with differences in seed size, color and growth habit. Sub-group D1 represented the more commercial types, while subgroup D2 presented less attractive (black or cream) colors, and more type 4 growth habits.

The Andean pool of cultivated bean displayed a surprisingly narrow genetic base (Beebe et al, 2001). Thus, races in the Andean pool have a different meaning that in the Middle American pool, and reflect relatively few loci that govern physiological adaptation and growth habit, and not broad evolutionary patterns at the genome level. However, about 10% of

accessions from the Andean zone that were assumed to be Andean types based on typical Andean phaseolin, presented significant introgression from Mesoamerican beans. Furthermore, this introgression was associated with detectable changes in phenotype. It is suggested that this phenomenon has implications when screening Andean germplasm for superior traits, for example, for disease resistance genes. One must be careful when assuming that genes are Andean in origin, since elite accessions might in fact contain introgression from Middle American beans.

The northern Andes and especially Colombia, is a region with a very complex genetic structure of common bean. Here one can find land races of both major gene pools that have been cultivated for many generations, introgressed types, local wild populations, and local domesticates. A wild-weedy-crop complex permits gene flow among all these groups (Beebe et al, 1997). Evidence suggests an incipient northern Andean cultivated pool, based on a broad analysis of morphological and biochemical traits (Islam et al, 2001a), and which presents a pattern of reaction to pathogens more typical of Andean beans (Islam et al, 2001b). A few accessions of cultivated bean are to be found with phaseolin types that are typical of Colombian wild beans (CH and L; Beebe et al, 1997).

Physiologically, Phaseolus from lower latitudes including Colombia and the northern Andes would appear to be quite different from accessions from Mexico or Argentina. At higher latitudes photoperiod response governs flowering of wild bean but no such daylength difference exists to stimulate flowering at low latitudes. We do not understand what stimulates flowering in this environment, but locally collected wild beans in Colombia are notoriously difficult to bring to flower in Colombia. Locally collected *P. polyanthus* shows a similar phenomenon with regard to difficulty of flowering and seed production in Colombia, and also separates from Middle American accessions in an analysis of AFLP. These observations highlight a knowledge gap with regard to *Phaseolus* genetic resources from low latitudes.

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Germplasm Enhancement in the United States: the Tropical Connection

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The utilization of tropical germplasm in U.S. bean improvement programs is not well documented. The USDA-ARS project in Mayaguez, Puerto Rico, under the tutelage of Dr. Freytag made many significant contributions of enhanced tropical dry bean germplasm that was utilized as parents to improve architecture and disease resistance in temperate breeding programs. Results of these efforts included the development of the upright small seeded navy bean Mayflower, first upright pinto varieties Sierra and great northern Matterhorn, rust resistant B-190, L-226 and L-227 lines, and improved germplasm, possessing multiple disease resistance released by Miklas in later years. The Puerto Rican location provided an alternative selection site for broadening the adaptation of previously locally adapted Durango race beans.

A historical perspective of the utilization of tropical and exotic germplasm during cultivar development of two traditional U.S. dry bean market classes, small red and great northern, is described in detail (reviewed by Miklas, 2000). Both market classes originate from landraces that were grown by the Indians and early settlers. Plant breeders in the early part of the twentieth century made selections from these landraces, for instance the great northern cultivars UI-1, UI-59, and UI-123 derive from the great northern landrace. Crosses between the great northern landrace selections and the common red landrace gave rise to small red cultivars UI-3 and UI-34 in the 1930's and the great northern cultivars UI-16 and UI-31 in the 1940's (Coyne 1999; Dean 1994). Crosses between UI-34 and UI-31 or UI-59 gave rise to UI-35, UI-36, and UI-37 small reds in the 1960's. Numerous crosses were made between these two market classes because the small red landrace possessed resistance to beet curly top virus (BCTV) but was susceptible to bean common mosaic virus resistance; whereas, the great northern landrace provided BCMV resistance but was susceptible to BCTV (Dean 1994). Both viral diseases were endemic to the Northwest region (ID, OR, WA) where small red and great northern beans were being grown at the time.

'NW-63' and 'Rufus' released in the 1970's (Burke 1982a) represent the first small reds with introgression of exotic germplasm (PI 203958). The landrace PI 203958 from Mexico contributed root rot (*Fusarium solani*) resistance to these cultivars, and subsequently to pink and pinto cultivars as well (Burke 1982b; 1982c). Small reds UI-239 and UI-259 (Myers et al., 2001) released in the 1990's with improved yield potential were derived from crosses conducted primarily among existing small red cultivars.

Recent small red cultivars LeBaron (Hang et al., 2000) and Merlot (Hosfield et al., 2004) developed by the ARS-MSU breeding program in E. Lansing, MI, derive from crosses among northwest small red cultivars, tropical small reds from Central America, and Sierra pinto (Kelly et al., 1990). The small red cultivars and germplasm lines emanating from ARS-MSU possess *Ur-3* gene for resistance to rust (*Uromyces appediculatus*), *I* and *bc-1*^{2} genes for resistance to BCMV, upright architecture, and better seed color. The development of these materials benefited from the shuttle-breeding program between MI and PR described above. Similarly 'AC-Scarlet' benefited from shuttle breeding between CIAT and Alberta, and likewise contains tropical germplasm.

'Emerson', released in 1971, was the first great northern cultivar with introgression of exotic germplasm (PI 165078). The PI 165078 from Turkey contributed bacterial wilt (*Curtobacterium flaccumfaciens*) resistance and improved seed quality. Aurora, a small white of tropical ancestry (Cornell 49-242 / Black Turtle Soup), contributed *Ur-3* and *I* genes to the great northern cultivar Beryl (1980). 'Alpine' (Kelly et al., 1992) with rust resistance and upright architecture from Sierra pinto and 'Starlight' with upright architecture, *I* gene, and rust resistance from Tacaragua, a tropical black bean, represent the next significant introgression events for the great northern class. Alpine and the recent cultivar Matterhorn (1999) were products of the MSU-UPR shuttle-breeding program.

In summary, few introgressions of exotic germplasm were made in the small red or great northern market classes prior to the shuttle-breeding efforts initiated in the early 1990's. Historically, small red and great northern beans have been grown in specific regions, Pacific Northwest for small reds and Idaho and Nebraska for great northern. The cultivars derived from shuttle breeding are more widely adapted and possess better disease resistance and architecture, which essentially enables them to be grown across a wider geographic area. These recent materials contribute genetic diversity, which will facilitate breeding for improved yield potential in the small red and great northern market classes, and may reduce vulnerability of the market classes to emerging diseases. The pioneering effort of George Freytag and Wayne Adams for establishing the shuttle-breeding program, and subsequent efforts by Jim Beaver and Jim Kelly to improve and maintain it, should be recognized.

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Use of Exotic Interracial and Wide Crosses for Common Bean Cultivar Development

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Introduction. There seems to be adequate useful genetic variation among cultivars for such traits as resistance to anthracnose, *Bean common mosaic virus* (BCMV), and rust. However, favorable alleles and quantitative trait loci (QTL) controlling these traits are not uniformly distributed across different market classes, races, and gene pools. Moreover, cultivars have inadequate resistance for angular leaf spot, ascochyta blight, *Bean golden yellow mosaic virus* (BGYMV), bruchids, common bacterial blight, leafhoppers, and white mold to mention a few. Thus, it is essential to identify, introgress, and pyramid favorable alleles and QTL from across different market classes, races, and gene pools within cultivars; from wild populations within the primary gene pool; and from related species in the secondary and tertiary gene pools. Use of exotic germplasm assures continuous availability of useful variation, maximizes selection gains, and helps develop new plant type, maturity group, and market classes. It also helps breed for durable pest resistance, especially when caused by variable pathogens and insect biotypes.

To facilitate introgression and pyramiding of favorable alleles and QTL from distantly related germplasm, it is essential to know the genetic distance of the donor germplasm in relation to the cultivars under improvement. The greater the distance, the more difficult it is to introgress and pyramid favorable alleles and QTL from exotic germplasm. Differences in growth habit, phenology, and seed traits affect the quality and quantity of useful genetic variation within hybrid populations and selection gains. For example, a large proportion of the tropical and sub-tropical germplasm is poorly adapted in the U.S., because of its sensitivity to longer day-lengths, and undesirable climbing growth habit. Consequently, the bi-parental crosses using conventional pedigree, bulk-pedigree, and single-seed-descent selection methods yield little or no progress. Instead, backcrossing or recurrent selection would be required.

A three-stage breeding strategy is often advisable for introgression and pyramiding of favorable alleles and QTL from exotic germplasm (Kelly et al., 1998; Singh, 2001). In the first step, favorable alleles and QTL from specific germplasm are introgressed in the cultivar to be improved. Secondly, favorable alleles and QTL from all other sources are pyramided in a similar adapted background. Finally, the resulting elite germplasm are crossed with other elite breeding lines and cultivars to develop new cultivars. Thus, the genetic variation within a population decreases from step one to step three. However the proportion of useful genetic variation increases accordingly. There are numerous examples of introgression and pyramiding of favorable alleles and QTL from exotic germplasm. Nonetheless, due to space limitations, only a few examples will be given here to highlight the importance of exotic germplasm for cultivar improvement. For further details please refer to Miklas (2000) and Singh (2001).

Introgression of Favorable Alleles and QTL Between Market Classes Within a Race. In-determinate upright growth habit Type II was introgressed from tropical black cultivars in determinate Type I navy beans in Michigan to increase yield and facilitate mechanical harvest. Type II growth habit was also introgressed into Type III Brazilian ‘mulatinho’ and ‘carioca’, and Central American small red cultivars. Resistance to BCMV was introgressed from red Mexican to great northern and pinto beans in the U.S., and from small red mottled (San Cristobal 83) to Central American small red.

Introgression of Favorable Alleles and QTL Between Races Within A Gene Pool. Growth habit Type II and resistance alleles *I* for BCMV, *Ur-3* and *Ur-11* for rust, and/or *Co-4* [2] for anthracnose were introgressed from race Mesoamerica into race Durango great northern, pinto, pink, and/or small red cultivars. Similarly, *bgm-1* allele for resistance to BGMV and BGYMV was introgressed from race Durango to Mesoamerica, BCMV and rust resistance from race Mesoamerica to Jalisco, and biological nitrogen fixation and *Apion godmani* resistance from race Jalisco to Mesoamerica cultivars.

Introgression of Favorable Alleles and QTL Between the Andean and Middle American Gene Pools. Resistance to anthracnose, BCMV, BGMV, BGYMV, and rust was introgressed from the Middle American to Andean germplasm. On the other hand resistance to anthracnose and rust was introgressed from the Andean to Middle American germplasm. Worthy of special mention is the project lead by Dr. J. Rennie Stavely at USDA-ARS in collaboration with the Michigan State University, North Dakota State University, University of Florida, and University of Nebraska. The Andean rust resistance alleles *Ur-4* and *Ur-6* were combined with the Middle American alleles *Ur-3* and *Ur-11* and BCMV and BCMNV resistance alleles *I* and *bc-3* into great northern, pinto, and other market classes (Pastor-Corrales, 2003).

Introgression of Favorable Alleles and QTL From Wild Common Bean. Introgression of high level of resistance to bruchids (*Zabrotes subfasciatus* Boheman) from wild common bean to cultivars is a singular well-documented example. Although over a half-dozen resistance alleles with varying effects exist at the arcelin locus, the multi-lines must be used to combine them in a cultivar. Use of electrophoresis and ELISA techniques have helped breeding for resistance to bruchids.

Introgression of Favorable Alleles and QTL From the Secondary Gene Pool. *Phaseolus* species in the secondary gene pool are known to possess such desirable traits as resistance to anthracnose, ascochyta blight, BGMV, BGYMV, BYMV, common and halo bacterial blights, and white mold. But, only moderate level of resistance to BGMV, BGYMV, common bacterial blight, and white mold have been introgressed into common bean. Instability of the interspecific genotype and fast reversal to common bean phenotype has been the major limitations.

Introgression of Favorable Alleles and QTL From the Tertiary Gene Pool. Some accessions of *P. acutifolius* possess high level of resistance to bruchids [*Acanthoscelides obtectus* (Say)], leafhoppers (*Empoasca kraemeri* Ross & Moore), drought, heat, and common bacterial blight, among other traits. But, only high level of resistance to common bacterial blight has been introgressed and pyramided into common bean.

Future Prospects. A relatively large proportion (>90%) of available variability within *Phaseolus* species still remains to be adequately evaluated and utilized for cultivar development. Despite substantial progress made thus far there are hardly cultivars with resistance to four or more abiotic and biotic stresses. Introgression of favorable alleles and QTL from exotic germplasm therefore must continue. Help from germplasm curators, and long-term federal, state, and private funding are essential for exotic germplasm conversion and enhancement, and cultivar development.

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SCREENING FOR BASAL ROOT SHALLOWNESS IN RESPONSE TO LOW PHOSPHORUS AVAILABILITY

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Abstract. Substantial genetic variation in the root architecture of common bean exists and has been associated with differences in plant performance and adaptation to low phosphorus (Lynch, 1995; Lynch & Beebe, 1995; Lynch & Brown, 2001). Phosphorus efficient bean genotypes have been shown to exhibit more shallow basal root growth angles and greater basal root angle plasticity in response to low phosphorus availability (Bonser et al., 1996; Liao et al., 2001). Using a 10-d, 2-dimensional pouch system, we have screened a recombinant inbred line (RIL) population for basal root shallowness in response to high and low phosphorus availability. This RIL population, developed by Dr. James Kelly at MSU, was derived from a cross between a drought resistant genotype from MSU, and a genotype adapted to low phosphorus from CIAT. Basal root angle, total root length, relative root length in the top 5-cm, and plasticity in response to P availability were determined. All parameters were found to segregate in this population, with values exceeding both of the parent genotypes. Basal root shallowness appears to be related to differences in genotypic tolerance to low phosphorus and inversely related to tolerance to drought, but further work needs to be done to verify these results.. RILs that are highly contrasting for basal root shallowness will be grown in the field in Honduras in a replicated factorial design of irrigated vs. non-irrigated , by P-fertilized vs. non-P fertilized treatments.

Introduction. Root architecture, the spatial configuration of roots in 3-dimensions, is an important factor determining belowground resource acquisition (Lynch, 1995). Plants typically grow in environments that have multiple constraints and must therefore co-optimize their carbon allocation for acquisition of several limiting resources. Spatial deployment of the root system determines the ability of a plant to exploit heterogeneous soil resources. For example, greater nutrient acquisition has been associated with increased soil exploration by roots in surface layers (Lynch & Brown, 2001), especially in the case of immobile nutrients such as phosphorus, while drought tolerance in common bean has been associated with depth of rooting (Sponchiado et al., 1989; White and Castillo, 1989;1992). In addition, root architectural plasticity, the ability to exhibit morphological and physiological responses to a changing environment, may play an important role in plant adaptation to heterogeneous environments. While root architectures that exploit topsoil resources efficiently may be advantageous in low-P soils, this may inadvertently result in reduced water acquisition, since water availability often increases with soil depth.. Thus, the primary objective of this work was to screen a RIL population that is segregating for tolerance to drought and low phosphorus conditions for root architecture traits. We hope to eventually correlate root architecture traits with plant performance in the field under different water and phosphorus stress conditions.

Materials and Methods. A 2-dimensional plastic pouch system was used to screen the L88 recombinant inbred population conditions for root shallowness under high and low phosphorus conditions. Five replicates of each genotype were grown for 10 days in the greenhouse. Intact root systems were then placed directly onto a scanner and a .TIF image was obtained at 400dpi. Basal root angle analyses was done using Image J version 1.31 (freeware from the National Institutes of Health, http://rsb.info.nih.gov/ij/) and root length and root area analyses was done using WinRhizo version 2002c.

Results and Discussion. We have demonstrated P availability regulates the orientation of basal roots with respect to gravity, and that genetic variation exists in the L88 population (Fig. 1). The P-efficient genotype has more shallow basal roots (smaller basal root angle) under low-P compared to the drought tolerant genotype (Fig. 1).

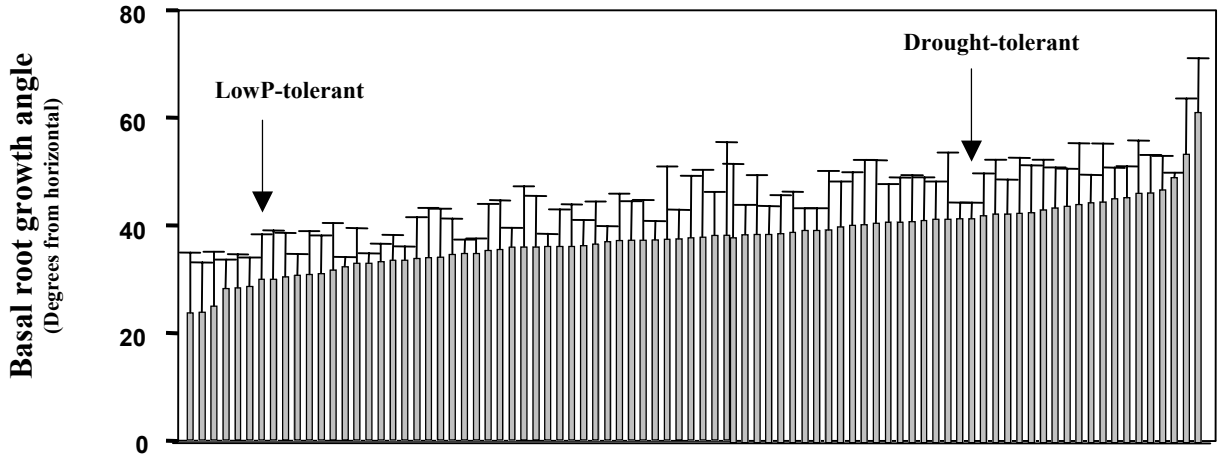
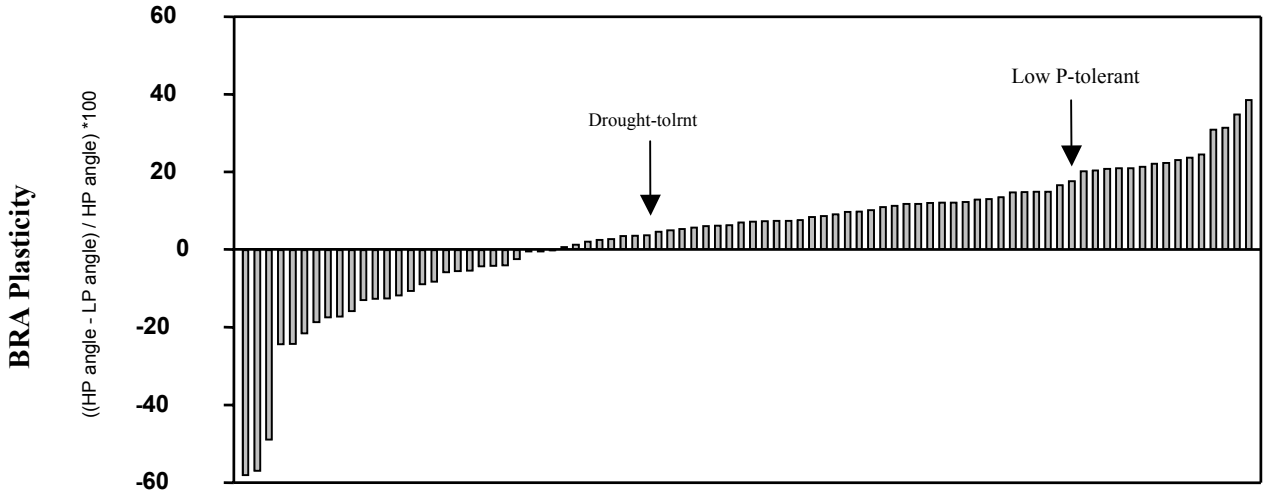


Fig. 1. Basal root angle variation for L88 RI population of common bean in 2-D pouch system grown under high phosphorus conditions. Basal root growth angle values for the two parents is shown. Transgressive segregation exists in this population.

We have also demonstrated that genetic variation for basal root angle plasticity exists in response to P availability for the L88 population (Fig. 2). The P-efficient parent genotype exhibits more basal root angle plasticity and becomes more shallow in response to low P availability, relative to the drought-tolerant parent. Transgressive segregation for this trait also exists for this population.



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Differential Responses of Common Bean Genotypes to High and Low Temperatures.

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Introduction

High temperatures (>30°C day and/or >20°C night) in tropical lowlands and production areas in temperate zones are a major limiting factor in the production of common bean (Singh, 2001). Temperate production areas experience brief and problematic seasonal heat waves during flowering resulting in blossom drop, and in the case of snap bean, a split set.

In order to identify the most heat tolerant common beans and those with differential reactions to high temperatures, yield components (YC) of 24 common beans were evaluated following exposure during reproductive development to 4 greenhouse (GH) day/night temperature treatments (TT) (24°C/21°C, 27°C/24°C, 30°C/27°C and 33°C/30°C) (Rainey and Griffiths, 2004). In a separate study, combining ability of YC for 10 snap bean parents (7 heat-tolerant) was calculated using a complete diallel mating design. The 90 F_1 progeny of these parents were screened at (32°C/28°C) and were also cold-tested (16°C/ 10°C) to investigate potential association between high and low temperature stress tolerances.

Materials and Methods

Both studies were conducted with replication in 90 m^2 GHs of the Dept. Horticultural Sciences, NYSAES, Geneva, NY in 2002 and 2003. All TT were imposed 2 weeks after seedling emergence, corresponding to microsporgenesis. All pods were harvested after complete plant senescence. For the diallel analysis 100 progeny were confirmed as crosses with RAPD markers unique to the parents.

Results

Of the 24 common beans tested under four TT, ‘Carson’, ‘CELRK’, ‘Cornell 503’, ‘G122’, ‘HB 1880’ and ‘Venture’ were found to be heat-tolerant through the 33°C/30°C treatment; ‘Barrier’, ‘Brio’, ‘Cornell 502’, ‘Opus’ and ‘PI 271998’ maintained yield through the less stressful 30°C/27°C treatment. Some lines, including ‘Hystyle’, were notable for their yield stability. Some lines such as ‘CELRK’ possessed heat-tolerant reproductive development as indicated by relatively high pod and seed set under heat stress, but were unable to maintain mean seed weight, perhaps due to a lack of somatic heat tolerance caused by such problems as photoinhibition or elevated respiration. In contrast, ‘Labrador’ lacked reproductive heat tolerance with decreased pod and seed set at high temperatures, but maintained mean seed weight (beans with this reaction are candidates for improvement of yield through crossing to lines possessing reproductive heat tolerance). 'Haibushi', 'IJR', and 'Tío Canela-75' were previously reported to be heat-tolerant but showed susceptible reactions in this study, perhaps emphasizing disparities between GH and field conditions. This study also determined optimal TT for assessing relative heat tolerance or susceptibility of common beans in a controlled environment (approximately 27°C night is crucial for discerning heat-tolerant material).

Results of the diallel analysis indicated yield improvements under high and low temperature stress are possible and progeny should be more heat-tolerant than parents in subsequent generations, as significant additive gene action ($P \leq 0.0001$) was found for pod number, seed number and seeds per pod under high and low TT. ‘Cornell 502’ was the only genotype with significant positive general combing ability (GCA) for all YC under high temperatures. Specific combining ability (SCA) was also significant for the three YC under both

TT ($P \leq 0.05$), although heterosis was not significant. Differential reactions to temperature were observed among the parents (**Figure 1**), which may indicate separate genes or alleles are controlling YC under temperature stress. For example, 'Brio' and 'Opus' were tolerant of heat and cold, while 'Barrier' was cold-tolerant only and 'Cornell 502' was heat-tolerant only. Lack of within-trait correlation between GCA and parental YC means, and incidence of significant negative GCA among parents with high yield potential under temperature stress, indicated GCA for temperature stress tolerance cannot be predicted from parental performance. Coincidence of cold and heat tolerance among parents was not seen, and significant GCA and SCA interaction with TT provides further evidence that temperature stress tolerances generally were not correlated. Diallel analysis also indicated reciprocal effects were not significant for any YC under temperature stress. Heat-tolerant inbred lines are being developed from select crosses.

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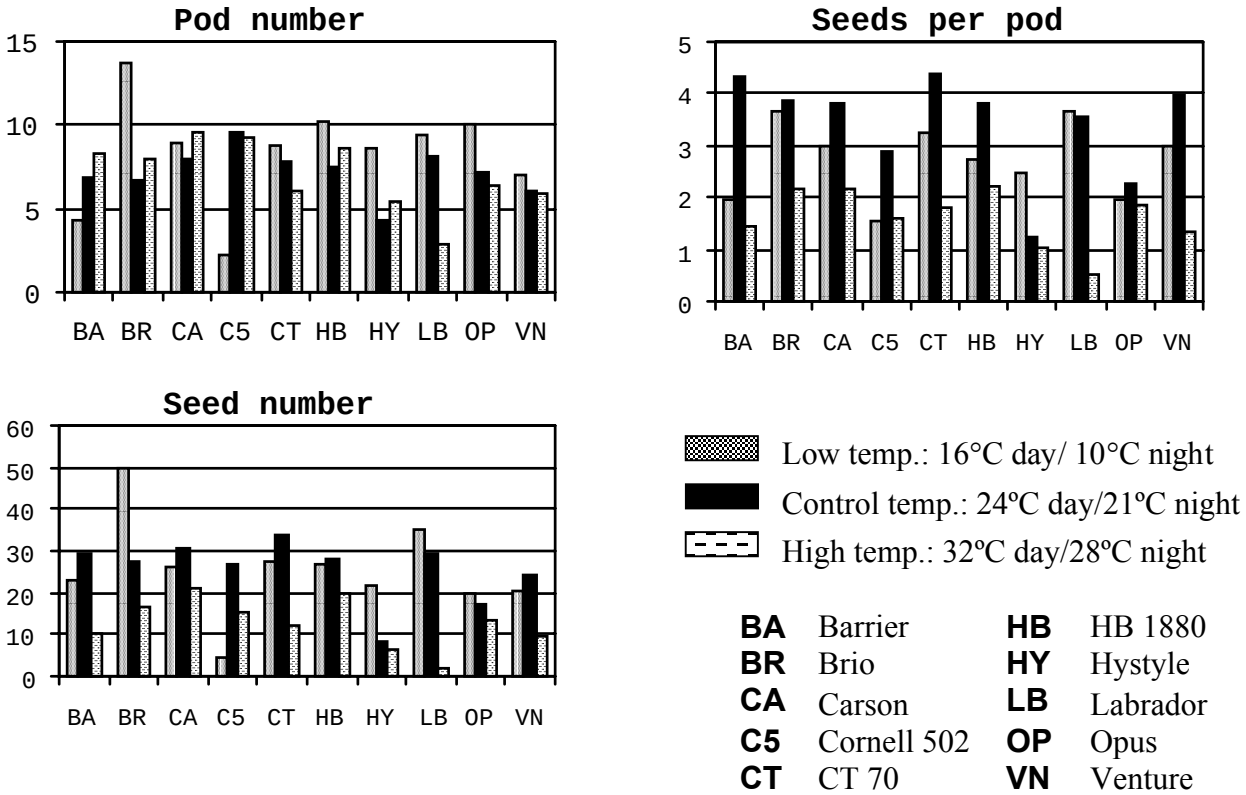
Figure 1. Yield component temperature reactions for the diallel snap bean parents. Pod number, seed number and seeds per pod were the only YC with significant GCA ($P \leq 0.0001$) under either high or low temperature stress in the diallel analysis. Included in the graphs as an indication of the yield potential of the parents are YC values from the control temperature treatment of the screen of 24 common beans conducted under similar conditions.



A New Gene (*Prp*^{i}-2) for Intensified Anthocyanin Expression Syndrome and Its Novel Expression in Flowers and Seeds

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The inheritance of intense anthocyanin expression syndrome has been previously reported (Bassett, 1994). The most obvious organ affected is the pod, expressing *purple* (*Prp*) pod, but purple flower buds, intense purple flowers, purple petioles and leaf lamina, and purple stems are also expressed. The gene [c^{u} *Prp*^{i}] controls the *intense* (superscript i) anthocyanin expression syndrome at the 'complex *C* locus', where *Prp*^{i} is very tightly linked (indicated by the brackets) to the gene for cartridge buff seed coat color, c^{u}. Other alleles at the *Prp* locus (all tightly linked to the dominant gene *C*) for various shades and patterns of purple pod have been reported (Bassett, 1994; Okonkwo and Clayberg, 1984). This paper proposes a second gene (*Prp*^{i}*-2*) for intensified anthocynin expression syndrome that is not linked to *C*.

CIAT common bean accession G07262 is heterogeneous, and one of its genotypes expresses dark purple seeds with a long white micropyle stripe and flowers with "blue" (methyl violet) veins on white wings (Bassett, 1998). The genotype for the long white micropyle stripe is *t p*^{mic} (Bassett, 1998, 2003). The cross G07262 x *v* BC_3 5-593 was made to derive an F_4 line with mineral brown seeds and flowers with bright red banner backs and white wings, with genotype hypothesis *T P v Prp*^{i}*-2*. The cross 'F_4 red banner back' x 5-593 (bishops violet flower) was made, and the F_2 segregation is presented in Table 1. Clearly, the color expression of *Prp*^{i}*-2* in flowers is a function of the genotype at *V*. The cross 'F_4 red banner back flower' (*Prp*^{i}*-2 v*) x *v* (*prp*^{i}*-2*) BC_3 5-593 was made, and the F_2 segregated for three classes: 24 with dark red banner back (*Prp*^{i}*-2*/*Prp*^{i}*-2*), 52 with pale red banner back (*Prp*^{i}*-2*/*prp*^{i}*-2*), and 22 with white flowers (*prp*^{i}*-2*/*prp*^{i}*-2*) (for the data 24, 52, and 22, the χ^2 (1:2:1) = 0.449, *P* = 0.80.). Those data support the hypothesis that the intensity of the red color of the banner back is controlled by the gene dosage at *Prp*^{i}*-2*.

Table 1. Segregation in the F_2 from 'F_4 red banner back flower' (*Prp*^{i}*-2 v*) x 5-593 (*prp*^{i}*-2 V*).

Prp^{i}*-2*/- *V*/-	*Prp*^{i}*-2*/- *v*/*v*	*prp*^{i}*-2*/*prp*^{i}*-2 V*/-	*prp*^{i}*-2*/*prp*^{i}*-2 v*/*v*-
Intense purple flower, black seeds	Red banner back and white wings, mineral brown seeds	Bishops (or cobalt) violet flowers, black seeds	White flowers, mineral brown seeds
196	80	65	21

For the data 196, 80, 65, and 21, the χ^2 (9:3:3:1) = 2.690, *P* = 0.44.

The cross 5-593 x G07262 was made, and selection was made in F_3 for plants with white flowers with blue veins on the wing petals, with genotype hypothesis *t V Prp*^{i}*-2 fib*. This F_3 stock was crossed with *t z* (*fib*) virgarcus BC_3 5-593 to develop a stock with the same flower phenotype as the female parent, but in BC_1 to 5-593. The cross F_1 [*t p*^{mic} *V Prp*^{i} *-2 fib* x 'F_3 red banner back' (*Prp*^{i} *-2 v*)] x c^{u} *b v rk*^{d} BC_1 5-593 was made to develop a stock with seeds having a black ventral side and a dark red kidney (DRK) dorsal side. The genotype hypothesis for the black/DRK seed is c^{u} *V rk*^{d} *Prp*^{i}*-2*. The cross 'Black/DRK' BC_1 5-593 x 5-593 was made, and the F_2 segregation is presented in Table 2. Clearly, the *Prp*^{i}*-2* gene segregates without any

indication of linkage with the *C* locus, and thus, the gene for anthocyanin expression intensification in G07262 is not a new allele at *Prp*^{i}*-1*, but an independent locus. Also, *Prp*^{i}-2 has the capacity to overcome the *unchangeability* of the c^{u} gene and express 1) purple color in the margo region of seed coats with genotype *Prp*^{i}*-2*/- c^{u}/c^{u} *Rk*/- and 2) black color in the ventral region and DRK color in the dorsal region of the seed coat with genotype *Prp*^{i}*-2*/- c^{u} rk^{d} (Table 2)

Table 2. Segregation in the F_2 from 'Black/DRK' BC_1 5-593 (c^{u} *V* rk^{d} *Prp*^{i}*-2*) x 5-593 (*C V Rk prp*^{i}*-2*)

Prp^{i}*-2*/- *C*/- -/-	*Prp*^{i}*-2*/- c^{u}/c^{u} *Rk*/-	*Prp*^{i}*-2*/- c^{u}/c^{u} rk^{d}/rk^{d}	*prp*^{i}*-2*/*prp*^{i}*-2* *C*/- *Rk*/-	*prp*^{i}*-2*/*prp*^{i}*-2* c^{u}/c^{u} rk^{d}/rk^{d}	*prp*^{i}*-2*/*prp*^{i}*-2* c^{u}/c^{u} rk^{d}/rk^{d}
Black seed	Purple margo/c^{u} seed	Black/DRK seed	Black seed	c^{u} seed (cartridge buff)	DRK seed
77	13	8	17	6	2

For the data 77, 13, 8, 17, 6, 2, the χ^2 (36:9:3:12:3:1) = 4.422, $P = 0.49$.

The breeding line (derived from G07262) with genotype *t* p^{mic} *V Prp*^{i}*-2 fib* BC_1 5-593 has white flowers with blue veins in the wing petals. When this line was crossed with *t fib* (*P V prp*^{i}*-2*) BC_2 5-593, the F_1 and F_2 progeny all had flowers with white banners. When the same female parent was crossed with *t Fib/fib* (p^{mic} *V prp*^{i}*-2*) BC_3 5-593, the F_1 progeny segregated for flowers with either 1) intensified anthocyanin expression, viz., medium blue banner petals and pale blue wings with dark blue veins or 2) white banner. The expression of anthocyanin in the banner petal is attributed to the *Fib* gene interacting with *Prp*^{i}*-2*. The F_2 from progeny of parents with medium blue banner segregated for dark blue banner (expressed by *Prp*^{i}*-2*/*Prp*^{i}*-2*) and medium blue banner (*Prp*^{i}*-2*/*prp*^{i}*-2*), a gene dosage effect.

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The Effects of Time, Temperature and Moisture on Nuña Popping

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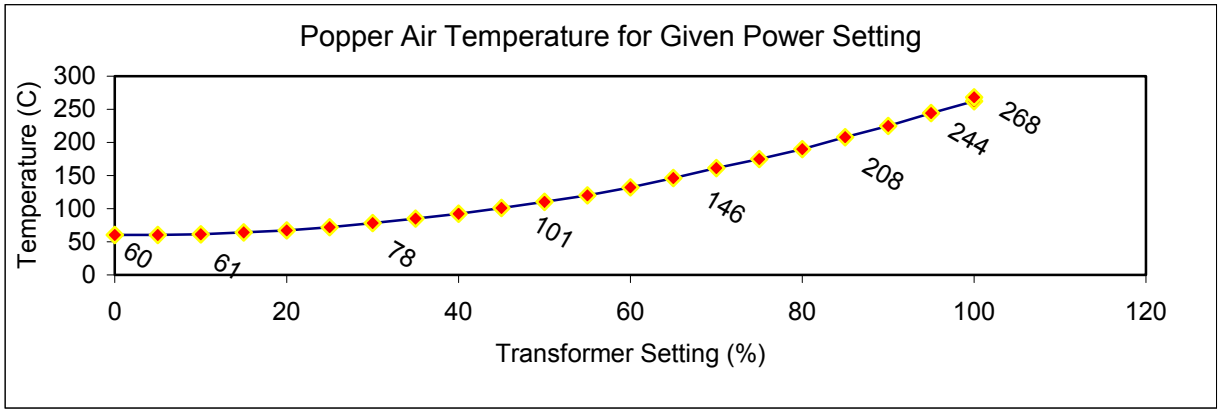
Nuña beans are Andean common beans with the unique property of popping in hot air or hot oil. In popcorn the endosperm liquefies, whereas in nuña beans steam rapidly expands in the cotyledons. To better understand the popping of nuña beans and to further the commercialization of nuñas, uniform popping procedures were examined. In terms of popping nuña beans, there are four parameters to consider including: variety, seed moisture, popping time, and popping temperature. In this study we explored the aspects of seed moisture, popping time and popping temperature.

Materials and Methods

The bean used in the popping trials was PB24. PB24 is a nuña bean with a day neutral adaptation developed by crossing Stockbridge Indian Bean x Ayacucho backcrossed to Ayacucho (Kmiecik and Nienhuis, 1997). Popping the beans at different temperatures required the construction of a variable temperature popper. To construct this popper a consumer model hot air popper was modified putting a variable transformer inline of the circuit of main heating element. The air temperature for the popper was measured at intervals of five-percent power through the transformer circuit and through the transformer bypass circuit. Five power setting were chosen from this procedure 45%, 65%, 85%, 95% and full power corresponding to the temperatures of 101 C, 146 C,
208 C, 244 C and 268 C, respectively (Fig. 1).

Figure 1. Graph of power setting and the air temperature produced.



Seed of PB24 was dried to approximately four-percent moisture in a forced air drier at 50 C. Moisture chambers were used to produce a range of seed moistures. The moisture chambers were constructed from one gallon glass Quantpro jars. Sample supports were made from an inverted 3 1/2 inch pot with the bottom removed and a piece of heavy plastic mesh (Bell and Labuza, 2000). Seven saturated salt solutions as well as a control (no salt) were used to produce the range of moisture levels (Table 1).

Table 1. Seed moisture and relative humidity with in moisture chambers.

Saturated Salt Solution	Relative Humidity*	% Seed Moisture
CONTROL	NA**	2.57
LiCl	11.30	3.08
$MgCl_2$	32.78	5.71
K_2CO_3	43.20	6.67
$Mg(NO_3)_2$	52.89	7.69
NaCl	75.29	12.36
KCl	84.34	15.13
K_2SO_4	97.30	19.77

***Reference values (National Institute of Standards and Technology, 2000)**
****No measured value**

Results and Discussion

The factors of time, temperature, moisture and the interactions between them had a significant effect on the popping of nuña beans (Table 2). Seed moisture had the largest effect on the mean square of popping. Moisture levels less than 6% had the largest percentage of full pops. Moistures above 6% yielded little to no popped product. There were no popped nuña beans at the temperature of 100 C and 146 C. The highest levels of popping were at temperatures above 244 C. Times of 1.5 and 2 minutes also yielded the highest popping percentages.

Table 2. Interactions between time, temperature and moisture.

Source	Degrees of Freedom	Mean Square	Significance
temperature	3	1490	**
time	2	118	**
moisture	6	2303	**
temp*time	6	59	**
temp*moisture	18	330	**
time*moisture	12	32	*

*Significance between .0001 and .0005
**Significance less than or equal to .0001

Acknowledgements

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ANTIOXIDANT ACTIVITY OF DRY AND COOKED BEANS

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Introduction. Dry beans play an important role in the traditional diet of Mexican people (13-15 kg per capita). Human diet includes a great variety of food components, that independently of their caloric and protein content, some may also have potential health benefits. A group of these compounds, the flavonoids, represent of great significance for consumers since these have antioxidant activity. This antioxidant capacity helps on reducing the risk of establishment of chronic-degenerative diseases (cancer, diabetes, osteoporosis, etc.). Consumption of natural antioxidants such as vitamin E and C, carotenes and flavonoids contribute to improve human defenses (Velioglu et. al., 1998; Jun Peng et al., 2000). Flavonoids have the capacity to function as antioxidants that neutralize free radicals (Aherne, 2000). Common bean grain has been studied to a great extent for its nutritional value. However, there are just a number of studies that have examined the content of substances with antioxidant capacity. There has been found a strong antioxidant activity either from crude extracts (Onyeneho and Hettiarachchy, 1991) or isolated pigments from colored and/or white beans (Tsuda, 1994). Much of this information has been obtained from fresh beans, but what occurs with cooked beans needs to be studied. The objective of this study was to assess the capacity of cooked bean extracts to inhibit free radicals.

Materials and methods. The antioxidant activity of bean seeds from 'Pinto Villa' cultivar was assessed using raw and cooked bean grain. Two different assays were used, the *in vitro* system of autoxidation of linoleic acid and the neutralization of the free radical DPPH (2,2-diphenyl-1-picrylhydrazyl). This report will focus on the scavenger capacity of cooked beans over the free radical DPPH. Seeds were boiled for 90 min until cooked, dried and grinded (Cyclotec, Tecator) to obtain a fine particle powder (0.5mm). Three 1.0 g powder samples were used for each of the bean extracts. The bean extracts were prepared with three different treatment solutions; pure water, absolute ethanol, and acidic ethanol comprised of trifluoride acetic acid 0.5%, 80% ethanol (v/v). Extracts were filtrated and vacuum dried at 40^{o}C. Bean samples were dissolved in 5ml methanol, then six (6) bean concentrations were prepared: 50, 100, 250, 1000, 1500, and 2000 µg, later adjusted to a final 100 µl volume required for the assay. To assess the antioxidant capacity, neutralization of the free radical DPPH was determined. DPPH has a maximum absorbance at 517 nm (violet color) in a methanol solution. The assay was carried out using 100 µl from each of the dried and re-suspended extracts. These samples were mixed with 3 ml from a 100 µmol DPPH absolute methanol solution (300 nmol per assay). Antioxidant activity was estimated by a color change and the absorbance drop at 517 nm (Cotelle, 1996).

Results and discussion. Table 1 indicates that bean extracts showed different capacity to inactivate the free radical DPPH, depending on concentration of cooked bean powder and the solution used for the bean extract (water, ethanol and acidic ethanol)

Table 1. Antioxidant capacity of cooked bean extracts measured as neutralized DPPH (μmol).

Bean conc. (μg)	Bean extracts Water	Ethanol	Acidic ethanol
50	3.0 *	5.0	17.0
100	5.0	9.5	33.0
250	8.0	17.5	48.5
500	4.0	29.6	57.2
1000	14.0	46.8	80.9
1500	42.0	53.0	119.7
2000	42.5	71.9	162.5

*** Average of two reps per sample.**

Antioxidant capacity exhibited a linear relationship between powder concentration and solution used for bean extract, water (r =0.948**), ethanol (r = 0.983**) and acidic ethanol (r=0.983**). Cooked beans contained chemical compounds with the capacity to neutralize free radicals. Evidently, these chemical compounds were not inactivated by heat during cooking. The fact that higher free radical neutralization was attained with ethanol and acidic ethanol bean extracts, these results might suggests that such bean compounds could correspond to a certain type of isoflavones as found in soybeans. Preliminary high-resolution liquid chromatography studies (data not shown) showed as principal component, a compound with time retention similar to a standard from the isoflavone genistein. More research needs to be undertaken not only for 'Pinto Villa' grain components but other commercial bean cultivars as well.

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Efficacy of New Vitaflo 280 (Carbathiin and Thiram) to Control Soil- and Seed Borne Diseases of Common Bean (*Phaseolus vulgaris* L.), Soybean (*Glycine max* (L.) Merrill), Pea (*Pisum sativum* L.), and Lentil (*Lens culinaris* Medicus) and Compatibility with Rhizobium Inoculants.

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Efficacy of Vitaflo 280 to Control Seed Rot and Seedling Blight of Pulse Crops.

The new Vitaflo 280 [Carbathiin (systemic) and Thiram (contact)] fungicide has a broad spectrum disease control and is registered on wheat, barley, oat, rye, triticale, dry common beans, snap common beans, soybean, corn, lentil, flax including edible oil flax and pea. This product is a water based flowable, low dusting and odor, soft settle and easy to reconstitute, easier to clean up due to change to a pigment vs a dye for the colorant, and safer on Rhizobium. Results of trials conducted in western Canada indicates that Vitaflo 280 at a rate of 260 mL 100 kg^{-1} of seed significantly increased percent emergence and grain yield of dry bean seed infected with *Anthracnose* when compared with the untreated control (UC). Vitaflo 280 significantly decreased dry bean *Anthracnose* leaf infection when compared with the UC and was similar to DCT (commercial control). On an average of six station years Vitaflo 280 significantly increased percent emergence of dry bean planted is soils infected with *Rhizoctonia solani* when compared with the UC.

On an average of four station years Vitaflo 280 at 260 mL 100 kg^{-1} significantly increased percent emergence of soybean seed infected with *Phomopsis spp*. Vitaflo 280 also significantly increased percent emergence of soybean planted in soils infected with *R. solani* and *Fusarium spp* on an average of six and ten station years, respectively.

Vitaflo 280 at a rate of 330 mL 100 kg^{-1} of seed significantly increased percent emergence (ten station years) and grain yield (five station years) of pea seed infected with *Mycosphaerella pinodes* (Ascochyta blight) when compared with the UC. Vitaflo 280 at a rate of 260 mL 100 kg^{-1} significantly increased percent emergence of pea planted in soils infected with *Rhizoctonia solani* (eight station years) and *Fusarium spp.* (12 station years). On an average of 16 station years, Vitaflo 280, significantly increased grain yield of pea planted in soils infected with *R. solani* when compared with the UC.

Vitaflo 280 at a rate of 330 mL 100 kg^{-1} of seed significantly increased percent emergence (12 station years) and grain yield (four station years) of lentil seed infected with *Botrytis cinerea* when compared with the UC. Vitaflo 280 significantly increased percent emergence (12 station years) and grain yield (four station years) of lentils planted in soils infected with *R. solani* when compared with the UC. In addition, Vitaflo 280 significantly increased percent emergence (nine station years) of lentils planted in soils infected with *Fusarium spp* when compared with the UC.

Vitaflo 280 Compatibility with Rhizobium Inoculants

Rhizobium inoculants can be applied as a tank-mix when the inoculant is mixed with the fungicide and then the mixture applied to the seed. Wet sequential if the inoculant is applied to the seed when the fungicide is still wet on the seed surface. Dry sequential if the inoculant is applied to the seed when the fungicide has dried on the seed surface. The maximum time between application of HiStick L soybean inoculant (Becker Underwood) or Cell-Tech soybean inoculant (Nitragin) and Vitaflo 280 on seed and planting is one day (24 hours) for tank-mix, wet-sequential, and dry-sequential. The maximum time between application of HiStick+ soybean inoculant (Becker Underwood) and Vitaflo 280 on seed and planting is one day (24 hours) for wet-sequential and two days (48 hours) for dry-sequential.

Fungicidal seed treatments had no effect on visual nodulation and percent Nitrogen derived from the atmosphere (Ndfa) of chickpea, dry bean, lentil, and pea. Visual nodulation and percent Ndfa of chickpea, dry bean, lentil, and pea treated with Vitaflo 280 and inoculated with Rhizobium was similar to that observed in these crops inoculated with Rhizobium and non-treated with fungicide. Rhizobium inoculation of chickpea, dry bean, lentil, and pea significantly increased visual nodulation and percent Ndfa when compared with the non inoculated control, suggesting that these soils may not have a native Rhizobium strain capable of efficiently nodulate these crops. Therefore, the new Vitaflo 280 at the recommended rates has no effect on visual nodulation or the ability of the Rhizobium to fix N_2 from the atmosphere, regardless of the inoculation time.

RECENT ADVANCES IN THE DEVELOPMENT OF BEAN GOLDEN YELLOW MOSAIC RESISTANT BEAN LINES

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Bean Golden Yellow Mosaic (BGYM), caused by a whitefly-transmitted geminivirus, can produce a range of symptoms in common bean (*Phaseolus vulgaris* L.) including intense foliar yellowing (chlorosis), pod deformation and severe plant stunting. Plant breeders have identified several sources of resistance to these symptoms (Beebe, 1994; Singh, 2000). The small red bean breeding line DOR 364, released in Central America as 'Dorado', has moderate levels of resistance to BGYM. When inoculated in the greenhouse with viruliferous whiteflies, Adames *et al.* (1996) found DOR 364 to have delayed symptom expression. Miklas *et al.* (2000) identified a major QTL and a RAPD for BGYM resistance in DOR 364. This marker has been converted by scientists at CIAT to the SCAR marker $SW12_{700}$. The most widely deployed resistance to BGYM is recessive gene *bgm-1* (Velez *et al.*, 1998) which confers resistance to leaf chlorosis. Urrea *et al.* (1996) identified a codominant RAPD marker linked to *bgm-1* that was converted by CIAT researchers to the SCAR marker SR-2. Molina Castañeda and Beaver (1998) and Acevedo Román (2003) identified the dominant gene *Bgp-1* that permits normal pod development in the presence of severe disease pressure. The expression of *Bgp-1*, however, requires the presence of the the recessive gene *bgm-1.* The small red CIAT bean breeding line DOR 482, released in Central America as 'Don Silvio', has the QTL linked to $SW12_{700}$, *bgm-1* and *Bgp-1*. The striped, light red kidney bean breeding line DOR 303 has the QTL linked to $SW12_{700}$ and a different recessive gene, *bgm-2*, that confers resistance to leaf chlorosis. Velez *et al.* (1998) reported that DOR 303 also has the dominant gene, *Dwf*, than can cause dwarfing when seedlings are infected with BGYMV. However, light red kidney lines such as PR9443-4, PR0003-384 and PR0003-390 have been developed in Puerto Rico that have the *bgm-2* resistance gene but no dwarfing reaction (Beaver *et al.*, 1999). The reaction of bean lines to BGYM that pyramid the recessive resistance genes *bgm-1* and *bgm-2* has not been documented. The *P. coccineus* accession G 35172 is resistant to both BGYM in Central America and the Caribbean and Bean Golden Mosaic (BGM) in Brazil (Singh *et al.*, 2000). Results from field trials conducted in Puerto Rico suggested that the BGYM resistance of G 35172 is controlled by two genes (Muñoz, 2002). Results from an allelism test suggested that the BGYM resistance genes in G 35172 were not *bgm-1* or *Bgp-1.* However, G 35172 does have the $SW12_{700}$ marker linked to the QTL for BGYM resistance. Osorno *et al.* (2003), studied an interspecific population with BGYM resistance derived from G35172 and found a recessive gene to confer resistance to leaf chlorosis and a dominant gene to confer resistance to pod deformation. Ferwerda, (2000), evaluating BGYM resistance in the interspecific cross 'G35172 x ICA Pijao', reported a recessive gene that conferred resistance to chlorosis and evidence of a second gene involved in the expression of resistance. Bianchini *et al.* (1994), studied the inheritance of resistance to BGM in an interspecific cross between *P. vulgaris* and *P. coccineus* reported a tendency for dominance for resistance to pod deformation and resistance to chlorosis appeared to be recessive. In Puerto Rico, three bean lines with BGYM resistance derived from *P. coccineus* are being considered for release as improved germplasm. The lines are highly resistant to BGYM but do not have the SR-2 marker linked to the recessive gene *bgm-1*. PR0157-4-1 is a small white bean line derived from

the cross 'Arroyo Loro // HP8437-95 / G35172 // HP8437-95'. PR0157-4-1 has an indeterminate (type III) growth habit, flowers at 35 days and reaches harvest maturity at 76 days after planting. PR0157-4-1 has the $SW12_{700}$ marker for the QTL for BGYM resistance and the SW-13 marker linked to the *I* gene for resistance to bean common mosaic (BCM). PR9771-3-2 is a small red bean line derived from the cross 'HP8437-95 / G35172 // HP8437-95'. PR9771-3-2 has an indeterminate (type III) growth habit, flowers at 35 days and reaches harvest maturity at 76 days after planting. PR9771-3-2 has the $SW12_{700}$ marker for the QTL for BGYM resistance and the SW-13 marker for *I* gene resistance to BCM. PR0247-49 is a shiny black-seeded line derived from the cross 'Morales // HP8437-95 / G35172 // HP8437-95'. PR0247-49 has an indeterminate (type III) growth habit, flowers at 38 days and reaches harvest maturity at 74 days after planting. PR0247-49 does not have the $SW12_{700}$ marker for the QTL for BGYM resistance. When screened in a greenhouse at the University of Nebraska, PR0247-49 was resistant to rust races 41,44 and 53.These lines could be used to broaden the genetic base of BGYM resistance in common bean.

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SOURCES OF RESISTANCE TO *Colletotrichum lindemuthianum* IN TRADITIONAL CULTIVARS OF COMMON BEAN FROM PARANÁ, BRAZIL

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Introduction: Anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams.-Scrib. is one of the most severe and widespread disease of common beans (*Phaseolus vulgaris*, L.). Given the wide variability of the pathogen and the potential for new virulent races to arise, the use of the genetically resistant cultivars is an effective way to control this disease (Kelly et al., 1994). The genetic resistance of the host to *C. lndemuthianum* is not durable, and the plant breeding programs demand a continuous work and require in a permanent and enlarging form of gene pools, in which new genes can be introduced in new cultivars or in cultivars already existent as a way to increase resistance (Pastor-Corrales et al., 1995; Young and Kelly, 1996). Paraná State is the main Brazilian bean producing State and is an area where highly variable traditional populations of beans can be found (Alberini, 2001). Evaluation of genetic resistance of landrace beans from Paraná to Andean and Mesoamerican races of *C. lindemuthianum* is needed prior to incorporation in new common bean cultivars as possible sources of resistance to anthracnose. The objective of this study was to characterize 26 landrace bean cultivars from Paraná State, for their reaction to a wide range *C. lindemuthianum* races present in the state.

Material and Methods: In the North, Northwest and West regions of the Parana State in Brazil were collected 26 landraces cultivars of common bean belonging to the commercial seed classes: Carioca, Preto, Navy, Rosinha, and Manteigão. The 26 landraces cultivars of common bean were inoculated with Andean (7, 19 and 55), and Mesoamerican (9, 31, 65, 69, 73, 81, 89, 95 and 453) races of *C. lindemuthianum*. The protocol for inoculation was as follows: 14-day-old bean plants with a fully developed first trifoliate leaf were spray-inoculated with a spore suspension (1.2×10^6 spores mL^{-1}) of each race of *C. lindemuthianum*. Twelve seedlings of each cultivar were spray-inoculated with standardized spore concentration (1.2×10^6 spores.mL^{-1}) of each race of *C. lindemuthianum*, using a De Vilbiss number 15 atomizer powered by an electric compressor. Spore concentration was adjusted to 1.2×10^6 spores.ml^{-1} using a hemacytometer. Seedlings were evaluated for their disease reaction (Van Schoonhoven and Pastor-Corrales, 1987) using a scale of 1 to 9.). A virulence index (VI) for each *C. lindemuthianum* race and a resistance index (RI) of each genotype was obtained (Balardin and Kelly, 1998).

Results and Discussion: The general mean virulence index (VI) of the races of C. *lindemuthianum* utilized in this study ranged from 12 to 85 %, where the races 7, 73, 89 e 65 were the most virulent, while the race 31 was least virulent, presenting an VI of 12% (Table 1). The resistance index (RI) of 9 Andean genotypes varied from 8 to 67 %, while the 17 Mesoamerican genotypes showed a RI which amplitude varied from 17 to 83 %. The more resistant Andean genotypes were Jalo Vermelho, Jalo de Listras Pretas, and Roxinho, while the more susceptible genotypes were Jalo Pardo, Jalo Pintado 1, and Bolinha (Table 1). Mesoamerican bean genotypes that showed higher resistance levels were Carioca Pintado 2, Carioca Pintado 1, Carioca 6 and Iapar 31, with values of resistance index were 83, 75, 58, and 58 respectively (Table 1). The results show that both Andean and Mesoamerican bean genotypes evaluated in this study have high genetic variability regarding to their response to different races *of C. lindemuthianum*. This material could be used in bean breeding program as source of resistance genes to *C. lindemuthianum* in the future.

Table 1. Reaction of nine Andean and 17 Mesoamerican genotypes of common bean, virulence and resistance index to Mesoamerican and Andean races of *C. lindemuthianum*[***]

Andean Genotypes	Races												Resistance Index (%)
	Mesoamerican									Andean			
	9	31	65	69	73	81	89	95	453	7	19	55	
Jalo de Listras Pretas	R	R	R	R	R	R	R	R	S	S	S	S	67
Jalo de Listras Vermelhas	R	R	R	S	S	R	S	S	R	S	S	S	42
Jalo Pardo	S	R	S	S	S	S	S	S	S	S	S	S	8
Jalo Pintado 1	S	R	S	S	S	S	S	S	S	S	S	S	8
Jalo Pintado 2	R	R	R	S	R	S	S	R	R	S	S	S	50
Jalo Vermelho	R	R	R	S	S	R	R	R	R	S	S	R	67
Jalo Mulato	R	R	R	S	S	S	S	R	R	S	S	S	42
Bolinha	S	R	S	S	S	S	S	S	S	S	S	S	8
Roxinho	R	R	R	S	R	S	S	R	R	S	S	S	50
Andean Virulence Index *(%)	**33**	**0**	**33**	**89**	**67**	**67**	**78**	**44**	**44**	**100**	**100**	**89**	
Mesoamerican Genotypes	**Races**												**Resistance Index (%)**
	Mesoamerican									**Andean**			
	9	**31**	**65**	**69**	**73**	**81**	**89**	**95**	**453**	**7**	**19**	**55**	
Carioca 1	S	R	S	R	S	S	S	S	R	S	R	R	42
Carioca 2	S	R	S	R	S	S	S	S	R	S	R	R	42
Carioca 3	S	S	S	R	S	S	S	S	R	S	R	R	33
Carioca 4	R	R	S	R	S	S	S	S	R	S	R	R	50
Carioca 5	R	R	S	S	S	R	S	S	S	S	R	R	42
Carioca 6	S	R	S	R	S	R	S	S	R	R	R	R	58
Carioca Claro	R	R	S	S	S	R	S	S	R	S	R	R	50
Carioca Pintado 1	R	R	S	S	S	R	R	R	R	R	R	R	75
Carioca Pintado 2	R	R	R	S	R	R	S	R	R	R	R	R	83
Carioca Pitoko	S	R	S	R	S	S	S	S	R	S	R	R	42
Iapar 31	R	R	S	S	S	S	R	R	R	S	R	R	58
Preto 1	S	S	S	S	S	S	S	S	S	S	R	R	17
Preto 2	R	S	S	S	S	S	S	S	S	S	R	R	25
Preto 3	S	R	S	S	S	R	S	S	S	S	R	R	33
Preto 4	S	R	S	S	S	S	S	S	R	R	R	R	42
Rosinha	R	R	S	R	S	S	S	S	R	S	R	S	42
Navy-UEM	S	R	S	S	S	S	S	S	S	S	S	R	17
Mesoamerican Virulence Index *(%)	**53**	**18**	**94**	**59**	**94**	**65**	**88**	**82**	**29**	**76**	**5,9**	**5,9**	
General Mean Virulence Index (%)	**46**	**12**	**73**	**69**	**85**	**65**	**85**	**69**	**35**	**85**	**38**	**35**	

*R = Resistant, S = Susceptible, * Virulence index = number of genotypes with susceptible reaction/ total number of genotypes; *General mean virulence index = total numbers of genotypes with susceptible reaction/26, the total number of genotypes.*Resistance index = total numbers of genotypes with resistance reaction/12, the total number of races used for inoculation.

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Inheritance of Anthracnose Resistance in the Common Bean Cultivar Widusa

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Genetic resistance is considered to be the most effective method of control of anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. et Magn.) Scrib. in common bean (*Phaseolus vulgaris* L.). In a previous report, one symbol, *Co-1*[5] was assigned to describe the gene that controls the resistance of the cultivar Widusa to race 7 of the pathogen. The inheritance of resistance in Widusa was confirmed to be monogenic by a single dominant gene (Alzate-Marin et al., 2002; Gonçalves-Vidigal et al., 2003), whereas Ferreira et al. (2003) affirmed that Widusa has two additional independent genes conferring resistance to race 38. In the present research we propose to confirm the inheritance in Widusa to races 7, 65, 73, and 453 through the evaluation of four F_2 populations and their respective families $F_{2:3}$, as well the allelism tests with previously characterized resistance genes.

Material and Methods

The common bean cultivar Widusa was crossed with Michigan Dark Red Kidney (MDRK), Cornell 49-242, TO, TU, BAT 93, and PI 207262. After this procedure the F_1, F_2, and $F_{2:3}$ families were grown in the greenhouse and tested on their disease reaction to the races 7, 65, 73, and 453 of the fungal pathogen *C. lindemuthianum*. The fourteen-day-old seedlings of the plants were spray-inoculated with a spore suspension (1.2×10^6 spores/ml) of each race.

Results and Discussion

The inheritance studies supported an expected 3R: 1S ratio presented in the four F_2 populations from the crosses Widusa x MDRK ($p = 0.79$), Widusa x BAT 93 ($p = 1.0$), Widusa x Cornell 49-242 ($p = 0.95$), and Widusa x TO ($p = 0.51$), when these populations were inoculated with races 7, 65, 73, and 453, respectively (Table 1). The results indicate that Widusa carries a single dominant gene that confers resistance to Middle American 65, 73, 453, and Andean race 7. The segregation 3R: 1S of the F_2 populations from the crosses Widusa x MDRK, Widusa x BAT 93, Widusa x Cornell 49-242 and Widusa x TO was confirmed by the analysis of the $F_{2:3}$ families, which showed a good fit to a segregation ratio of 1:2:1. These results confirm that one dominant gene is responsible for the anthracnose resistance in Widusa to races 7, 65, 73 and 453.

Allelism tests in the cross (R x R), involving Widusa and Cornell 49-242, fitted a 15R:1S ratio in the F_2 population, when inoculated with race 7. The F_2 populations derived from the crosses Widusa x TO, Widusa x TU, and Widusa x BAT 93, were inoculated with races 7, and 73 to determine whether Widusa carries other resistant genes. The segregation ratio fitted was 15R: 1S and the p value varied from 0.53 to 0.92, indicating that each of the parents carries an independent dominant resistant gene. In the F_2 population derived from the cross Widusa and PI 207262, inoculated with race 65, the segregation fits a 15R:1S (269R:18S; $p = 0.98$) ratio, indicating the presence of two independent dominant genes, one of them is *Co-4*[3] in PI 207262 (de Arruda et al., 2000), and the other *Co-1*[5] gene in Widusa (Gonçalves-Vidigal, 2003). Otherwise, Alzate-Marin et al. 2003, reported that no segregation was observed in F_2 population from the cross Widusa x BAT 93 inoculated with race 65 (Bioagro). However, we observed that BAT 93 has a compatible reaction when inoculated with the race 65 collected in Parana State. The race 65 showed wide genetic variability, through the RAPD analysis of the isolates of this race, demonstrating an intra-race molecular variability (Gonçalves-Vidigal and Thomazella, unpublished data). This fact could be explained due to the origin of the race 65 and its different levels of virulence. After the inoculation with race 65, the F_2 population of the cross Widusa x PI 207262 segregated in ratio of 15R:1S, suggesting that PI 207262 possesses one dominant gene and Widusa another one. Since PI 207262 has previously shown to possess two dominant genes, thus the second gene in PI 207262 must have been defeated by race 65. Studies carried out by Ferreira et al. (2003) demonstrated results that do not agree

with the ones obtained by this experiment. The authors concluded that Widusa has two additional genes resistant to race 38. Besides that, they obtained in the F_2 population of the cross Widusa x A1183 (*Co-2)* a segregation ratio of 61R:3S with a p value = 0.71. In the meantime, if the observed data ratio had been considered to be 15R: 1S, it would have had a p value = 0.64. Furthermore, in the cross Widusa x TU, the authors considered an expected ratio of 61R:3S (p = 0.08), but if the data had been fitted a 15R:1S ratio, consequently the p value would have been 0.47. In addition, the segregation was fitted to a ratio 15R:1S in the F_2 population derived from the crosses Widusa x TO, and Widusa x SEL 1308 when inoculated with race 65 (Alzate-Marin et al. 2002).

In our experiment 200 individuals of the F_2 population derived from the cross Widusa x MDRK did not present segregation when inoculated with race 65.This result indicated that the dominant gene in Widusa is located at the same locus as the *Co-1* gene in MDRK. The inheritance studies supported an expected 3R:1S ratio observed in the four F_2 populations and confirmed by a ratio of 1:2:1 in their $F_{2:3}$ families for resistance to races 7, 65, 73, and 453 of the *C. lindemuthianum*. In addition, allelism tests in F_2 populations derived from crosses Widusa and following cultivars: Cornell 49-242 (race 7); TO, TU, and BAT 93 (races 7 and 73), and PI 207262 (race 65), showed segregation ratio of 15R: 1S. This allelism test indicated that the single dominant resistance gene in Widusa is independent and located at a different locus from the *Co-2*, *Co-4, Co-5*, *Co-9*, and *Co-4*^{3} genes. Since Widusa has a different resistance spectrum from all the other characterized *Co-1* alleles based on its position in the differential series, these data would indicate that it carries a new allele at this locus. The authors reaffirm that the anthracnose resistance allele in Widusa should be designated *Co-1*^{5}.

Table 1. F_2 and $F_{2:3}$ reactions and expected ratios of resistant (R) and susceptible (S) to *C. lindemuthianum* in cross R x S

Cross	Race	Phenotypic evaluation in the F_2					Phenotypic evaluation in the $F_{2:3}$			
		Expected Ratio	R-	rr	χ^2	*P value*	RR	R-	rr	*P value*
Widusa x MDRK	7	3:1	164	57	0.074	0.79	23	43	24	0.90
Widusa x BAT 93	65	3:1	252	84	0.0	1.0	26	36	20	0.80
Widusa x Cornell 49-242	73	3:1	68	23	0.036	0.95	25	43	23	0.83
Widusa x TO	453	3:1	151	43	0.435	0.51	50	101	43	0.65

***R = Resistant; S = Susceptible; MDRK = Michigan Dark Red Kidney.**

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Phenotypic and Genotypic Characterization of the Bean Rust Pathogen from Bean Fields in the Americas

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Common bean is recognized to have two major gene pools - Andean and Middle American. *Uromyces appendiculatus*, cause of bean rust, has been reported to have Andean-specific and nonspecific pathotypes (using virulence and RAPD fingerprints) (1). However, this and other bean pathogen population studies were based on small numbers of isolates. The present study uses 90 isolates collected from bean fields throughout the Americas where the host was subdomesticated. The objectives were to determine the pathogenic relationship of *U. appendiculatus* isolates using landrace differentials representing the two main centers of domestication and standard differentials with some known resistance genes and genotypic variation using RAPDs.

Materials and Methods

A metapopulation of single uredinium isolates of *U. appendiculatus* from the Andean (Argentina, Brazil, Boliva, Ecuador and Peru) and Middle American (Cost Rica, Cuba, Dominican Republic, El Salvador, Guatemala, Honduras, Jamaica and Mexico) regions was evaluated on the standard set of 19 bean differentials according to the procedure described by Stavely et al. (2). A second set of differentials made up of nine Andean and six Middle American bean landraces obtained from S. Singh were also used to determine specificity of the isolates from both centers of domestication. DNA was extracted from this population and analyzed using 50 polymorphic bands generated by the RAPD technique.

Results and Discussion

From 90 single uredinium isolates 206 virulence patterns found: 94 on standard differentials, 46 on Andean landraces, 66 on Middle American landraces. Mean virulence on Middle American landraces was higher than on Andean landraces. Isolates from Middle American countries e.g. Honduras and Mexico, were more virulent than those from Andean countries, e.g. Ecuador and Bolivia (Table 1). Individual Andean isolates had higher disease scores on Middle American landraces than some Middle American isolates. However, in general, most Middle American isolates showed high to medium disease scores on both Andean and Middle American landrace differentials. Bean rust pathogen populations from Middle America are both more genetically variable and more virulent than populations from temperate or Andean regions.

Three major groups of *U. appendiculatus* isolates which represented the major gene pools and a pre-ancestral pool from the Americas were confirmed using phenotype (virulence) and genotype (RAPD) analysis (Table2); 84% of isolates were classified as Andean, Middle American or mixed: Andean/Middle American. While molecular analysis using RAPD-PCR did not group these isolates by geographic origin, they were placed in the same three clusters which represent virulence pathotypes.

Ongoing adaptation between pathogen and host is responsible for characterization of these major groups. The large number of virulence patterns, some of which were unique to

certain countries, requires use of specific resistance genes in different regions, and knowledge of their specificity or lack of specificity will be necessary for rust resistance gene deployment.

Table 1: Comparison of means of average disease score of *Uromyces appendiculatus* from Latin America and the Caribbean on two sets of bean landrace differentials

Differential Code [a]	Landrace Differential	Mean of Disease score and standard deviation		
		Andean isolates [b]	Middle American isolates [c]	Total population [d]
A-1	Jatu Rong 558	4.5" 1.2	4.7 "0.9	4.6"1.0
A-2	Jalo EEP	2.4 "1.1	3.6" 1.8	3.1"1.6
A-3	Tortolas Corriente	1.9" 1.3	3.3" 1.8	2.7"1.7
A-4	Blanco espanol	2.5" 1.7	4.4 "1.4	3.5"1.8
A-5	Bolon Rojo	1.6" 1.2	2.7" 1.8	2.1"1.7
A-6	Bolon Bayo.	4.0 "1.8	3.4 "1.7	3.6"1.8
M-1	Carioca	5.3" 0.3	5.1" 0.3	5.1"0.3
M-2	Riotobaga	5.5" 0.4	5.1" 0.4	5.3"0.5
M-3	Zacatecano	5.2" 0.6	3.7 "1.6	4.4"1.5
M-4	Guanjuato,	3.3" 1.2	3.6 "1.2	3.4"1.3
M-5	Flor de mayo	5.0" 0.3	4.4" 0.8	4.7"0.7
M-6	Amarillo 154	5.1" 1.2	4.4" 1.2	4.5"1.2

[a]A-1 to A-6 are Andean landrace differentials and M-1 to M-6 are Middle American Landrace differentials; [b]N=42 Andean isolates; [c]N=48 Middle American isolates; [d]N= 90 isolates.

Table 2: Matrix comparison values (r) for Andean and Middle American isolates based on virulence on standard and landrace differentials and RAPD markers

	Standard Differentials	Landrace Differentials	RAPD Markers
Standard Differentials	1.00	0.35	0.30
Landrace Differential	0.35	1.00	0.42
RAPD Markers	0.30	0.42	1.00

At p #0.05, N=90 and r = 0.031.

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Evaluation of Bean Classes for Root Traits Differences Associated with Root Rot Resistance

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Introduction. Detecting differences in root architecture and growth patterns among common bean (*Phaseolus vulgaris* L.) genotypes may provide unique selection criteria for genetic resistance to root rot caused by *Fusarium solani* (Mart) Sacc. f.sp. *phaseoli* (Burk.) W.C. Snyder & H.M. Hans. The pathogen infection acts to reduce root density by killing roots and may attenuate the functional efficiency of the remaining infected roots, leading to yield loss. When the primary root dies due to infection, promoting lateral and adventitious roots may contribute to plant survival in the presence of root rot organisms (Snapp et al., 2003). Understanding mechanisms of *Fusarium* root rot resistance in common bean, especially kidney beans, is a major goal of breeding programs. Quantitative information on root system traits associated with root rot resistance would improve selection criteria. Moreover knowledge of the genetic determinants of root traits and how they influence yield would allow for a more targeted breeding approach utilizing technologies such as QTL analysis. The objectives of this study were to: 1) characterize genetic variation of root architecture in contrasting bean classes expected to vary in reaction to *Fusarium* root rot and root system traits, under field and greenhouse conditions; and 2) identify root system characteristics that may be associated to root rot resistance in common bean.

Materials and Methods. Ten genotypes representing, four bean seed classes (kidney, cranberry, blacks, and snap beans), were evaluated for reaction to *Fusarium* root rot (Schneider and Kelly, 2000) and root system traits during the summer 2002 and summer 2003. The field study was conducted at the Montcalm Research Farm located near Entrican, MI, with an alfisol soil, series name Montcalm/ McBride loamy sand, arrange in a lattice design. The greenhouse study was conducted at Michigan State University using Treepots™ (a mixture of coconut coir and perlite (1:2) was used), arranged in a RCBD with two replications of the study each summer. Temperature in the containers for the greenhouse was monitored using a Watchdog™ and it ranged from 25° C at day and 20° C at night. Temperature for the field trial varied from 20º C to 26º C. The field environment was heavily and naturally infested with root rot while the greenhouse was disease free. A total of 180 roots were excised from the shoot and placed in ice to prevent dehydration while harvesting and preparing samples for analysis. Root architectural traits were analyzed by scanning the root samples into a digital image using the image analysis system WinRHIZO™ , following the procedure of Yabba and Foster (2003) and Frahm et al. (2003). Root were divided in ten root length classes based on diameter and classified as A-J respectively ranging from 0.1 to >4.5 mm, these were grouped in three root diameter classes: fine roots or secondary roots (A-C), intermediate roots or laterals (D-G), and taproots (H-J). One way analysis of variance using SAS was performed, evaluating genotype effect (SAS Inst., Inc., Cary, N.C.).

Results and Discussion. Genetic variation existed in root architecture among common bean classes and was highly significant under field environment at 30 days after planting (DAP) (Fig. 1). Plant breeders interested in enhancing root rot resistance and over all root health could focus on evaluating adventitious roots, root dry weight, and lateral roots in a breeding population since these traits are relatively easy to quantify under field conditions. Selection for root dry weight

would appear to be useful in the greenhouse (Fig. 2), but should be delayed to flowering around 45 DAP to allow greater expression of root trait differences as plants mature. In the field, by contrast, edaphic stress may have enhanced differences earlier as shown by the markedly greater number of adventitious roots. The potential for improving root characteristics in common bean exist, and it appears that breeders have been effective in introgressing desirable rooting traits from black bean into kidney and snap bean as is the case of Chinook 2000 and FR266 which could serve as valuable parental lines to further enhance the root rot resistance of susceptible commercial kidney and snap bean varieties.

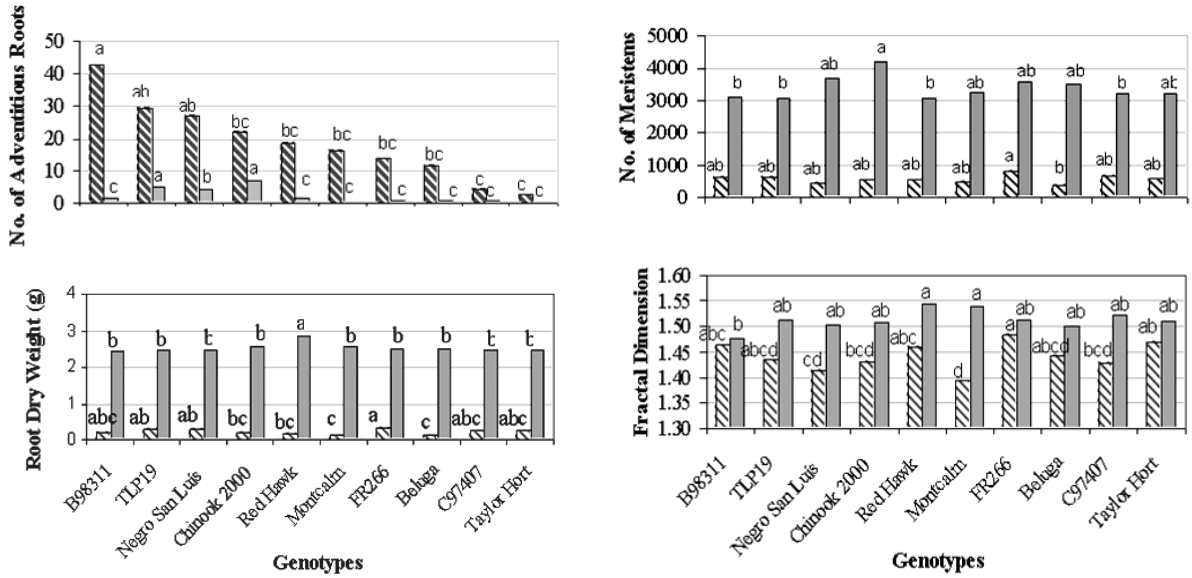

Figure 1. Illustration of differences between genotypes 30 days after planting under field (hatched boxes) and greenhouse conditions (solid boxes) for root traits such as adventitious rooting (A), number of meristems (B), root dry weight (C), and fractal dimension (D). (Different letters on columns represent statistical differences at $P< 0.05$)

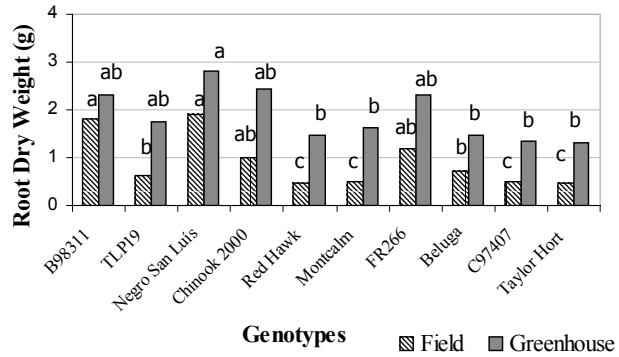

Figure 2. Differences between genotypes 45 days after planting under field and greenhouse conditions for root dry weight. (Different letters on columns represent statistical differences at $P<0.05$)

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CONTROL OF DOWNY MILDEW AND WHITE MOLD IN LIMA BEANS.

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Yields of lima beans (*Phaseolus lunatus*) produced in Delaware are threatened by Downy mildew (*Phytophthora phaseoli*) and White mold (*Sclerotinia sclerotiorum*). Over the past five years, two new races of *P. phaseoli* have been detected in Delmarva lima bean fields. White mold has been a chronic problem for decades, but incidence seems to be increasing in recent years.

Downy mildew

During the 2000 season race E of *P. phaseoli*, caused losses in excess of one million dollars. The 2003 growing season was similar climatologically to the 2000 season. A number of lima beans fields suffered losses caused by *P. phaseoli* and the prevalent genotype for the 2003 season was determined to be race F. These losses were lessened in party due to the timely application of copper or Ridomil-Gold fungicides. Field studies were established in 2002 and 2003 to determine resistance of lima bean varieties to races E and F at separate locations. Varieties were planted in rows 30 inches apart and the middle ten feet of each row was evaluated for the percent of plants infected approximately 2 weeks after inoculation and percent pods infected at harvest. The plots were irrigated as needed. Sporangial suspensions of race E and F were applied on 29 Aug for both locations and s second inoculation of race F on 11 Sept. The plots were misted nightly after inoculation to increase humidity and leaf wetness. The results of the 2003 studies are reported in Tables 1 and 2.

Table 1. Reactions to *P. phaseoli* race E – Newark, DE. 2003.

Variety	Type	Resistance(E)	% plants Infected[a]	% pods infected
M-15	Baby	S[b]	13.8 b[c]	25.5 c
Eastland	Baby	S	20.0 a	83.1 a
8-78	Baby	S	12.3 bc	75.7 a
184-85	Baby	R	0.0 f	5.4 d
Cypress	Baby	R	0.0 f	4.3 d
C-elite select	Baby	R	0.0 f	2.4 d
Sussex	Fordhook	MS	5.5 de	50.3 b
Dixie Butter Pea	*	MS	2.3 ef	50.6 b
Early Thorogreen	Baby	MS	8.0 cd	43.4 b

[a] Percent plants infected includes infection of any plant part including racemes, petioles, or pods.

[b] Resistance reactions: HS = highly susceptible (>85% infected plants), S = susceptible (>40% infected plants), MS = moderately susceptible (5-25% infected plants), R – resistance (<2% infected plants).

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

* Indicates neither a baby nor a Fordhook type of lima bean.

Table 2. Reactions to *P. phaseoli* race F – Georgetown, DE. 2003[a]

Variety	Type	Resistance(F)	% Plants Infected	% Pods Infected
M-15	Baby	R	0.0 e	1.8 d
Eastland	Baby	R	0.0 e	2.2 d
8-78	Baby	R	0.0 e	0.0 d
184-85	Baby	S	85.5 a	23.7 ab
Cypress	Baby	MS	34.0 cd	9.1 cd
C-elite select	Baby	S	79.0 cd	24.5 ab
Sussex	Fordhook	MS	3.5 de	1.5 d
Dixie Butter Pea	*	MS	50.5 bc	18.1 bc
Early Thorogreen	Baby	MS	80.0 ab	29.3 a

[a] Refer to explanations at the bottom of table 1.

The results obtained in 2002 are consistent with those obtained in2003. The cultivars 184-85, Cypress, and C-elite select are resistant to race E, while M-15 and 8-78 are resistant to race F. The variety Sussex was determined to be moderately susceptible to both race E and F. It should be noted that the variety Cypress, which was determined to be susceptible to race F, was determined to have a "slow mildewing" characteristic. When large plots of only Cypress were inoculated with race F, it performed like a resistant variety, presumably because of the reduced levels of secondary inoculum.

Three experiments were conducted in 2003 to evaluate the efficacy of several preventative fungicides, the proper timing of application, and the impact of post-infection fungicide applications. The disease severity in the field was very high, all plants in the control plants were infected. Sixteen treatments were evaluated in the efficacy trial. Ridomil Gold/Copper WP 2.0 lb was the only treatment that significantly increased yield compared to the controls. Phostrol 4.0 pt, Phostrol 2.0 pt, and Champ DP 2.0 lb all performed well and were found to be significantly better than the controls for percent plant and percent pod infection.

In the timing trial, the best was Ridomil Gold//Copper 2.0 lb applied one time followed by three applications of Champ DP 2.0 lb every seven days. The two best post-infection application schedules tested were Ridomil Gold/Copper WP 2.0 lb applied two times, seven days apart and Ridomil Gold/Copper WP 2.0 lb applied one time followed by one application of Champ DP 2.0 lb.

White Mold

White mold consistently causes reduced yield in lima beans and contamination during processing. The causal agent of white mold, *Sclerotinia sclerotiorum*, overwinters in residue or as sclerotia in and near bean fields. The sclerotia survive in the soil for many years. When soil is moist for a period of 6 to 10 days, the sclerotia in the top two inches of the soil germinate, form apothecia, and release spores that are carried to plants by wind. The spores may infect senescing leaves and flowers, and spread to other plant parts.

Studies were initiated in 2002 and 2003 to evaluate Contans WG, a new bio-fungicide (biocontrol agent, *Coniothyrium minitans*), which attacks the white mold sclerotia. Contans is incorporated into the upper two inches of the soil, three to four months prior to disease development. In a 2002 trial in Delaware, Contans WG applied once prior to planting, once at

the seedling stage, and at both times, significantly reduced the number of infected pods at harvest. The test was not definitive because disease level was low, however, the results indicate Contans WG has potential to manage white mold in lima beans.

A fungicide evaluation study was established with the fordhook lima bean cultivar 'Sussex', seeded in 30 in. rows on 27 Jun, 2003. Fungicides were applied on 6 Aug with a CO_2 backpack sprayer equipped with 4 nozzles spaced 18 in. apart. White mold severity was high in the field. On 17 Sep, plots sprayed with Endura 70WG alone or in combination with Penetrator Plus had significantly fewer pods infected with white mold than nontreated plots. Endura 70WG plus HyperActive, Serenade 10WP, Switch 62WG at 11 and 14.1 oz/A and Omega 4SC had intermediate levels of pods infected with white mold that were not significantly different than the nontreated plots. There were no significant differences in the number of infected pods at harvest. Plots sprayed with Endura 70WG, alone, with HyperActive, or Penetrator Plus, and Pristine 38WG had significantly higher yield than nontreated plots. The active ingredient (a.i.) in Endura is boscalid. Pristine contains the a.i. boscalid and also pyraclostrobin. Yield in plots sprayed with Topsin M 70WP, Topsin M 4.5F, Switch 62WG and Omega 4SC was intermediate, and due to field variability, not significantly different than the nontreated plots.

Table 3. Results of white mold fungicide evaluation study - 2003.

Treatment and rate/A *	Infected pods/A 17 Sep		Infected pods/A at harvest	Yield (T/A)	
Endura 70WG 7 oz + HyperActive10.5floz	9332	cd**	11258	0.79	a
Endura 70WG 7 oz + Penetrator Plus 2.7 pts	6652	d	13543	0.86	a
Endura 70WG 7 oz	7354	d	4875	0.86	a
TopsinM 70WP 2 lbs.	20570	ab	9536	0.71	ab
TopsinM 4.5F 3.1 pts.	15948	abcd	12826	0.71	ab
Serenade10WP 6 lbs.	11242	bcd	10297	0.62	ab
Sonata F 8 pts	15895	abcd	14282	0.53	b
Switch62WG 11 oz	10582	bcd	12166	0.68	ab
Switch62WG 14.1 oz	11279	abc	9466	0.67	ab
Omega 4SC 8 fl oz	9321	bcd	11946	0.72	ab
Pristine38WG/lb	18614	cd	15644	0.79	a
Serenade 10WP 4 lbs plus TopsinM70WP 1 lb	26546	a	11872	0.54	b
Nontreated	19229	abc	12645	0.54	b
LSD ($P = 0.05$)	10,689		n.s.	0.24	

* Fungicides were applied on 6 Aug.

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

A cloned *R* gene from *Phaseolus vulgaris* confers a systemic necrosis response to *Cucumber mosaic virus* in *Nicotiana benthamiana*

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Bean dwarf mosaic virus (BDMV) is a single-stranded DNA virus (Genus *Begomovirus*, Family *Geminiviridae*) that infects common bean (*Phaseolus vulgaris* L.) and causes stunted growth and mosaic symptoms. A Toll-interleukin-1 receptor (TIR)-nucleotide-binding site (NBS)-leucine rich repeat (LRR) resistance (*R*) gene analog (*RT4-4* gene) was cloned, using reverse transcription-PCR with degenerate NBS primers, from common bean (*Phaseolus vulgaris* cv. Othello) tissues undergoing a resistance response to BDMV. Northern blot and RT-PCR analyses revealed that *RT4-4* was expressed and upregulated in BDMV-infected tissues after inoculation of BDMV. A functional analysis of *RT4-4* was performed by generating transgenic *Nicotiana benthamiana* plants (susceptible to BDMV and many other viruses) and inoculating with DNA and RNA viruses. The RT4-4 transgenic plants were not resistant to BDMV. However, these plants developed a systemic necrosis phenotype in response to infection by *Cucumber mosaic virus* (CMV; Family *Bromoviridae*, Genus *Cucumovirus*), a tripartite positive-sense RNA virus. These plants developed a systemic necrosis response to all the CMV isolates tested, except for a bean-infecting strain (strain 67). Of all the CMV strains instead, only strain 67 systemically induced cv. Othello, causing mosaic and dwarfing symptoms. Thus, the development of the systemic necrosis response of RT4-4 transgenic *N. benthamiana* plants to infection with CMV strains was consistent with the relative susceptibility of cv. Othello from which the *RT4-4* gene was cloned. The finding that the CMV defense response elicited by *RT4-4* in *N. benthamiana* was very different from that in common bean, i.e., systemic necrosis versus no obvious symptoms, likely reflects the interaction of the RT4-4 protein with different host factors and/or signal transduction pathways in these two plant species. Segregation analysis of T_1 transgenic plants indicated that the CMV systemic necrosis phenotype conferred by *RT4-4* acted as a single dominant gene. *Agrobacterium*-mediated transient expression experiments revealed that the CMV *2a* protein (replicase) of the non-bean-infecting CMV strains was the elicitor of necrosis in the RT4-4 transgenic *N. benthamiana* plants, whereas the 2a of the bean-infecting strain (67) did not induce necrosis. A single amino acid change in the 2a of a non-bean-infecting strain, changing phenylalanine to tyrosine at position 631, abolished the necrosis phenotype. These results are consistent with *RT4-4* acting in a gene-for-gene manner. Thus, *RT4-4* is a CMV *R* gene from common bean, which is functional across plant families.

The Search for Resistance to the Soybean Aphid Virus Complex in Snap Beans

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Introduction

In 2003, approximately 188,000 acres of snap beans were harvested in the United States of which 66,000 acres were grown in Wisconsin with an estimated crop value of $30 million (Wisconsin Ag Statistics, 2004).

Since first detected in Wisconsin in 2000, a virus complex thought to be transmitted predominantly by the soybean aphid (*Aphis glycines*) has resulted in significant economic losses to the Midwestern snap bean industry. It is not clearly understood to what extent a single virus or combination of viruses is responsible for these economic losses. Visual symptomatology and ELISA (Enzyme-Linked Immunosorbent Assay) results indicate that the current virus complex consists primarily of cucumber mosaic virus (CMV) and alfalfa mosaic virus (AMV); however, other viruses such as Bean Common Mosaic Virus (BCMV) and Clover Yellow Vein (CYVV) have been observed. 2001, 2002 and 2003 research indicates that there are currently no commercial cultivars available with resistance to the virus complex (Stevenson and Grau, 2004). In addition, insecticides have proven ineffective in controlling the soybean aphid with respect to virus spread (Wyman, 2004). Identifying new sources of resistance continues to be the most desirable long-term solution to this problem.

Materials and Methods

A replicated field trial was planted at Arlington, WI Agricultural Research Station (ARS) on July 14th. Fourteen days prior to planting the trial, spreader rows, consisting of a soybean and a virus susceptible snap bean cultivar (Hystyle), were planted. Three hundred accessions were evaluated at four levels as follows:

1. 200 *Phaseolus vulgaris* accessions from the USDA Regional Plant Introduction (PI) Station in Pullman, WA.
2. 32 commercial and heirloom (pre-1950) cultivars.
3. 34 recombinant inbred lines (RIL) from a cross of Eagle x Puebla 152. These lines were excluded in the 2002 trial due to lack of seed.
4. 34 individual plant selections. Germplasm was carried forward from 2002 in which 147 individual plants tested ELISA CMV and AMV negative (Sass, et al., 2003). We harvested seed from 77 of these individuals. Six replications of this material was hand inoculated with CMV and AMV at Walnut Street Greenhouses in winter 2002 and spring 2003 in cooperation with Dr. Craig Grau and narrowed to 34 lines.

On the same day the trial was planted, snap beans within the spreader rows were hand inoculated at random throughout the trial using infected CMV and AMV leaf tissue (Larsen, et al., 2002). This design optimizes disease transmission because it provides a highly compatible host for prolific soybean aphid reproduction and insures that the specific viruses of interest are present in the field.

At 56 days after planting (DAP), a composite sample of 10 leaves from each of the 600 plots was taken for ELISA. CMV and AMV antibody specific ELISA kits (Agdia, Elkhart, IN) were used.

Results

Composite sample ELISA results indicated that all but six entries tested positive for AMV and 100% of the entries tested positive for CMV including the 34 individual plant selections from 2002.

Visual ratings of virus symptoms were taken three times at 38, 45 and 53 DAP. Twenty-six entries remained symptomless throughout the growing season. These plots were resampled to confirm the positive CMV ELISA results on an individual plant basis and to also confirm the negative AMV ELISA results for 12 entries. One leaf per plant in each plot was taken for individual plant ELISA. All 260 individual plants harvested tested positive for CMV and absence of AMV was confirmed.

Although CMV resistance was not identified, these results suggest that genetic variability for tolerance to CMV and possible resistance to AMV exist within *Phaseolus vulgaris*.

We were able to harvest mature seed from 10 of the 16 individual plant selections. This material may serve as a source of CMV tolerance and possible AMV resistance in the future. This seed is currently being increased in cooperation with Kenneth Kmiecik, Seminis Vegetable Seeds for evaluation at West Madison ARS in 2004 and a large-scale evaluation in 2005.

Table 1. 2003 ELISA results for 300 accessions.

Accessions	# Evaluated	Visually Symptomless	ELISA (-) AMV and CMV	ELISA (-) AMV
PIs	200	9	0	6
Cultivars	32	1	0	0
E x P RILs	34	0	0	0
Individ. plants	34	16	0	6

Acknowledgements

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Review of Coevolution Studies Between Pathogens and their Common Bean Hosts: Implication for the Development of Disease-Resistant Beans

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Diversity of the common bean. The genetic diversity of the cultivated common bean (*Phaseolus vulgaris* L.) is organized into two distinct gene pools, Andean and Middle American (Gepts et al., 1986). These gene pools appear to have originated separately in the Andes region of South America, and in Central America and Mexico, respectively. Morphological, biochemical, and molecular attributes differentiate these two gene pools. For example, the Andean pool is comprised of large-seeded beans while the Middle American pool is constituted of small and medium-seeded beans (Singh, 2001). It is significant that the diversity of cultivated common bean cultivars parallels the diversity of their wild bean ancestors.

Diversity and evolution of certain common bean pathogens. Starting in the mid-eighties, bean scientists speculated about the probable existence of two groups of bean pathogens that paralleled the gene pools of the common bean (Gepts and Bliss, 1985). Subsequent research results revealed that the diversity of the pathogens causing angular leaf spot - ALS - (*Phaeoisariopsis griseola*), anthracnose (*Colletotrichum lindemuthianum*) and rust (*Uromyces appendiculatus*) segregated into two distinct groups, Andean and Middle American, that mirrored the diversity of their bean host. The separation of isolates of these pathogens into two different groups has been attained with virulence assays that included Andean and Middle American bean differential cultivars, isozymes, RAPDs, random amplified microsatellites, and restriction fragment length polymorphism of the amplified ribosomal intergenic spacer region (Beebe and Pastor Corrales, 1991; Correa, 1988; Geffroy et al., 1999; Guzman et al., 1995; Mahuku et al., 2000; Pastor-Corrales et al., 1993; Pastor-Corrales, 1996; Sandlin et al., 1999). This separation has been documented for isolates from wild beans from South America and Mexico, as well as for isolates from cultivated beans from South, Central and North America, the Caribbean and Africa (Chacon et al., 1997; Mahuku, et al., 2000).

It is significant that isolates of these three pathogens are virulent only on certain bean genotypes, revealing their high affinity for specific types of beans. Research results using bean differential cultivars show that the Andean isolates of these pathogens are associated under field conditions with large-seeded Andean beans. In addition, Andean isolates exhibit a narrow host range and are compatible only or mostly with beans of the Andean gene pool. On the other hand, Middle American isolates are usually associated under field conditions with small or medium-seeded Middle American beans. Their host range is much wider than that of Andean isolates and it includes Middle American and Andean bean genotypes; however, Middle American isolates tend to be more virulent on Middle American than on Andean beans. Our recent results with isolates of *U. appendiculatus* from different parts of the world also reveal the presence of two groups of isolates.

The intimate relationship between Andean and Middle American isolates of the rust, ALS, and anthracnose pathogens with certain bean cultivars appears to have resulted from a long adaptation process. Andean group isolates appear to have evolved with and adapted to Andean beans in South America. Middle American isolates would have evolved with and adapted to Middle American beans in Central America and Mexico. This parallel organization between the bean host and these pathogens suggests coevolution. The resulting specialization may explain why the rust pathogen is a biotrophe comprised of different populations (i.e., races) with very

specific virulence for certain bean genotypes only. Although the ALS and anthracnose pathogens are not true biotrophes, they behave as such and are comprised of races that also exhibit specific virulence for certain bean genotypes only. Research results using molecular markers also separate the isolates of these pathogens into two groups corresponding to those obtained using virulence assays. It is also significant that diversity among Middle American beans and Middle American races of these three pathogens is appreciably higher than that of Andean beans and Andean races of these pathogens.

The high specificity found among the ALS, anthracnose and rust pathogens for certain bean cultivars suggested that disease resistance genes from Andean beans could be used to manage Middle American races of these pathogens. Similarly, genes from Middle American beans could be used to manage Andean races of these pathogens. Thus, bean cultivars having appropriate combinations of Andean and Middle American disease resistance genes, attained through gene pyramiding, could provide durable resistance to all races of these pathogens. We have released bean germplasm lines with two Andean and two Middle American rust resistance genes that have been evaluated as resistant to rust under field conditions in different parts of the world (Pastor-Corrales et al., 2001).

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BIOTIC AND EDAPHIC FACTORS AFFECTING BEAN ROOT ROT

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Management practices have a significant impact on the occurrence of root rot in dry bean grown in North Central Minnesota. Soil impedance, soil pH, reduced tillage, cropping sequences, excessive soil moisture, low soil temperatures and excessive nitrogen fertilization have been conducive for root rot produced by *Fusarium solani, Rhizoctonia solani* and *F. oxysporum* (Estevez de Jensen et. al., 2003). High soil bulk density has shown to increase root rot severity (DS). Under greenhouse conditions bulk densities of 1.5 and 1.7 g/cc that are similar to those found in compacted soils increased DS of root rot caused by *Fusarium solani* f. sp. *phaseoli* (6.9 and 7.5, respectively) when compared to bulk density of 1.3 g/cc (DS 4.4) found in uncompacted soils. Experiments were conducted in grower's fields with previous history of root rot at Park Rapids and Verndale, Minnesota. Cultivar 'Montcalm' was sown at 70 kg ha^{-1}. The experiment was arranged in a split-split-split design with tillage in the main plots, liming in the subplots and inoculation with *Rhizobium* and *Bacillus subtilis* plus *Rhizobium* in the sub-subplots. No nitrogen fertilizer was applied at either location. Deep tillage (45 cm depth) improved plant emergence and yield and decreased disease severity at both locations (Table 1). Seed treatment significantly decreased DS at Park Rapids but did not have an influence on plant emergence (Table 2). A non significant yield increase was recorded at both locations (Table 2).

Under greenhouse conditions in a Verndale soil (untreated seed, original pH = 5.3), liming with calcium carbonate (30 g/Kg soil) increased soil pH (pH = 7.0), decreased DS from 5.6 to 3.5 and increased plant dry weight (PDW) from 1.0 to 1.8 g/plant. In a soil from Park Rapids (untreated seed, original pH = 4.8) similar effects were observed, DS was decreased from 5.5 to 3.8. Even though addition of lime to a Staples soil (original pH = 6.8 pH of limed soil pH = 8.2) decreased DS from 6.8 to 3.6, PDW was decreased from 1.9 to 1.3 g/plant, due to a resulting greater than the pH of 6.5 to pH 7.0 that is optimum for dry bean production (Robertson and Frazier, 1978). Under field conditions at Park Rapids (original pH 5.2) application of agricultural lime (3 MT/A), deep tillage and inoculation with *Rhizobium tropici* resulted in reduced when compared to a combined treatment of reduced tillage, no liming and no inoculation (DS 3.2 vs. 4.7). Yields were significantly increased also at Park Rapids (Table 3). At Verndale no significant change was observed in plant emergence, DS, or yield after the addition of 2 MT/A of lime.

Management practices recommended to minimize stress factors that increase severity of root rots include deep tillage to disrupt compacted layers; seed treatment with *Bacillus* and inoculation with *Rhizobium* spp. in fields with previous history of root rot; delayed sowing dates to avoid planting before soil warm up and liming of acid soils to maintain optimum soil pH for bean growth and development. Some of these recommendations have been reported elsewhere but need to be practiced according to conditions in particular sites.

Table 1. Effect of tillage on disease severity, emergence and yield of dry beans in 2003.

	Disease severity (1-9)	Emergence # plants	Yield
Park Rapids			
Reduced tillage	4.1 a*	25 b	1414 a
Deep tillage (45 cm depth)	3.9 a	30 a	1619 a
Verndale			
Reduced tillage	5.0 a	15 b	697 b
Deep tillage (45 cm depth)	4.3 a	20 a	973 a

* Values with different letters in the same column are significantly different at $P = 0.05$.

Table 2. Effect of seed treatment on disease severity, emergence and yield of dry beans, in 2003.

	Disease severity (1-9)	Emergence # plants	Yield
Park Rapids			
Untreated	4.4 a*	29 a	1384 a
Rhizobium tropici	3.6 b	27 a	1649 a
Verndale			
Untreated	4.8 a	19 a	768 a
Rhizobium/Bacillus	4.6 a	17 a	902 a

* Values with different letters in the same column are significantly different at $P = 0.05$.

Table 3. Effect of liming on disease severity, emergence and yield of dry beans, in 2003.

	Disease severity (1-9)	Emergence # plants	Yield	Soil pH
Park Rapids				
No Lime	4.3 a*	27 a	1250 b	5.2
Lime 3 TM/A	3.8 a	28 a	1458 a	5.9
Verndale				
No Lime	5.2 a	18 a	816 a	6.2
Lime 2 TM/A	4.1 a	18 a	854 a	6.3

* Values with different letters in the same column are significantly different at $P = 0.05$.

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DEVELOPMENT OF A GENOME-WIDE ANCHORED MICROSATELLITE MAP FOR COMMON BEAN

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Introduction:
Microsatellites are polymerase chain reaction (PCR) based markers that have been developed for a wide range of plant species, including many commercial crops. Among the grain legumes, microsatellite markers are now available for soybeans, chickpea, cowpeas, peanuts and more recently common beans. Microsatellite markers have been developed for common beans from both non-coding (genomic) and coding (genic) sequences containing simple repeats. Our principal objective in this study was to map the both types of microsatellites in a single mapping population derived from the cross DOR364 x G19833 and to integrate this map with the genetic maps developed by Freyre et al. (1998) and Vallejos et al. (1992).

Materials and Methods:
Two populations of recombinant inbred lines (RILs) were used for this study: the first population was based on 87 RILs from the cross DOR364 x G19833 (DG population). The second population was based on 91 RILs from the cross BAT93 x JaloEEP558 (BJ population) whose development and origins are described by Freyre et al. (1998). Total genomic DNA for each of the recombinant inbred lines in both populations was isolated from bulked leaf tissues of eight greenhouse-grown plants per line, using a CTAB extraction method. We used three sets of markers in this study: 1) genomic microsatellites developed in this laboratory by Gaitán-Solís et al. (2002); 2) gene-coding microsatellites developed by Yu et al. (1999, 2000) and 3) additional gene-coding and non-coding microsatellites from searches for SSR containing Phaseolus sequences deposited in the Genbank database before July 15, 2001. Primers were designed using Primer 3.0 software to produce PCR amplification fragments that were on average 150 bp long. PCR primers with consistent melting temperatures of 55ºC or above and an average length of 20 nucleotides. Polymorphisms between the mapping parents were determined on parental survey gels. Standard microsatellite PCR conditions were used throughout and the PCR reaction was carried out in 20 mL final volumes. Gel staining and image capture are as described in more detail by Gaitan et al. (2002). The sizes of the parental alleles were estimated based on 10 bp and 25 bp molecular weight ladders. Segregation data was used to place the microsatellites on the established genetic maps for the DG and BJ populations (Beebe et al. 1998; Freyre et al. 1998) and the two maps were linked by RFLP markers with the map described by Vallejos et al. (1992).

Results:
A total of 150 common bean microsatellites were used in this study. Of these, 81 were anonymous genomic or non-coding microsatellites and 69 were gene-derived microsatellites. All the microsatellite markers were screened for amplification products and polymorphism in the parents of the DG and BJ populations and no overall difference in band intensity was observed and a majority of the microsatellites produced single bands. Polymorphism rates for the DG and BJ populations were 65.4 and 63.2% for the genomic microsatellites, and 46.3 and 46.2% for the genic microsatellites, respectively. Overall the percentage of polymorphism between the parents of both populations was very similar: a total of 84 out of the 150 microsatellites tested for the parents of the DG population were polymorphic (56.0%), while a total of 68 out of the 122 microsatellites tested for the parents of the BJ population were polymorphic (55.7%). A total of 100 new microsatellite loci were placed on the two genetic maps (78 on the DG population and 22 on the BJ population). Microsatellite loci were found on each of the eleven chromosomes of the species and each chromosome was tagged with at least five or more microsatellite

(Table 1). The average number of microsatellite loci per chromosome was 10 and the average distance between microsatellite loci in this map was 19.5 cM; however the distribution of loci was variable and several large gaps between microsatellites remained on the map while for some genomic microsatellites the loci were clustered together.

Discussion:
This study brings to a total of 115, the microsatellite loci located on the bean genetic map and provides coverage for every chromosome in the genome with from five to twenty markers each. As single-locus markers, the microsatellites in this study were specific to a given place in the genome and this allowed them to be used for comparative mapping across both the DG and BJ populations. This comparative mapping showed the consistency of microsatellite location on both populations: with all the microsatellites mapping to the same individual chromosome and equivalent map locations in each of the populations. Comparative mapping allowed us to determine the identity and orientation of each linkage group and to obtain a more accurate position for each of the microsatellites. This set of microsatellite markers provides the basis for anchoring and aligning genetic maps one to each other based solely on PCR-based markers and therefore make ideal second-generation markers for whole genome analysis, gene tagging and quantitative trait loci studies.

Table 1. Distribution of microsatellite markers across two genetic maps of common bean

Chromosome	DOR364 x G19833		Total	BAT93 x JALO		Total	Grand Total
UCD (UF)	Gene-based	Genomic		Gene-based	Genomic		Mapped MS
b01 (h)	3	2	5	0	2	2	7
b02 (d)	5	8	13	4	0	4	17
b03 (c)	1	8	9	1	0	1	10
b04 (b)	9	1	10	0	3	3	13
b05 (e)	5	2	7	0	2	2	9
b06 (g)	1	3	4	0	3	3	7
b07 (a)	0	8	8	0	3	3	11
b08 (f)	1	3	4	0	1	1	5
b09 (k)	2	5	7	0	1	1	8
b10 (i)	0	4	4	0	2	2	6
b11 (j)	3	4	7	0	0	0	7
Average	2.7	4.4	7.1	0.5	1.5	2.0	9.1
Total	30	48	78	5	17	22	100

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Molecular Marker Assisted Selection Technique in Improvement of Multiple Disease Resistance in Common Bean: A Plant Breeder's Perspective

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Application of molecular marker assisted selection (MMAS) technique to cultivar/germplasm development excites plant breeders because it is a more effective and reliable tool for indirect selection for desired characteristics than the conventional technique. We applied MMAS technique for germplasm improvement for multiple disease resistance.. Disease resistance with corresponding SCAR markers used in our study were anthracnose (*Co-4* ^{2} gene, SAS13 marker), bean common mosaic virus (*I* gene with SW13 marker) and common bacterial blight (QTL genes derived from XAN 159, UBC420 marker). Single plants were selected from several sets of multiple crosses made to bring together all the gene sources for navy and red kidney beans. Some verification of the genes was done by the conventional screening tests. This project taught us a few lessons as plant breeders. This note presents our study conducted 1) to develop cultivar/germplasm carrying multiple disease resistance in navy and coloured beans by 2) applying molecular markers in crossing and selection, and 3) lessons learned about the suitability of MMAS technique in bean breeding.

Materials and Methods

Recurrent cultivars used were AC Compass, AC Cruiser, HR100 navy, AC Calmont DRK, AC ELK LRK, AC Harblack, HR106, AC Pintoba pinto and SVM Taylor Hort cranberry beans. **Germpasm** as donor parents of the disease resistance were HR45 or HR67 (both from XAN 159 source) and XAN159, OAC Rex (OAC 95-4, U. Guelph) for *Xanthomonas phaseoli*, Sel 1308 (provided by Dr. J. Kelly) for *Collectotrichum lindemuthianum* (*Co-4*^{2} gene) and bean common mosaic virus (BCMV) "*I*" gene from HR45 and HR67.

Molecular markers (SCAR) used for the diseases were UBC 420 for XAN159 source (Yu, et al. 2000), SAS13 for Co-*4*^{2} gene of Sel1308 (Young et al. 1998), SW13 for BCMV "I" gene (Haley et al. 1994; Melotto et al. 1996), and BC73 for CBB of OAC Rex (Bai et al. 1997).

Crosses were prepared initially in 1999 by using the above parental materials. For **navy bean**s, H4514 (HR67//Envoy/Sel 1308), H4515 (HR67//AC Compass/Sel1308), H4642, HR67/H4514; for **red kidney bean**, H4628 (Isle///Montcalm//(AC Darkid //Camelot/XAN159)/Sel1308), H4827 (AC ELK/H4628) and H4856 (AC Calmont/H4836), and other classes like cranberry, pinto and black beans crosses were similarly prepared.

Results and Discussion

Out of many crosses used in our bean program, a few representative navy and DRK crosses were tested for the molecular markers and screened for the respective disease resistance. About 60-72% of navy and 7-20% of DRK plants detected as positive UBC420 marker carriers were resistant to CBB (Table 1). Anthracnose marker (SAS13) carriers showed only 43% resistant plants and we experienced some difficulty in detecting the marker in red kidney bean (Table 1).

Molecular marker assisted selection technique (MMAS) is a useful tool for bean breeders, particularly when the target trait is controlled by qualitative genes or even a few QTL genes, and for pyramiding several traits. However, in the application of MMAS consideration

should be given to the following issues: a) polymorphism of parental lines (marker is cross specific); b) unclear expression of markers in some market classes (i.e. SAS13 marker for C0 4^2 gene in red kidney bean crosses). At this time, we are not sure if there is an effect of the origin of the bean type but it appears to be difficult to determine SAS13 markers for C0 4^2 gene in kidney and cranberry beans; c) false positive markers depending on association between target gene and marker (map distance); d) discrepancy between the presence of marker and target gene which requires testing the gene with conventional method (i.e. disease screening); and e) multiple QTL genes scattered on several linkage groups (QTL markers should be determined on the basis of good G x E trial data).

In addition to the above listed precautions**, good laboratory practices,** especially in carrying out crossing and progeny testing should be exercised as listed below: a) Probability of transmission of target genes gets smaller as number of genes increases, therefore, more hybrid seed are required to recover all the genes (at least 3 times gene transmission probability, considering false positive markers), b) not be too ambitious in planning (minimize no. of target cultivars in B/C and multiple crossing), c) good record-keeping is essential (as in pedigree system, remember it takes at least 4 B/C and progeny testing at end), d) conduct disease screening test concurrently (with MMAS) if possible to reduce risk of loosing genes in the course (phenotypic confirmation), e) toward end of B/C, large number of crossing to recover recurrent parental characteristics is required.

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Table 1. Brief summary of molecular markers and screening test results of a few navy and dark red kidney crosses during 1999-2003

Year/ Population		No. tested	SW13 (I)	UBC420 (CBB)	SAS13 (Co-4^2)
2000	H4514 navy	13F_1	10	3	7
	H4628 DRK	40 F_1	34	11	*
2001	H4642 navy	62 F_2 (452 pl. 3 marker carriers)			
	CBB test	45 $F_{2:3}$ (334 pl.)		72.5% CBB resistant	
	H4642	28 $F_{2:4}$ (342 pl.)	330	248	244
	CBB/ Anthracnose			61% R	43% R
2002	H4628 DRK	60 F_2 (280 pl.)	60	60	29
	CBB test			20% R	
2003	H4836 DRK	58 F_2 (all 2 markers, UBC420 and SAS13 carriers)			
	CBB test			7% R	0 **

*Experienced difficulty in detecting the marker, ** No or false positive marker

Mapping Quantitative Trait Loci for Green Bean Traits of Horticultural Importance

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Oregon Bush Blue Lake (BBL) green beans are uniquely different from other snap beans. They possess dark blue-green pods with very low fiber that process well but retain their quality even after long duration in a commercial kitchen. The plants are high yielding and have good cold tolerance during germination and emergence. However BBL beans have poor growth habit and potentially higher off-type frequency than snap beans of Midwest origin. Oregon BBL beans are difficult to recombine with other snap beans. For example, it took Frazier about 20 years to convert Blue Lake beans from pole to bush growth habit (Myers and Baggett, 1999). We initiated this research to determine why BBL beans were difficult to recombine with other snap beans, and how the process might be facilitated.

We determined from a phylogenetic molecular marker study that Oregon BBL cultivars are of Mesoamerican center of domestication (Davis & Myers, 2002). Not only do they possess S phaseolin, but also cluster with Mesoamerican dry beans compared to most other snap beans, which are predominantly from the Andean center of domestication. The objectives of the current work was to develop a molecular marker map for the BBL background and to identify molecular markers linked to important quantitative traits in green bean. We would use markers to facilitate introgression of desirable traits into the BBL background.

The recombinant inbred (RI) mapping population was developed from the cross 'Minuette' x OSU 5630 where 80 lines in the F_2:F_7 and subsequent generations were used. The Harris-Moran cultivar Minuette typifies Andean snap bean types. The population segregated for characters important to processing (pod length, color, shape, size, fiber, and shine [*ace*]), plant traits (height, branching angle and length, leaf size, and lodging resistance) as well as phaseolin seed storage protein (*phs*) and hybrid incompatibility between the two centers of origin (conditioned by *lcr*).

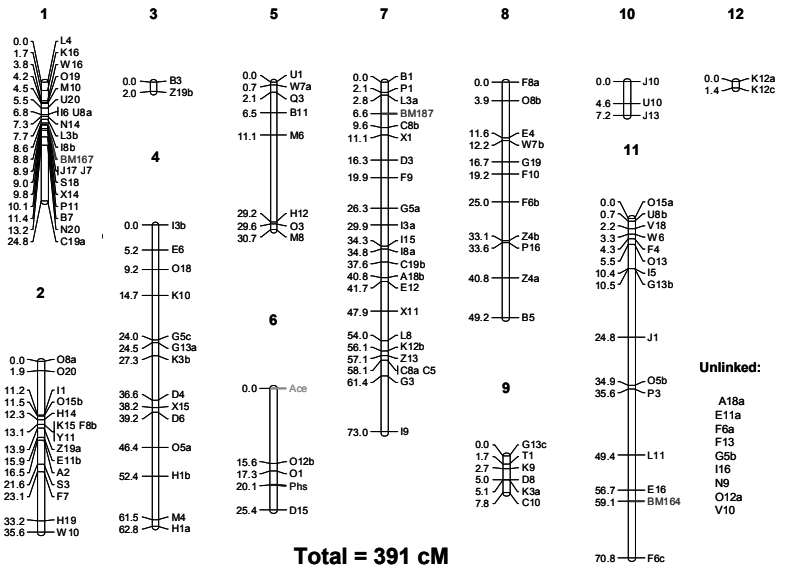

Figure 1. Linkage map from the cross Minuette x OSU 5630.

The population was evaluated in a trial (replicated twice) with pods from a single harvest being graded, processed and analyzed at the OSU Pilot Plant.

Of the three qualitatively scored traits, *ace*, and *phs* exhibited normal Mendelian segregation, but *lcr* did not fit any known segregation ratio. We discovered that *ace* and *phs* are loosely linked (Fig. 1). We also mapped *lcr* as a quantitative trait, tentatively placing it in linkage group 1 (Table 1). Much more transgressive segregation was observed in the RI population than expected, and in some

cases, was indicative of gene complementation. For example, both parents possess stringless pods, but some progeny had partial to full strings. Both parents have round pods, but progeny segregated for oval to creaseback pod crossectional shape. With the exception of percent sieve size distribution, most traits showed normal distributions, but often exceeding the parental values. Percent sieve size showed a bimodal distribution suggesting major gene control of this trait.

Table 1. MQM QTL analysis of major quantitative trait loci segregating in an RI population from the cross Minuette x OSU 5630.

Quantitative Trait*	Linkage Group	% V_G Explained
Stem thickness	5	22.4
Pod length	11	30.9
Pod width	7	37.7
Pod height	7	22.2
Pod straightness	11	16.3
Pod strings	7	25.9
lcr (LOD = 2.69)	1	14.8
Mean sieve distn.	6, 7	15.7, 42.8
Pod distn. skewness	7	41.8
%1 - 4 sieve size	6, 7	8.9, 57.5
% 1-3 sieve size	6, 7	22.8, 20.9
Color- L (LOD = 2.98)	7	18.4
Color- b (LOD = 2.86)	4	17.6

*LOD 3.0 (unless indicated otherwise)

For the map, 77 progeny, and 133 markers (predominantly RAPD with three microsatellite markers) were used. The map was constructed with Joinmap 3.0 using a minimum LOD of 3 and a maximum linkage distance of 30.0 cM. In all, 124 loci in 12 linkage groups were placed for a total length of 391 cM. Two microsatellites were mapped. One of these (BM 164) has been placed on the common bean consensus map (Blair et al., 2003). Table 2 shows tentative correspondence between the Oregon snap bean map and the common bean consensus map as published in Blair et al. (2003). Linkage group matching was based on microsatellite markers and where two to several RAPD markers of the same molecular weight corresponded. QTL for 13 traits were placed on the snap bean map. A QTL with major effect on a number of traits was observed on linkage group 7. Several of the traits at this location are involved with fiber, such that the QTL may represent gene(s) affecting lignin biosynthesis. Study of this QTL may shed light on the nature of oval and stringy podded off-types. Overall, the large amount of transgressive segregation suggests that co-adapted gene complexes for snap bean phenotype may be associated with different centers. Such a hypothesis could explain the difficulty of introgressing traits from Andean snap beans into Mesoamerican BBL types.

Table 2. Putative correspondence between consensus map and Oregon snap bean map based on common microsatellite and RAPD markers.

Consensus map	Oregon map
Linkage Group	
2	11
3	9
4	8
5	2
6	7
7	6

References:

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Cloning Genes for Secondary Metabolites that Affect Seed Colour, Plant Defense, Nodulation and Human Health in Beans

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Because of its low fat/high protein content, dietary fiber, complex carbohydrates and vitamins including folic acid, the dry bean is characterized as the nearly perfect food. It also contains dietary phytoestrogens, secondary metabolites such as isoflavonoids and lignans, which may have significant impacts on human health by preventing some types of cancer, cardiovascular disease, osteoporosis and menopausal symptoms.

Secondary metabolites are compounds that are restricted to a specific plant species or specific plant organs that participate in interactions between the plant and its environment. A major class of secondary metabolites are the phenylpropanoids. Plants produce more than 8000 phenylpropanoids, which are derived from the amino acid phenylalanine through the action of various enzymes in the phenypropanoid pathway.

These phytochemicals play significant roles in the bean plant, including: to give seed coats their colours, signaling nitrogen fixing bacteria in the initiation of nodules, as defense compounds against a variety of pathogens and as UV sun screens.

Selection and/or molecular manipulation for increased levels of these compounds in bean requires information about the genes that control their synthesis. Although a great deal of information exists in other species, only a few gene sequences for genes that code for enzymes and regulatory molecules in the phenylpropanoid pathway were available for bean at the beginning of the current study.

Our objective in the current work was to clone, sequence and map approximately 30 structural and regulatory genes in the phenylpropanoid pathway in bean. This information will be used to assay the activity of the genes coding for phenylpropanoid pathway enzymes and regulatory proteins in a variety of beans using DNA microchip technology.

Since the information on bean phenylpropanoid pathway gene sequences is fragmentary, literature and databases were searched for the appropriate gene sequences from related species such as soybean, *Phaseolus coccineus* and *Medicago sativa.* The sequences were aligned and the conserved regions were used to design PCR primers. The primers were used in RT-PCRs with bean seedling RNA to amplify fragments of a number of structural genes coding for enzymes of the general, lignin/lignan and flavonoid branches of the phenylpropanoid pathway (including: PAL2, PAL3, C4H, 4CL-1, 4CL-2, COMT, LAC, IFS, CHS, CHI, DFR, F3H and F3'H; Fig 1). From thirty-seven primer sets for structural genes 23 gave PCR products that gave sequence that matched the expected gene; but only 5 out of 31 PCR primers designed for regulatory genes were successful. The construction of a test microarray of bean phenylpropanoid genes is underway. The array will be used to screen bean germplasm for variation in the levels of genes in the phenylpropanoid pathway.

Cloned Phenylpropanoid Gene Fragments in Bean

Enzyme	Gene	Source sequence			PCR product (bp)
		species	accession number	source	
Phenylalanine ammonia lyase	PAL2	*P. vulgaris*	P19142	DNA	398
	PAL3	*P. vulgaris*	P19143	DNA	397
Cinnamate 4-hydroxylase C4H	*P. vulgaris*	Y09447	mRNA		359
4-coumarate CoA ligase	4CL-1	*G. max*	AF279267	mRNA	545
	4CL-2	*G. max*	AF002259	mRNA	402
	4CL-3	*G. max*	AF002258	mRNA	550
Cinnamoyl CoA reductase CCR	*G. max*	BI426824	EST		447
Cinnamyl-alcohol dehydrogenase	CAD	*M. sativa*	L46856	mRNA	306
Caffeate O-methyltransferase	COMT	*M. sativa*	M63853	mRNA	392
Ferulate 5-hydroxylase	F5H	*G. max*	BM527849	EST	468
Laccase	LAC	*G. max*	AF527604	DNA	492
Lignin peroxidase	FBP4	*P. vulgaris*	AF149279	mRNA	695
Chalcone synthase	CHS	*P. vulgaris*	X06411	mRNA	778
Chalcone isomerase	CHI	*P. vulgaris*	Z15046	DNA	215
Flavanone 3-hydroxylase	F3H	*M. sativa*	X78994	DNA	550
Flavonoid 3'-hydroxylase F3'H	*G. max*	AB061212	mRNA		800
Dihydroflavonol 4-reductase	DFR	G. *max*	AF167556	mRNA	800
Leucoanthocyanidin reductase	LAR	*M. trunc*	AY184243	mRNA	800
2-hydroxyisoflavanone synthase	IFS	*G. max*	AF195818	DNA	535
7-O-methyltransferase	IOMT	*M. sativa*	AF000975	mRNA	404
Isoflavanone reductase	IFR	*M. sativa*	U17436	DNA	1400
Anthocyanin 5-acyltransferase	AAT	*P. cocc*	CA900148	EST	407
Vacuolar transporter	VT	*P. cocc*	CA907034	EST	453
LIM domain protein WLIM1 (lignin)	LIM1	*G. max*	BU764417	EST	1350
R2R3-MYB trans-factor AtMyb4	Myb4	*P. cocc*	CA902489	EST	315
homeodomain protein, GL2 like 1	HD-GL2	*P. cocc*	CA902455	EST	324
R2R3-MYB trans-factor AtMYB15	Myb15	*P. cocc*	CA902486	EST	600
trans factor KAP -2 (CHS)	KAP2	*P. vulgaris*	AF293344	mRNA	504

We anticipate that the information will accelerate and simplify breeding for increased levels of phenylpropanoid compounds in bean that might benefit human health and play important roles in the seed coat colour, disease resistance, stress tolerance and nitrogen fixation.

Potential Application of TRAP Markers for Tagging Disease Resistance Traits in Common Bean

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The TRAP (Target Region Amplified Polymorphism) technique is a simple but powerful PCR-based system useful for generating polymorphic markers around targeted candidate gene sequences (Hu and Vick, 2003). TRAPs for disease resistance genes in common bean were targeted in this study.

TRAPs are amplified by one fixed primer designed from a target EST sequence in the database, and a second arbitrary primer designed to anneal with either an intron or exon sequence. The fixed primers were selected by using the web-based PCR primer designing program "Primer 3" with the following parameters: primer optimum, maximum, and minimum sizes set at 18 nt; and primer optimum, maximum, and minimum T_m at 53°C, 55°C, and 50°C, respectively. Fixed primers were either designed against the sequenced ESTs homologous to disease resistance genes in the Compositae Genomics database (Michelmore, personal communication, 2002), or against sequenced RGAs from common bean in the National Center for Biotechnology Information database.

For development of arbitrary primers the general principles of PCR primer design were upheld such as to avoid self-complementarity and maintain proper GC content (40 to 60%). In addition, the following three parts were incorporated in each arbitrary primer: (1) the selective nucleotides, 3 to 4 nts at the 3' end, (2) the "core", 4 to 6 nts with AT or GC rich regions, and (3) the filler sequences which make-up the 5' end (Li and Quiros 2001). The arbitrary primers were 3' end-labeled with IR dye 700 or IR dye 800 for autodetection by the Li-Cor Global DNA Sequencer, or 5' end-labeled with 5-FAM dye for autodetection by the ABI Avant 3100 DNA sequencer.

PCR was conducted with a final reaction volume of 15 µl in 96-well microtiter plates with the following components: 2 µl of the 30 to 50 ng/µl DNA sample, 1.5 µl of 10X reaction buffer (Qiagen), 1.5 µl of 25 mM $MgCl_2$, 1 µl of 5 mM dNTPs, 3 pmol each of 700- and 800-IR dye labeled random primers, 10 nmol of the fixed primer, and 1.5 units of *Taq* DNA polymerase (Qiagen). The PCR was performed by initially denaturing template DNA at 94°C for 2 min; then 5 cycles at 94°C for 45 s, 35°C for 45 s and 72°C for 1 min; followed by 35 cycles at 94°C for 45 s, 50°C for 45 s and 72°C for 1 min; then a final extension step at 72°C for 7 min.

Two mapping population were used to generate and map TRAP markers. The RIL (recombinant inbred line) mapping population BAT 93/Jalo EEP 558 (BJ) was obtained from P. Gepts (University of California-Davis). The BJ population has been widely used to integrate common bean linkage maps (Freyre et al. 1998; Kelly et al. 2003). The Dorado/XAN 176 RIL (DX) population was obtained from J. Beaver (Univ. of Puerto Rico-Mayaguez) and has been used previously to map loci conditioning resistance to diseases caused by bacterial, fungal, and viral pathogens in common bean (Miklas et al. 2000).

For the DX population 21 TRAPs were generated from eight multiplex PCR reactions (1.3 TRAPs per reaction). This low level of polymorphism is due to the relatedness of the Dorado and XAN 176 parents, both predominately of Race Mesoamerican origin. Conversely, for the BJ population derived from a wide cross between parents from the Middle American and Andean gene pools, 107 TRAPs were generated from eleven PCR reactions (9.7 TRAPs per reaction: four multiplex and three simplex).

For the 21 TRAPs in DX, eight were unlinked, four incorporated into existing linkage groups, and nine formed three linkage groups of 2, 3, and 4 TRAPs. None of the TRAPs were associated with previously identified QTL (eight of them) conditioning resistance to common bacterial blight, bean golden yellow mosaic virus (BGYMV), or ashy stem blight, or two resistance genes for rust previously mapped (Miklas et al., 2000). However, all three of the partial linkage groups consisting of just TRAP markers and two of the unlinked TRAP markers detected new QTL as listed here:

TRAP	Linkage group	Common blight	BGYMV	Ashy stem blight	Web blight
G64.680	partial-2	14.5 *		14.0 **	
F64.405	partial-2				16.0 *
H64.245	partial-3	30.0 **	8.2 *		
B64.230	partial-4				15.7 *
F64.770	partial-4	9.5 *			
G64.700	unlinked		9.6 **		
G64.345	unlinked		9.8 *	15.9 **	

R^2 value for amount of phenotypic variation explained followed by level of Significance: $P < 0.05$ or $P < 0.01$ for * and **, respectively.

For the BJ population, 88 of the 107 TRAPs mapped to the eleven linkage groups, ranging from 3 to 12 per linkage group. The TRAPs tended to cluster and some mapped in the vicinity of resistance gene loci (data not shown). Overall, these preliminary data suggest that TRAPs will be useful for tagging and mapping disease resistance traits in common bean.

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Identification of a RAPD Marker Linked with Resistance to *Beet Curly Top Virus* in Common Bean

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Introduction. Beet curly top virus (BCTV) is a member of the family *Geminiviridae,* genus *Curtovirus* and is a persistent disease problem for bean production in the Pacific Northwest region and other dry areas where the virus and its leafhopper vector (*Circulifer tenellus*) is present. Breeding for resistance to BCTV has been difficult because screening of selected lines in the field results in usually sporadic non-uniform infections across and within test plots. These difficulties support the development and use of marker-assisted selection (MAS) for developing curly top disease-resistant bean cultivars in the absence of the pathogen. Hence, our objectives were to identify tightly linked DNA markers with application for MAS of the resistance gene(s), and locate the gene(s) on the molecular map.

Materials and Methods. Ninety-four F_5-derived F_7 ($F_{5:7}$) RILs were obtained from a cross between susceptible snap bean cultivar Primo and the highly resistant Moncayo. Utilizing a randomized complete block design, replicated field experiments consisting of Primo, Moncayo, the 94 $F_{5:7}$ RILs, and Taylor Horticultural cranberry bean included as susceptible check, were planted in three locations in the Columbia Basin region of Washington State in 2002. Once occurrence of a severe BCTV epidemic was evident at Prosser, one F_1 seed from Primo/Moncayo and five residual F_1 seed from the reciprocal cross Moncayo/Primo were germinated in individual pots in the greenhouse and subsequently transplanted to the field at the primary leaf stage. Because none of the plants expressed intermediate reactions to infection with BCTV, individuals were rated either resistant or susceptible. The presence or absence of infection by BCTV in test plants was verified by DAS-ELISA.
DNAs bulked from eight BCTV-resistant and eight susceptible RILs, respectively, were extracted from bean leaves at the first trifoliate stage using FastDNA spin columns (BIO 101, Vista, CA). After adjusting DNA concentrations to 10 ng/µl, 750 random decamer primers (Operon Technologies, Inc. Alameda, CA) were screened for RAPD DNA markers detected as amplified fragments present in one bulk but absent in the other as viewed on agarose gels. RAPD markers that were present across all R but not S RILs were cloned and SCAR primers designed based on the RAPD sequence information (1).

Results and Discussion. Primo, Taylor Horticulture and 29 $F_{5:7}$ RILs from Primo/Moncayo exhibited severe curly top symptoms at the seedling stage in the field. Moncayo and 65 $F_{5:7}$ RILs from the Primo/Moncayo cross showed no symptoms and were categorized as resistant. All six F_1 plants (Primo/Moncayo and reciprocal) exhibited no disease symptoms indicating that resistance was dominant. The tentative symbol *Bct* was assigned to this resistance allele given its dominant expression in the F_1 generation and ultimate derivation of the dominant resistance gene in Burtner as described by Schultz and Dean (2)

Three dominant RAPD markers, AS8.1550, AH10.950, and I14.1700, detected between the resistant and susceptible bulked DNAs had greater than 85% cosegregation with disease reaction among individual RILs comprising the bulks. Mapped across the entire population of 94 $F_{5:7}$ RILs, the three RAPDs were tightly linked in coupling (*cis*) with a single locus conditioning resistance to BCTV (Fig. 1). SCAR marker SAS.1550 mapped within a cluster of disease resistance genes on linkage group B7 of the core map. The SCAR marker assay matched the known phenotype for 15 of the 16 cranberry, kidney, and miscellaneous dry beans surveyed of Andean origin. Although limited in survey size, all of the representative dry beans sampled of Middle American origin possessed the marker regardless of disease reaction.

The proximity of the begomovirus *Bean golden yellow mosaic* (BGYMV) QTL to *Bct* on the *Phaseolus* linkage map suggests that *Bct* may have a pleiotrophic effect by conditioning partial resistance

to other geminiviruses. In addition to *Bct* and BGYMV resistances, a major gene for resistance to anthracnose (*Colletrotrichum lindemuthianum*), and QTL for resistance to common bacterial blight (*Xanthomonas axonopodis phaseoli*), white mold (*Sclerotinia sclerotiorum*), and ashy stem blight (*Macrophomina phaseolina*) map in the same region of B7, which supports the presence of a resistance-gene cluster in this region of B7 (Fig. 1).

The ubiquitous presence of SAS.1550 in resistant Middle American germplasm indicates that MAS of *Bct* using this SCAR marker will be limited to the Andean gene pool, fortunately, where it is most needed. The SCAR will be applicable for high throughput marker detection systems. Therefore, marker-assisted selection for *Bct* resistance to BCTV using the SAS8.1550 marker should considerably reduce the time currently required for screening germplasm using field trials or *Agrobacterium*-mediated inoculation, and expedite development of resistant cultivars.

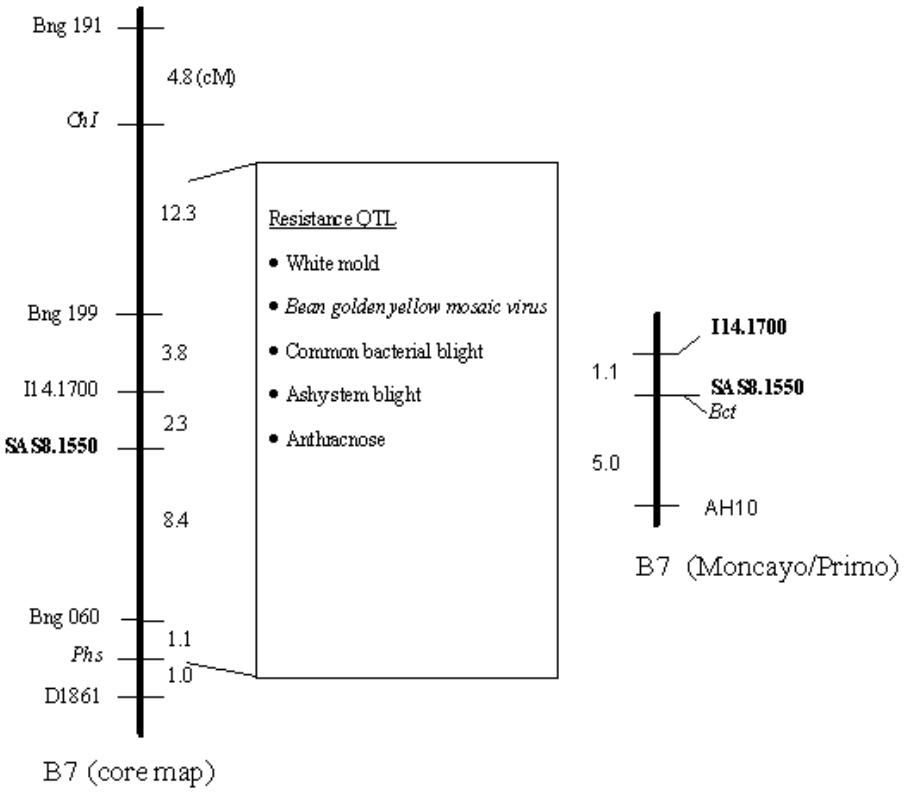

Figure 1. Partial linkage group of SCAR SAS8.1550, RAPDs AH10.950 and I14.1700, and the dominant allele *Bct* conferring resistance to *Beet curly top virus*; *and* position of I14.1700 RAPD and SAS8.1550 SCAR markers on linkage group B7 of the core map. Quantitative trait loci (QTL) conditioning resistance to bacterial, fungal, and viral pathogens and a major allele for anthracnose resistance map within the region shown (box insert).

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IDENTIFICATION AND MAPPING BEAN ROOT ROT RESISTANCE IN AN 'EAGLE X PUEBLA 152' POPULATION[1]

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Introduction

Root rot is a major constraint of snap bean production in the Central Sands of Wisconsin, where snap beans are grown on irrigated, well-drained soils. Crop rotation has traditionally been the only control for this disease. *Pythium ultimum* and *Aphanomyces euteiches* f. sp. *phaseoli* are the most damaging root rot pathogens in the region (Pfender and Hagedorn, 1982). *Pythium* is important at temperatures under 20ºC, while *Aphanomyces* causes damage between 16-24ºC; when combined, they cause more damage than individually (Pfender and Hagedorn, 1982). Development of resistant varieties is the best long-term solution to root rot. Our objective is to identify lines and molecular markers associated with resistance to facilitate breeding programs.

Materials and Methods

Plant Material and Design: 72 lines of an Eagle x Puebla 152 (EP) recombinant inbred population were evaluated for root rot resistance in the summers of 2001-2003 at the Hancock ARS, in Central Wisconsin. Eagle is an Andean, root rot susceptible snap bean variety. Puebla 152 is a resistant Mesoamerican black bean cultivar. A blocks within replication design planting lines in single row of 1.52m (22 plants) separated 0.91m was used. The plot was planted with beans since 1991 for high and uniform inoculum level. *Pythium* and *Aphanomyces* have been consistently associated to root rot at the test site.

Disease Evaluation: Each year, three replicates of each line were rated 17 days after planting (E_1 for vigor and emergence), and at flowering time (E_2 for vigor) using a 1-9 scale. A severity index (DSI) was computed to represent the importance of this disease in causing loss at emergence and decreased plant vigor. The DSI was equal to $0.6E_1 + 0.4E_2$; if the resulting DSI was lower than E_1, then E_1 was used as the DSI of that plot.

Marker Analysis: Quantitative trait analyses (QTL) were done using an Eagle x Puebla 152 molecular map previously developed in our laboratory by Skroch (1998) and by using composite interval mapping (Zeng, 1994). QTL analyses on transformed DSI (Box and Cox, 1964) were done using Windows QTL Cartographer (Wang et al., 2003).

Results and Discussion

Test site uniformity was demonstrated by low experimental errors compared to the lines (blocks) source of variance for the $DSI^{1/2}$ analyzed each year (Table 1). The design employed explained 89% of the variability for $DSI^{1/2}$ in 2001. Eagle, Puebla 152 and the check varieties performed consistently to their expected root rot reactions over years (Fig.1). A set of EP showed high resistance (low DSI, see Fig. 1). Variation among EP lines for plant architecture and poor snap bean quality traits make the choice of a line for introgression of root rot resistance to snap bean varieties a compromise.

[1] This research was possible thanks to the financial support from the Federal Multistate (W150) HATCH projects W1504257 and W1503897, and grants from the MidWest Food Processors Association, Inc.

Fig.1 Root rot severity index (DSI) for Eagle x Puebla 152 lines averaged over 2001-2003

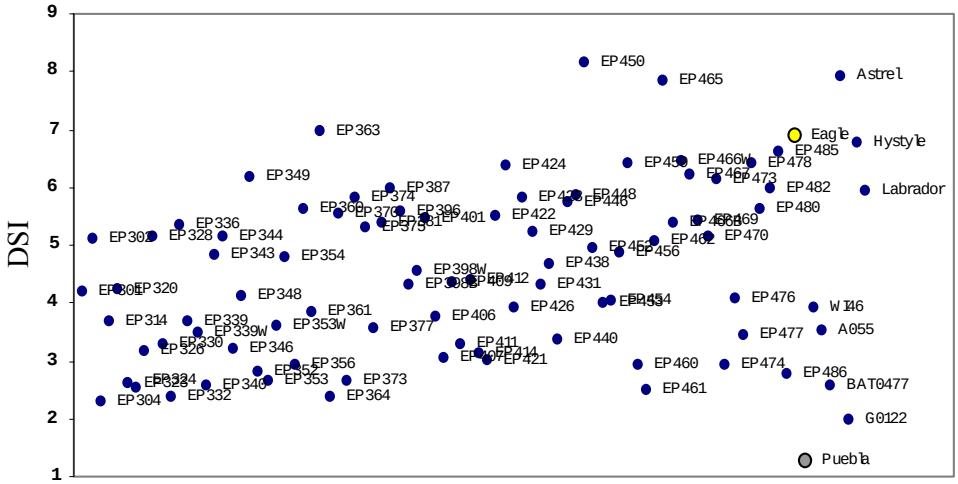

Table 1. Mean squares of analyses of variance for DSI[1/2], Eagle x Puebla 152, 2001-2003

Sources of Variation	Mean Square 2001	Mean Square 2002	Mean Square 2003
reps	0.014^{NS}	0.354^{NS}	1.109*
blocks(reps)	0.752**	0.272^{NS}	1.984^{NS}
lines(blocks)	0.617**	0.642**	4.247**
Exp. error	0.048	0.179	0.476
R^2	0.89	0.68	0.83

Table 2. Best supported root rot marker-QTL associations, Hancock, WI ARS

Year	LG	Flanking RAPD Marker	LOD Score	R^2
2001	6	S18.1500/ AD09.950	10.2	0.46
2003	6	AD09.1050	2.4	0.12
2001	6	010.650	2.4	0.06
2002	6	010.650	4.1	0.18
Other candidate QTL				
2003	3	AD04.1000	2.7	0.14
2002	7	F08.1250	5.2	0.26
2002	7	AM13.350	4.2	0.22

Note: in Table 2, boldfaced marker is a Puebla 152 marker for resistance, others are inherited from Eagle. LG= linkage group

The most consistent marker-QTL associations for root rot resistance were found in linkage group 6 (Table 2). In 2001, S18.1500 and AD09.950 were linked to a QTL that explained 46% of the variation for root rot, with a high LOD score. AD09.950 is a marker for root rot resistance inherited from Puebla 152 that co-segregates with S18.1500 inherited from Eagle. Other markers from linkage groups 6, 3 and 7 are also significantly associated with variation for resistance. The use of germplasm and markers associated with *Pythium* and *Aphanomyces* resistance may represent tools for improving beans.

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INTRON-BASED SEQUENCE DIVERSITY STUDIES IN *PHASEOLUS*

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Molecular markers are useful for diversity studies in plants. In turn, diversity studies can address agrocentric interests, such as identifying variable parents for hybridization or fingerprinting cultivars. Diversity studies can also address such evocentric interests as describing the genetic architecture of a species and the relationship of that architecture to the organization of diversity in the genus. In particular, such studies have shown that *P. vulgaris* landrace diversity is a subset of that found in the species (Gepts and Bliss 1986; Tohme et al. 1996), that common bean contains two major genepools (Gepts and Bliss 1986; Kami and Gepts 1994), and that these genepools are divided into races (Singh et al. 1991; Beebe et al. 2001. At the genus level, diversity studies have demonstrated the monophyletic origin of the genus *Phaseolus* (Delgado-Salinas et al. 1999).

To date, most of the diversity studies have relied upon molecular marker systems such as proteins (Gepts and Bliss 1986), RFLP (Becerra Velasques and Gepts 1994), RAPD (Beebe et al. 2001), and AFLP (Tohme et al. 1996). Yet DNA sequence analysis has an advantage over traditional marker studies because it can uncover rare genomic changes that can facilitate both shallow and deep diversity studies. To date, sequence-based diversity studies have been limited in common bean (Kami and Gepts 1994; Kami et al 1995; Rivkin et al. 1999; Vallad et al. 2001). The target sequence for diversity studies can be either exons or introns. In general, introns are a rich source of variation that can potentially uncover structure within races and reveal ancestral relationships that have previously gone unrecognized. From a population genetics perspective, such rich variation can address questions such as the roles of selection and mutation/drift in developing adaptive variation, the extent of recombination in the history in the species, and the degree of linkage disequilibrium along the length of the chromosomes within the genome.

We have recently begun using intron sequence data to study variation within *Phaseolus*. Our standard approach is to select a gene of interest, obtain the corresponding sequence data from *Glycine*, *Medicago*, and *Arabidopsis*, identify conserved nucleotide sequences, align the sequences with the genomic (exon+intron) sequence of the *Arabidopsis*, and design primers that span the intron space. The feasibility of this approach is based upon the observation that for all genes that we have studied (including but not limited to dihydroflavonol reductase, chalcone isomerase, anthocyanin synthase, and flavonol 3' hydroxylase), the exon/intron borders are conserved between *Arabidopsis* and all other species for which genomic sequence data is available. The primers are used to amplify DNA fragments that are directly sequenced. If the fragment proves polymorphic, it is cloned, and multiple clones are sequenced.

A recent publication (McClean et al. 2004) describes variation at intron one of dihydroflavonol reductase (DFR) among 95 heirloom or ancestral varieties, ancestors of modern cultivars, modern elite cultivars, current snap beans, mapping parents, and landraces that represent all of the common bean races. It was observed that cultivar and landrace diversity was equal, while Middle America landrace diversity was greater than among Andean landraces. Several test statistics suggest that selection was acting upon the intron in the Middle America

gene pool. Further, it was discovered that race Durango and Jalisco genotypes are monomorphic whereas the greatest variability was noted among race Mesoamerica genotypes. Finally, signature recombination events suggest that an ancestral population existed that contained the variability currently observed in the Middle America and Andean gene pools.

We report here the application of sequence data at the DFR intron 1 locus to study relationships within the *Phaseolus* genus. We investigated 31 samples in addition to the *P. vulgaris* genotypes described above. This included 18 *Phaseolus* species that represented all nine clades defined using rDNA data (Delgado-Salinas et al. 1999). *Macroptilium erythroloma* was included for comparative purposes. Our first observation is that the intron is modular in nature. All species contain conserved a 21 nt 5' and a 11 nt 3' module. Internal to these modules, *P. vulgaris*, *P. dumosus*, and some *P. coccineus* genotypes contain two copies of an element ~175 nt in length. The other *P. coccineus* genotypes only contain a single copy of this element. All of the remainder of the species in this study lacked this element. Further, *P. glabellus*, *P. grayans*, *P. microcarpus*, *P. xanthorichus*, *P. angustissimus*, *P. augustii, P. leptostachyus*, *P. lunatus*, *P. maculatus, P. micranthus*, *P. parvalus*, *P. polystachios*, and *P. ritensis* contain a related element between 66 and 96 nt in size. *P. actuifolius* and *M. erytholoma* were uniquely different from each other and from all other species in the analysis. Neighbor-joining cluster analysis strongly supported a relationship between Middle America *P. vulgaris* landraces and *P. coccineus*. Additionally, clustering of *P. dumosus* with this clade is highly supported. The *P. vulgaris* Andean landraces are only distantly related to this large clade. Among the species without the repeated element, a Microcarpus group consisting of *P. glabellus*, *P. grayanus*, *P. microcarpus*, and *P. xanthorichus* was highly supported. Finally, a *P.lunatus* and *P. polystachios* group was supported.

To further test this model, we analyzed diversity at intron 3 of chalcone isomerase among the same set of species. All of the relationships described for DFR intron 1 were observed using data from this intron except one. In that case, support for the Microcarpus group was not observed. Thes results suggest that gene trees at other genes are necessary to better understand the relationship among *Phaseolus* species. The results also point to the value of intron data for diversity studies in *Phaseolus*.

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A Preliminary Molecular Marker Map for *Phaseolus coccineus.*

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Within *Phaseolus*, the scarlet runner bean (*P. coccineus*) has highest levels of white mold resistance. This species is in the secondary gene pool for common bean, and it is possible to introgress genes without using extraordinary measures. Previous researchers have partially transferred resistance, but the resulting germplasm appears not to have been widely used to develop elite common bean cultivars. There are several possible reasons: accessions used were not the most resistant, resistance is quantitatively controlled and not all resistance factors were transferred, and linkage drag hindered transfer. Molecular tools integrated into a breeding program provide new avenues through which the genetic architecture of white mold resistance can be understood and transferred.

Following a screen of the *P. coccineus* USDA Plant introduction germplasm collection (Gilmore et al., 2002), we focused on a few accessions, including PI 255956. This accession has high levels white mold resistance and has been analyzed for physiological mechanisms of resistance. We crossed PI 255956 to the white mold susceptible 'Wolven Pole' *P. coccineus* parent to examine inheritance in the F_2 generation. We intended initially to use bulked segregant analysis to identify molecular markers linked to resistance, but it became apparent from the distribution of progeny (Fig. 1) that resistance was inherited quantitatively. We then created a molecular marker map to place quantitative trait loci (QTL) for resistance.

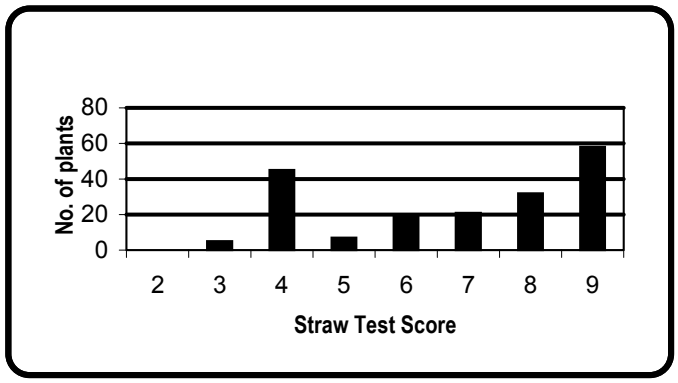

Figure 1. Disease reaction of an F_2 population of 188 individuals from the cross PI 255956 (R) x Woven Pole (S) when inoculated with white mold in the greenhouse. Scale of 1 to 9 where 1 = immune, and 9 = dead. (A score of 4 or less indicates that *Sclerotinia* mycelia failed to penetrate a node.)

Resistance of the parents and F_2 progeny was assessed using the straw test (Petzoldt & Dickson, 1996) except plants were read after four weeks rather than eight days. Resistant individuals were retested and disease was allowed to progress for another four weeks to identify escapes. DNA was isolated as described by Kobayshi et al., (2000). Random Amplified Polymorphic DNA (RAPD) markers were generated using protocols described by Myers et al. (2004). Bean microsatellite markers (Gaitan-Solis et al., 2002) were also used. Microsatellite primers were synthesized by the MWG-Biotech and PCR amplification protocols of Gaitan-Solis et al. (2002) were used. The scaffold map was constructed in Joinmap (Van Ooijen & Voorrips, 2001) and Windows QTL Cartographer (Basten et al., 2003) was used to place QTL for white mold resistance on the scaffold map.

PI 255956 had a straw test score of 4 while Wolven Pole was 5. The F_1 had a straw test score of 4. Forty-eight F_2 progeny had scores of 4 or less while 144 F_2s had scores greater than 4. The Wolven Pole x PI 255956 map was constructed from an F_2 of 188 individuals. From an initial screen of 600 RAPD primers, 111 were used in the total population. Twenty-four of the 111

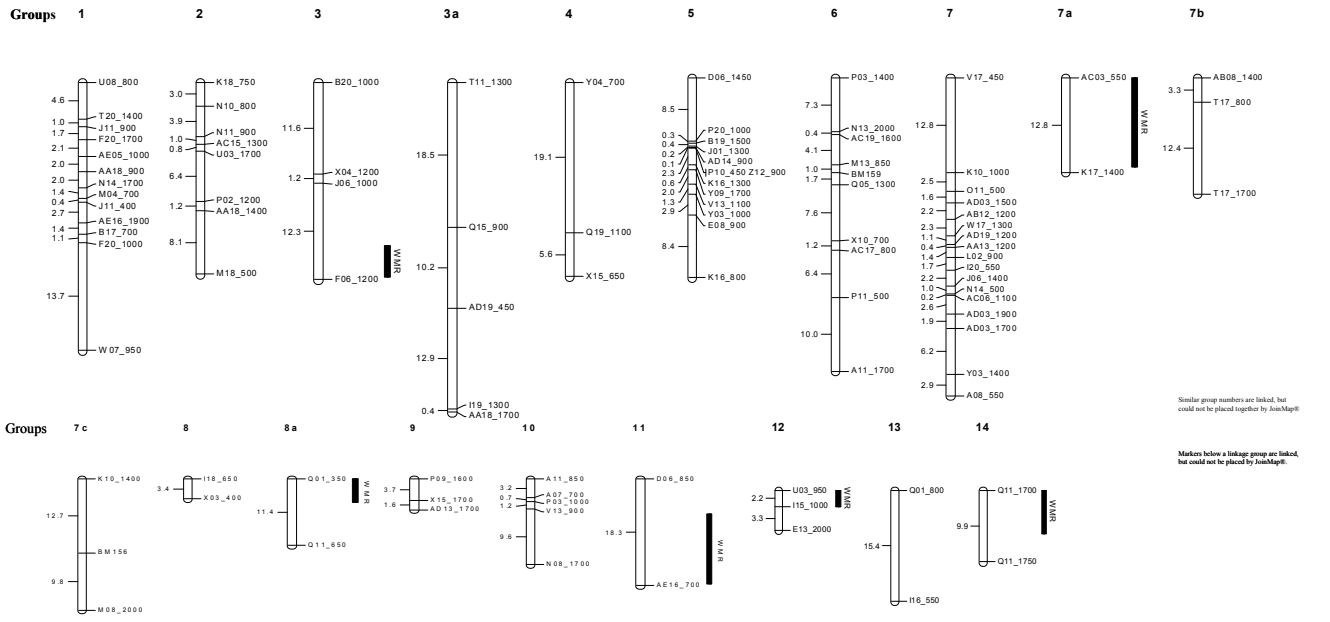

Figure 2. RAPD and microsatellite based linkage map for *P. coccineus* from the cross Wolven Pole x PI 255956. QTL for white mold resistance are indicated by black bars to right of linkage groups.

primers were used to screen 188 individuals and the F_1 yielded 28 markers. Eighty-seven primers screened with 94 individuals and F_1 yielded an additional 92 markers. Three microsatellite marker primers produced polymorphic bands when tested with the two parents, the F_2 population of 92 individuals and F_1. The map has 102 linked RAPD and microsatellite markers on 14 linkage groups for a total length of 395 cM (Fig. 2). Ten markers are unlinked including one microsatellite. Interval mapping in QTL Cartographer revealed six statistically significant (LOD $\geq$ 4.0) QTL on linkage groups 3, 7, 8, 11, 12, & 14 (Fig. 2). Percent additive genetic variation explained by QTL individually range from 9 to 12%), while in combination they explain 63%. The two microsatellites mapped here have been placed on the *P. vulgaris* consensus map with our linkage groups 6 and 7c corresponding to consensus linkage groups 2 and 3, respectively.

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Barriers to Interspecific Hybridization in *Phaseolus* Backcrosses

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Introduction

In high latitudes and high altitudes, periods of low, but above zero temperatures and frosts during the growing season are the major abiotic constraints to common bean (*Phaseolus vulgaris*) production. *Phaseolus angustissimus*, a species of the tertiary gene pool is resistant to both spring and fall frosts (Balasubramanian et al. 2003). Seedlings of *P. angustissimus* at the third trifoliolate growth stage had a percent survival of 89 in response to a fall frost (–5^{o}C), and 55 in response to a spring frost (–7^{o}C). Frost resistance, if successfully introgressed into common bean germplasm, may reduce the risk of bean seedling death on the Canadian prairies and expand the distribution of the bean crop, possibly to higher altitudes in the tropics. The objectives of this study were to develop F_1 interspecific hybrids of *P. vulgaris* with *P. angustissimus*, and investigate barriers to hybridization in the backcross of the F_1 interspecific hybrids to parents.

Materials and Methods

1. Development of the F_1 interspecific hybrids: Dry bean cultivars ICA Pijao and CDC Nighthawk, a breeding line 5-593, *P. vulgaris* var. *mexicanus* (G11024) and *P. vulgaris* var. aborigineus (PI 266910) were crossed as female to *P. angustissimus* (PI 535272). Reciprocal crosses were unsuccessful. Aborting embryos were rescued at 18 to 20 days after pollination. Embryos were cultured in three-quarter strength MS medium supplemented with 0.125 µM benzyladenine, 0.7 µM glutamine, 0.8% (w/v) agar and 3% (w/v) sucrose. Embryos were incubated in dark and then transferred to full-strength MS medium at 23/18^{o}C (12 h photoperiod). Vigorously growing plants were transferred to pots containing Redi-earth and gradually acclimated to ambient growing conditions over a period of seven days. Number of pollination, number of pods, number of pods with at least one culturable embryo, number of embryos cultured and number of plants acclimated were determined. Data were subjected to the chi-square test of independence of proportions.

2. Cytogenetic characterization of the F_1 interspecific hybrids: Pollen fertility was assessed by acetocarmine staining. Also, flower buds at the appropriate development stage were fixed and stained, and chromosome pairing in the pollen mother cells was observed under the microscope.

3. Barriers to interspecific hybridization in the backcross of the F_1 interspecific hybrids:

Pollen tube growth: Surface sterilized flower buds of the F_1 interspecific hybrids were either selfed or crossed in-vitro, as female, to ICA Pijao and *P. angustissimus,* in petri plates containing the L-6 medium (Mallikarjuna 1999) supplemented with 0.3% (w/v) phytagel. Ten pistils for each self and cross combination were fixed at 24 h after pollination and observed under the microscope for pollen tube germination and growth.

Embryo Development: Experimental procedure was the same as above except that pistils were fixed at 24, 48, 72 and 96 h after pollination. Fixed pistils were subjected to paraffin sectioning, stained and observed under the microscope. Pistils of ICA Pijao and *P. angustissimus*, selfed in-vitro were the controls. Ten pistils were sectioned for each treatment combination.

Results and Discussion

1. Development of the F_1 interspecific hybrids: The chi-square test indicated the number of pods and number of hybrid plants acclimated were dependent on the female parent used in the cross combinations (Table 1). ICA Pijao and PI 266910, when crossed to *P. angustissimus,* were the only parents that resulted in hybrid embryos which developed into plants. The acclimated plants grew to produce flowers, but failed to set any seed. The F_1 interspecific hybrids were intermediate between

the parents for leaf size and shape. The flower size of the hybrids was similar to that of the female parent whereas, the growth habit was indeterminate prostrate and similar to that of the male parent.

Table 1. Chi-square test of independence for mean number of pods, pods with at least one culturable embryo and plants acclimated for crosses with *Phaseolus angustissimus* (PI 535272) as the male parent.

Crosses	No. of pollination	No. of pods	Pods with at least one culturable embryo	No. of embryo cultured	No. of plants transplanted
Female parents					
CDC Nighthawk	9	5	5	7	0
5-593	11	9	2	4	0
ICA Pijao	20	6	5	13	11
G11024 (*mexicanus*)	5	2	2	3	0
PI 266910 (*aborigineus*)	12	4	4	10	9
χ^2_{obs}		**6.1**	**0.4**		**26.5**
P value		**0.01**	**0.53**		**0.00**

2. Cytogenetic characterization of the F_1 interspecific hybrids: Sterility of the F_1 interspecific hybrids was confirmed by a preponderance of lightly or unstained pollen grains (representing sterile pollen). Based on over a 1000 random pollen sample count from various plants of the same hybrid combination, pollen fertility was estimated to be 4.3% in *P. vulgaris* var. *aborigineus* x *P. angustissimus*, and 2.4% in *P. vulgaris* x *P. angustissimus* hybrids. Also, a wide range in pollen grain size was observed for any hybrid plant, indicating a strong genomic imbalance during meiosis. Germination of pollen grains on boron containing nutrient agar medium indicated low pollen viability values. All hybrid plants were sterile owing to low pollen fertility and pollen viability.
Chromosome Pairing: Results indicate many unpaired chromosomes, an array of meiotic abnormalities, and formation of micronuclei which lead to the observed distinct range in pollen size. During meiosis of the pollen mother cell, the *P. vulgaris* x *P. angustissimus* hybrids, on average showed a mean chromosome pairing of 12.72 univalents, 3.45 bivalents, 0.45 trivalent and 0.18 quadrivalent.

3. Barriers to interspecific hybridization in the backcross of the F_1 interspecific hybrids:
Pollen tube growth: In the selfed pistils of the F_1 interspecific hybrids, ovules abort within 24 h after pollination. No pollen germination or pollen tube growth was observed, therefore, confirming the poor pollen fertility of the F_1 interspecific hybrids. However, in pistils of the F_1 interspecific hybrids backcrossed to either parent, pollen tubes were observed entering the ovule region and the ovule itself.
Embryo Development: Preliminary results show the formation of a 4 to 8 cell backcross embryo at 4 days after in-vitro pollination of the F_1 interspecific hybrids with pollen from ICA Pijao. This indicates the absence of pre-fertilization barrier.

Conclusions: Pods abort at 4 days after pollination in the backcross of the F_1 interspecific hybrids to parents. In-vitro pollination studies indicate the presence of post-fertilization barriers in the backcross of the F_1 interspecific hybrids to parents. A combination of pod, ovule and embryo culture protocols may be required to enable continued growth and development of the backcross embryo.

Acknowledgements: CIAT, USDA and Dr. Basset for providing seeds of bean lines.
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Most Promising Bean Varieties from AAFC-Lethbridge and Morden

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IRRIGATED Alberta / Saskatchewan

1. AC Redbond, 'small red' (Red Mexican), registered April 1999

'AC Redbond' is a high-yielding(2.82 t/ha vs. NW63 2.42), upright, early-maturing (99 d vs 102 for NW63), small red dry bean cultivar with moderate resistance to white mold, well adapted to wide or narrow rows. AC Redbond has the type IIb, indeterminate growth habit with erect stem and branches, in contrast to the type IIIb of NW63, with weak to prostrate stems.

2. AC Black Diamond, registered Nov. 2000

'AC Black Diamond' is a high-yielding (6% higher than UI906), large-seeded, shiny black dry bean cultivar. It was developed from a series of crosses at CIAT, Colombia, on contract to AAFC, Lethbridge. AC Black Diamond is well adapted to the Canadian prairies, yielding significantly more than the check cultivar, UI 906, at 122% in narrow-rows and 106% in wide-rows. AC Black Diamond is moderately susceptible to white mold and resistant to Bean Common Mosaic Virus (BCMV). The seed of AC Black Diamond turns to harvest colour prior to the other two cultivars, the risk of fall frost should be less for AC Black Diamond. AC Black Diamond has the type IIa.

3. AC Polaris, great northern, registered March 2000

'AC Polaris' (PI 613178) is a high yielding (compared to US1140 and CDC Nordic) great northern dry bean. In irrigated trials,. AC Polaris has Type IIB growth habit. Pods are higher on plants of AC Polaris and more widely distributed than on US 1140. The seed mass of AC Polaris averaged slightly lower than that of US 1140. AC Polaris is resistant to strains 1 and 15 of BCMV, while both checks, US1140 and CDC Nordic, are susceptible. In contrast to the susceptible check, US1140, and similar to the moderately susceptible check, CDC Nordic, AC Polaris is moderately susceptible to white mold.

MANITOBA

4. AC Scarlet, 'medium red', registered Nov. 2000

'AC Scarlet' is a high-yielding small red dry bean cultivar. It was developed from a series of crosses at CIAT, Colombia, on contract to AAFC, Lethbridge. AC Scarlet is well adapted to the eastern Canadian prairies, yielding significantly more than the check cultivar NW 63. AC Scarlet is moderately susceptible to white mold. The seed of AC Scarlet is significantly greater than that of NW 63. AC Scarlet has moderately good lodging resistance. AC Scarlet has the type IIa, indeterminate growth habit.

5. AC Alert, 'great northern', registered Feb. 2002
'AC Alert' is a common dry bean with an upright growth habit and high bearing pods. AC Alert, maturing at the same time as US1140, is a high-yielding cultivar suited specially to southern Manitoba, the higher heat-unit eastern prairies in Canada. AC Alert has better lodging resistance than US1140. AC Alert carries the pods in the upper part of the plant. The hydration coefficient and percentage drained wet weight of AC Alert are significantly greater than those of US1140, giving Alert an improved cooking quality over US1140. AC Alert has the type IIa, indeterminate growth habit. The seed mass of AC Alert is greater than that of US1140. AC Alert is resistant to strains 1 and 15 of BCM*V*. AC Alert is moderately resistant to white mold compared to the susceptible US1140. AC Alert was resistant to the delta race of *Colletotrichum lindemuthianum*.
6. **AC Black Violet**, (opaque) black, registered March. 2003
'AC Black Violet' is a high yielding (in narrow rows), large-seeded, purple-podded, black dry bean, with moderate resistance to white mold, specially suited to the longer growing season regions of Manitoba, Canada. AC Black Violet has good lodging resistance and a type IIa, indeterminate growth habit. It is resistant to the delta race of anthracnose and moderately resistant to race 1096. AC Black Violet is resistant to race 15 of BCMV, and has variable resistance to race 1, with a necrosis, followed by death resulting in over half the infected plants.

7. AC Morden003, 'navy', registered March 2003
'AC Morden003' is an early-maturing (101 d vs. 106 d for Envoy) navy bean cultivar suited for production specially in narrow-rows in southern Manitoba. AC Morden003 is upright and has the type I, determinate growth habit, with strong erect stem and branches.AC Morden003 is moderately resistant to white mold; and resistant to alpha-Brazil, race 173, and race 1096, moderately resistant to the delta race of anthracnose, Morden003 is resistant to strains 1 (with some plants displaying necrotic symptoms which may lead to death of plant) and 15 of BCMV.

ACROSS PRAIRIES
8. AC Early Rose, 'pink', registered April 2003
'AC Early Rose' is a high-yielding, very early-maturing cultivar suited for production specially in narrow-rows across the Canadian prairies. The seed of Early Rose is greater than that of Viva. AC Early Rose is moderately upright having a type IIa, indeterminate growth habit, with strong erect stem and branches. AC Early Rose is resistant to both the yellow and orange strains of bacterial wilt, while the check, Viva is moderately resistant to the yellow strain and also resistant to the orange strain . AC Early Rose is moderately susceptible to white mold, as is the check, Viva. AC Early Rose is resistant to races 1 and 15 of BCMV. AC Early Rose is moderately susceptible to the delta race of anthracnose.

PRELIMINARY EVALUATION OF COMMON BEAN LANDRACES FROM LEÓN (SPAIN)

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Common bean (*Phaseolus vulgaris* L.) is potentially the most valuable source of plant protein in many parts of South Europe, and contributes significantly to the sustainability of traditional cropping systems, because of the predominance of small-scale farmers who cultivate bean in these areas. The socioeconomic peculiarities of the Northwest of Spain, the use of traditional varieties, grown in smallholdings and by the own supplying or sale in local markets, have made possible the maintenance of these traditional culture systems, although as a consequence of new technology and market opportunities, common bean landraces are being replaced by bean improved varieties.

European Community regulations have introduced the possibility to attribute marks of origin and quality to local typical products. These marks can be an important support to *on farm* maintenance of elite landraces of principal crops (Piergiovanni and Laghetti, 1999). In the frame of collaboration between Department of Agrarian Engineering (University of León, Spain) and an association of farmers and canners of bean, common bean landraces from province of León are being studied.

The objective is can choose common bean landraces which would be included in an European Community mark of origin and quality.

The evaluation of these landraces has been focused on agronomic performance as well as on quality traits of seed. This paper shows the best common bean landraces of each one of the principal market classes (Amurrio, et al., 2001) from the province of León.

Food quality data were measured on dried, soaked and cooked bean seeds. These included, dry and soak seed weight (determined on 100 seeds per plot after soaking for 18 h), seed length and width (determined on 10 random seeds per plot after drying for 72 h at 80 ºC), proportion of coat (defined as the relation in weight between coat and cotyledon plus coat, after removing the coat from the cotyledon and keeping them for 24 h at 105 ºC), and water absorption (measured as the amount of water dried seeds absorb during soaking). Detailed methodology concerning to the calculation of each trait has been published (Santalla et al., 1995; Escribano et al., 1997). Hardshell describes a condition in which the seed fails to imbibe water within a reasonable time after when moisture is applied (Bourne, 1967). Bean cooking time was estimated with a 25-seed Mattson cooker pin drop cooker (Jackson and Varriano-Marston, 1981). Cooking time was calculated as the elapsed time from initiation of cooking until the time when 13 of the 25 pins (52%) of the instrument had dropped and penetrated seeds in the cooker.

According with the results (Table 1 and 2) it could be point out some landraces (Canela, Favada, Small White Kidney and White Kidney) with appropriate attributes to be produced in this area, which could be included in a European Community mark of origin and quality.

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Table 1. Morpho-agronomical characteristics of landraces from León

Market Class	Landrace	Growth habit[a]	Seeds/pod	Pods/plant	Seed yield (kg/ha)	Seed length (mm)	Seed width (mm)
Canela	Ca 13-9	1	4.4	16.1	2695	18.1	9.0
Cramberry	Pi 5-2	3	5.1	14.5	3864	13.8	10.0
Favada	Fa-gen	3	3.8	14.6	2122	18.6	7.2
Large Great Northern	Pd 2-1	3	5.4	16.5	2713	15.7	8.4
Pinto Red	Pm 11-1	3	5.7	18.1	3161	12.8	8,4
Small White Kidney	Ri men	2	5.8	17.4	3564	14.7	7.5
White kidney	Ri 8-3	1	4.8	16.8	2687	16.2	8.0

[a] According to Singh 1982

Table 2. Seed quality characteristics of landraces from León

Market Class	Landrace	100-SW (g)	WA (%)	HS (%)	CP (%)	CT (m:s)
Canela	Ca 13-9	70.86	108.83	0.00	5.73	18:54
Cramberry	Pi 5-2	69.74	98.82	0.64	5.99	16:11
Favada	Fa-gen	78.80	111.50	1.03	6.71	17:58
Large Great Northern	Pd 2-1	56.91	93.95	13.97	7.02	16:50
Pinto Red	Pm 11-1	51.53	70.65	20.27	7.06	19:34
Small White Kidney	Ri men	51.42	105.90	0.27	6.16	15:44
White Kidney	Ri 8-3	63.23	112.87	0.30	6.27	21:58

SW (100 seeds weight); V10 (seed volume); WA (water absorption); HS (Hardshell);
CP (Coat proportion); CT (Cooking Time).

Elucidation of the Genotype of a Virgarcus Seed Coat Pattern Mutant of Red Hawk Dark Red Kidney Bean

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Introduction

The soldier bean line, with proposed name Redcoat, originated from a few off-type seeds discovered in a Foundation Seed lot of Red Hawk, a dark red kidney bean variety. Unlike Red Hawk, which has totally colored seed, Redcoat possesses white seed with a red virgarcus pattern. The only other observed phenotypic difference between the two varieties is in flower color. Redcoat's flowers are pure white, whereas Red Hawk's flowers are white with faint red veins in the wing petals.

The inheritance of partly colored seed coat patterns, like that of Redcoat, is controlled by at least five interacting loci: *T, Z, Bip, Fib,* and *J*. A dominant *T* allele results in totally colored (also called self-colored) seed, whereas the recessive genotype *t/t* allows the other seed coat pattern genes to be expressed. This *t/t* genotype also has a pleiotropic effect resulting in white flower color. The other seed coat pattern loci determine the shape and extent of the colored area (Bassett and McClean, 2000). Since two different genotypes could confer Red Hawk's self-colored seed (*T z* or *t Z*), this study was undertaken to elucidate Red Hawk's genotype and determine which gene in Red Hawk had mutated to express the soldier pattern of Redcoat.

Materials and Methods

Crosses were made between Redcoat and Red Hawk and between both of these varieties and each of three genetic testers for seed coat pattern developed by Bassett: *t* self-colored BC_3 5-593, *t cl z g b v* virgarcus BC_3 5-593, and *t z bip* bipunctata 5-593 (Bassett and Blom, 1991; Bassett, 1996). For all crosses, the seed coat pattern of each F_2 plant was recorded, and the flower color of a random sample of the F_2 plants was also noted. No F_1 data were recorded.

Results and Discussion

The 273 Redcoat/Red Hawk F_2 plants segregated 3:1, self-colored to virgarcus (p=0.382). A random sample of 37 plants was classified as follows: 28 with white flowers having red veins and self-colored seed; 9 with pure white flowers and virgarcus pattern seed. Our genetic hypothesis is that Red Hawk has *T z* and Redcoat has *t z*. Segregation at *T* appears to affect expression of red veins on white wing petals, where *t v* rk^d fails to express the red veins expected from the genotype *v* rk^d. The latter result is contrary to the observations of Prakken (1972).

The flower colors in the F_2 generation of the Red Hawk and Redcoat by seed coat pattern tester lines are given in Table 1. The tester lines are all white flowered since they carry the recessive *t* allele. However, the RH/self-colored and RH/bipunctata F_2 populations included plants with violet, white, and red-veined white flowers. The RH/virgarcus F_2 plants had either white flowers or red-veined white flowers. This suggests that Red Hawk carries the dominant *T* allele, since violet flowers (conferred by *V*) would not be expressed were Red Hawk carrying *t*. Violet flowers were not present in the RH/virgarcus F_2 plants since the tester line carries *v* and Red Hawk apparently does as well.

Seed coat pattern frequencies in the RH/tester F_2 populations also support the theory that Red Hawk carries *T* (Table 2). If Red Hawk's self-colored seed coat were conferred by the genotype *t Z Bip*, no partly colored patterns other than expansa would be expected in the RH/self F_2 population, since the self-colored tester has this same genotype. However, a few individuals

Table 1. Flower Color in the Red Hawk & Redcoat by Seed Coat Pattern Tester F_2 Populations and Inferred Genotypes for Red Hawk and Redcoat

	Cross*					
Flower Phenotypes	RH *T v* rk^d/			RC *t v* rk^d/		
and Genotypes	self *t V Rk*	vir *t v Rk*	bip *t V Rk*	self *t V Rk*	vir *t v Rk*	bip *t V Rk*
Violet T/- V/-	X		X			
White/Red Veins *T/- v/v* rk^d/rk^d	X	X	X			
Pure White *t/t*	X	X	X	X	X	X

Table 2. Seed Coat Pattern in the Red Hawk & Redcoat/Seed Coat Pattern Tester F_2 Populations

	Cross*					
Seedcoat Pattern	RH/self	RH/vir	RH/bip	RC/self	RC/vir	RC/bip
Self	78	110	63	42	0	0
Expansa	4	0	0	36	0	0
Ambigua	0	17	5	26	27	0
Red Coat Virgarcus	3	11	9	13	31	58
Tester Virgarcus	2	20	0	6	24	0
Weak Virgarcus	1	5	5	5	13	40
Bipunctata	0	0	3	0	7	22
TOTAL	88	163	85	128	102	120

* RH denotes Red Hawk; RC, Redcoat; self, *t* self BC_3 5-593; vir, *t cl z g b v* virgarcus BC_3 5-593; and bip, *t z bip* bipunctata 5-593

in this population expressed the ambigua and vigarcus patterns. Additionally, Red Hawk carries the *z* gene for Redcoat's virgarcus pattern cryptically, since all of the RH/tester F_2 populations contained some plants expressing Redcoat-like virgarcus patterned seed.

The data from the RC/tester F_2 populations suggest that the alleles conferring Redcoat's virgarcus pattern may be different from those of the virgarcus tester. The RC/virgarcus F_2 population contained plants with ambigua and bipunctata patterned seed. If Redcoat and the virgarcus tester had the same seed coat pattern genes, all the progeny would be expected to express the virgarcus pattern. Redcoat's seed coat pattern is different from the virgarcus tester. Plants expressing the tester virgarcus pattern occurred in the RH/self, RH/virgarcus, RC/self and RC/virgarcus F_2 populations, suggesting that the difference between the tester and Redcoat virgarcus patterns is controlled at the *Bip* locus since the tester virgarcus pattern only occurred in populations from crosses where the tester carried the dominant *Bip* allele.

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AGRONOMIC AND NUTRITIONAL CHARACTERISTCS OF SNAP BEAN PLANTS (cv UEL-2), INFLUENCED BY SOURCES AND DOSES OF NITROGEN

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Introduction

Nitrogen fertilization is very important for snap bean once there are no commercial inoculants with specific indication to biological nitrogen fixation. The root nodulation normally is due to the native strains of Rhizobium (Franco & Balieiro, 1999), not always efficient. The covering nitrogen application, may improve the bean yield even when inoculated (Franco et al., 1979, Vidor et al., 1989). When the inorganic nitrogen soil content is low, the yield reduction may occur (Castelane et al. 1988). The trial set in order to evaluate the effect of doses and sources of nitrogen applied in covering on the agronomic and nutritional characteristics of snap bean plants.

Material and methods

The experiment was carried out in greenhouse. The substrate was collected from surface layer of clay Oxissol from Experimental Station of State University of Londrina, PR, Brazil. The vessels had 3,5 liters of capacity. During the experimental period the humidity was kept in 70% of the maximum retention capacity, through daily reposition of water lost by evapotranspiration. Each vessel was fertilized with an equivalent rate of 400 kg ha^{-1} of the 04-14-08 fertilizer. The crop used was the snap bean (cv UEL-2), leaving two plants in each vessel. The experimental design was completely randomized with four replication in 4x2 factorial arrangement, with 4 doses of nitrogen applied in covering (0, 30, 60 and 90 kg ha^{-1}) and 2 sources (ammonium sulphate (AS) and urea (U). Nitrogen fertilization was applied at the 23 days after emergence (DAE). The harvest was made at the 43 DAE. Plants high (PH), number of commercial pods (NCP), dry matter mass of commercial pods (DMCP), nitrogen content of leaves (NCL) and nitrogen content of commercial pods (NCCP) were evaluated. The data were submitted to the variance, regression and correlation analyses.

Results and discussion

Plant high was not influenced by either nitrogen sources or doses. However, NCP increased linearly with the nitrogen doses when AS was used. When U was used, the adjust was

Figure 1. Number of commercial pods

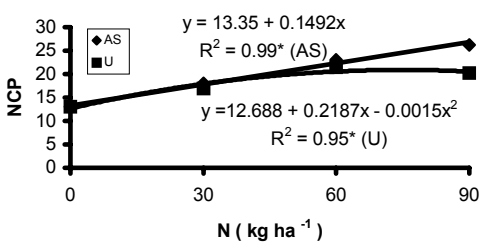
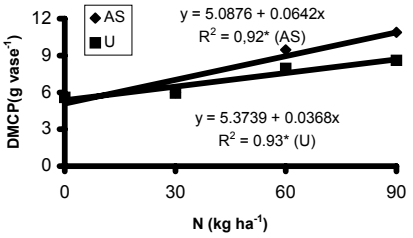


Figure 2. Dry matter production of commercial pods



significant (P<0,05) for the second degree polynomial regression with maximum point in 78.1 kg ha^{-1} of N (Fig.1).

For DMCP was observed linear increase in function of nitrogen doses regardless of the sources (Fig.2). The results suggest that the yield can be increased with the increase of nitrogen application in covering.

Figure 3. Nitrogen content in dry matter of leaves

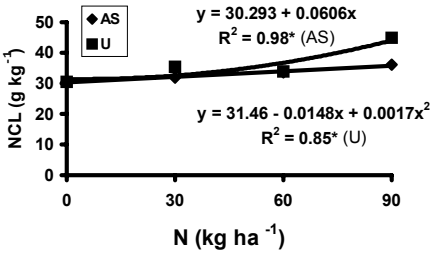
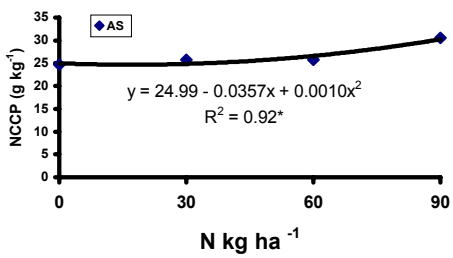


Figure 4. Nitrogen content in dry matter of commercial pods



NLC increased linearly for AS source (r^2=0.98) and it was estimated a minimum content in 4.35 $kgha^{-1}$ of N, when U source was applied (Fig. 3)

For data of NCCP the equation of adjust was $Y= 24.99 – 0.0357x + 0.0010x^2$ (r^2=0.92*) with minimum in 17.85 $kgha^{-1}$ of N, when AS source was employed, however this variable did not influenced by U source (Fig.4).

In the correlation analyses between NCL and another variables studied, it was observed that only for NCCP occurred positive and significant coefficient correlation (r=0,69*), when U source was used.

Finally, it can be observed that except for PH, all available variables were influenced by studied treatments. NCP increased linearly with ammonium sulphate. However, when urea was used, the maximum NCP was obtained with 78,1 kg ha^{-1} of N. NLC and NCCP were influenced of different forms by N-sources. NLC was correlated with NCCP, only for U source. DMCP increased linearly with covering nitrogen application for the two studied sources.

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EVALUATION OF SEED-Fe CONCENTRATION IN DRY BEAN

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Introduction

Iron deficiency is a serious public health problem, affecting approximately two billion people throughout the world (Della Pena,1999). Due to its substantial iron content the common bean plays an important role among foods (Pennington and Young, 1990). Highly consumed by populations in Latin American and African countries, common bean is one of the main sources of protein, calorie and iron for these populations. Thus, issues related to improving the nutritional quality of foods, such as the common bean, should be addressed genetically through plant breeding programs (Koehler and Burke, 1981).

Material and Methods

The experiment was carried out during 2003 spring, in the green house of University of Londrina, Paraná, Brazil. Two cultivars Ruda and Perola were used as females, and Xamego, Rio Tibagi, IAC –Uma and FT Nobre were used as males.

After the harvest, the pods were hulled to obtain the seeds that were placed separately (by plot) in a stove with forced air circulation, at 50º- 55ºC and dried until constant weight was reached. After drying, the material was grounded in a rotating mill and sieved through a 1 mm mesh. Three 400 mg samples were then weighed from each experimental plot and nitroperchloric digestion was performed until the samples were lightened. Each sample was then diluted in distilled and deionized water up to a 100 ml. A representative aliquot was taken from this sample and the iron present in the ground bean grains was quantified by an atomic absorption spectrophotometer.

Results and Discussion

Genetic variability for iron content was observed in the common bean seeds (Table1). There was favorable complementation of alleles, therefore the averages of the iron concentration in the seeds from the crosses were greater than the averages of the respective parents (Table1 and 2). In the general combination, Rudá was the parent that provided the best average in all the crosses, when compared with Perola (Table 2 and 3). It is important to consider that Rudá was derived by crossing of Carioca with Rio Tibagi. In the specific combination, Rudá and Rio Tibagi produced highly favorable effect, raising the average of the F2 population (Table 2 and 3).

Table 1. Means of Seed-Fe concentration (mg $100g^{-1}$) in dry bean parents grown at Londrina-PR. Brazil, in 2003

Genotype		Seed-Fe Concentration (mg $100g^{-1}$)
Males	Rudá	12.4
	Pérola	10.4
Females	Xamego	6.8
	Rio Tibagi	5.5
	IAC-Uma	5.1
	FT Nobre	6.2

Table 2. Means of Seed-Fe concentration (mg $100g^{-1}$) in F2 population derived from dry bean crosses at Londrina-PR. Brazil, in 2003.

Genotype	Xamego	Rio Tibagi	IAC-Uma	FT Nobre	Means
Rudá	10.6	13.4	9.5	11.2	11.2
Pérola	9.5	10.3	9.7	9.7	9.8
Means	10.1	11.9	9.6	10.5	10.5

Table 3. Heterosis means of Seed-Fe concentration in F2 populations derived from dry bean crosses at Londrina-PR. Brazil, in 2003.

Genotype	Xamego	Rio Tibagi	IAC-Uma	FT Nobre
Rudá	1.00	4.45	0.75	1.90
Pérola	0.90	2.35	1.95	1.40

Conclusions

It is possible to increase iron concentration through breeding.

It was demonstrated that there was genetic variation in iron content among bean seed genotypes with a favorable complementation of the alleles.

'Rudá' was the parent that raised the average effect the greatest and when crossed with 'Rio Tibagi', produced a highly favorable effect.

Acknowledgement

We are grateful to Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq) and Fundação Araucária.

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EFFECT OF DOSES OF NITROGEN AND MOLYBDENUM ON COMMON BEANS, IN LICHINGA PLATEAU, NORTHERN MOZAMBIQUE

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INTRODUCTION

The current production levels of common beans in Mozambique are insufficient to meet the country's consumption requirement. Soil fertility is the major problem. The use of fertilizer in Mozambique is limited to very small number of producers and they use only macronutrients (NPK). The use of micronutrients, such as Mo is not a common practice. This study was conducted to evaluate the effect of nitrogen and molybdenum on common beans in the Niassa plateau, Northern Mozambique.

MATERIAL AND METHODS

The experiment was carried out at Lichinga Research Station, in northern Mozambique, in a soil classified as ferralsol. The soil texture was loam to clay. A randomized complete block design with four replications was used. The treatments were in a factorial 4 x 4, with 4 additional treatments. The factors comprise four levels of N (0, 30, 60 and 90 $kg.ha^{-1}$) and four levels of Mo (0, 40, 80 and 120 $g.ha^{-1}$) applied as side dressing. The sources of N and Mo were ammonium sulfate and sodium molybdate, respectively. Molybdenum fertilizer was sprayed only once on the foliage, 22 days after emergence (DAE). Nitrogen fertilizer was split as it follows: the level of 30 $kg.ha^{-1}$ was applied once at 22 DAE; the level of 60 $kg.ha^{-1}$ was split in two applications each with a rate of 30 $kg.ha^{-1}$ and applied 22 and 29 DAE; and the level of 90 $kg.ha^{-1}$ was split in three applications each at a rate of 30 $kg.ha^{-1}$ and applied at 15, 22 and 29 DAE. All experimental plots received basal N, P and K at rates of 20 $kg.ha^{-1}$, 90 $kg.ha^{-1}$ and 60 $kg.ha^{-1}$, except for the four additional treatments where basal N was not applied. These four additional treatments were as it follows (Basal N, Side dressing N and Mo at foliage): (0-0-0), (0-0-50), (0-60-0) and (0-60-50). Each plot comprised four rows of 5 meters long.

RESULTS AND DISCUSSION

The effect of N-fertilizer application to the yield of beans is shown in Figure 1 and the effect of Mo is presented in Figure 2. The quantity of Mo necessary to get the maximum yield varied according to the rate of N applied as side dressing. It was observed that in the absence of N-fertilizer as side dressing, the maximum productivity was obtained with the application of 80 kg/ha of Mo. With the application of 30 kg/ha, the rate of Mo to attain maximum yield was of 79,6 g/ha, while the application of 60 kg/ha of N the corresponding rate of Mo was of 79,4 g/ha; and when the level of N applied was of 90 kg/ha of N, the corresponding rate of Mo was of 79,3 g/ha. This variation was very small when compared with the results obtained by Berger et al (1996) and Amane et al. (1999) in experiments conducted at Zona da Mata of Minas Gerais in Brazil. Regarding the four additional treatments, it was observed that the application of only 20 kg/ha of N at the planting stage, the yield of bean increased by 35% (Table 1). When N was not applied at planting, the application of 60 kg/ha as a side dressing increased the yield of beans by

68%. The application of 50 g/ha of Mo increased the yield by 74%. Without N application at planting, the combined application of 60 kg/ha of N and 50 g/ha of Mo increased the yield by 125%. These results show the importance of N applied at planting time and also the positive effect on yield when N is applied in combination with Mo.

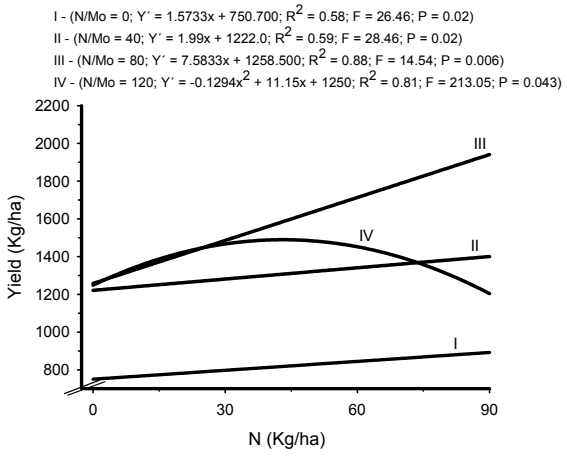

Figure 1. Response of common bean to different levels of N under four levels of Mo

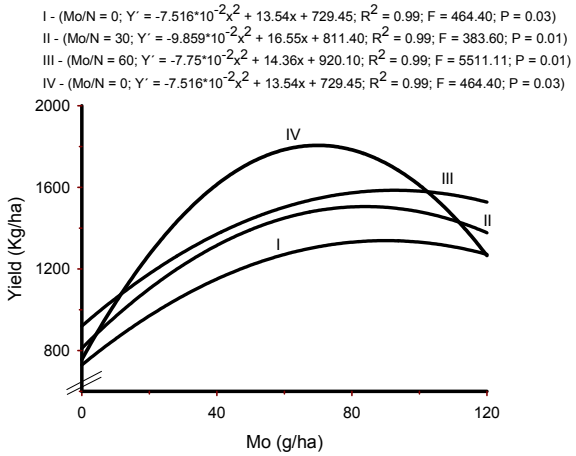

Figure 2. Response of common bean to different levels of Mo under four levels of N

Table 1. Response of common bean to basal N, N as side dressing and Mo, in Lichinga plateau.

N (kg/ha)	Mo (kg/ha)	Yield* (kg/ha)
0	0	539
0	50	937
60	0	908
60	50	1,211
C.V.(%)		14,24

* Means followed by the same letter didn't differ significantly among them by Duncan means test

CONCLUSIONS

1- Basal N is necessary for high yielding of common beans
2- High levels of Mo appears to have a detrimental effect on yields of this bean varivariety.
3- The effect of Mo is high under high levels of N

ACKNOWLEDGEMENT

To INIA, the National Agronomic Research Institute for supporting this study.
To the staff of Lichinga Research Station for providing facilities for the implementation of this study.

GENES CONDITIONING HALO BLIGHT RESISTANCE TO RACES 1, 7, AND 9 OCCUR IN A TIGHT CLUSTER

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Halo blight is a seed-borne bacterial disease (caused by *Pseudomonas syringae* pv. *phaseolicola*) that infects common bean (*Phaseolus vulgaris* L.) worldwide. Genetic resistance is the most effective control method. A host/pathogen differential series developed by Taylor et al. (1996a; 1996b) identifies five resistance genes (Table 1).

Table 1. Race differentiation of *P. syringae* pv. *phaseolicola* on 8 differential lines

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

+, compatible (susceptible); -, incompatible (resistant)

Our goal is to further characterize and map halo blight resistance genes in bean and tag them for marker-assisted selection (MAS). An inbred population (BelNeb-RR-1/A55) consisting of 77 RILs was challenged by Races 1, 3, 4, 5, 7, and 9. BelNeb-RR-1 is resistant to Races 1, 5, 7, and 9 and A55 is resistant to Races 3 and 4. This population was used previously to map QTL conditioning resistance to an unknown *Psp* strain and putative Race 7 *Psp* strain (Ariyaranthe et al., 1999). Five plants of each RIL were inoculated with each race. Each plant represented a replicate in a RCBD (five replicates). Inoculum [10^8 CFU/ml] was applied to 7- to 10-d-old seedlings with fully expanded primary leaves using the method of Taylor et al. (1996a). Inoculated plants were kept in a humidity chamber (19^{o}C, RH=100%) for 48 h before being transferred to a greenhouse (18^{o}C night/25^{o}C day, RH=70%). Plants were rated for infection 10 DAI on a 1 to 5 scale with 1 being highly resistant and 5 being highly susceptible (Taylor. et al., 1996b). Lines rated between 2 and 5 were considered susceptible.

Three major resistance genes were mapped in the BelNeb-RR-1/A55 mapping population (Fig 1). The *Pse-1* gene that conditions resistance to Races 1, 7, and 9 is a putative cluster of individual genes conditioning resistance to Race 1 (*Pse-1*), Race 7 (*Pse-7*, a newly proposed symbol) and Race 9 (*Pse-9*, a newly proposed symbol). The *Pse-1* gene cluster is located on

linkage group B4 near RAPD marker B10.520 (Ariyaranthe et al., 1999). This genomic region is the same location of a cluster of genes conditioning resistance to anthracnose (*Co-9* and other resistance loci), ashy stem blight (QTL), bacterial brown spot (QTL), BGYMV (SW12 QTL), and rust (*Ur-5* gene block and rust resistance genes from Ouro Negro and Dorado). The B10.520 RAPD marker has been converted to a SCAR marker for MAS of *Pse-1*. Sequence for the 520 bp fragment closely aligns with the sequence of a resistance gene analog (RGA) mapped in the same genomic region.

The *Pse-4* gene conditions resistance to Race 5 and is also located on linkage group B4, 14.7 cM from the *Pse-1* gene cluster (Fig. 1). *Pse-3* gene conditioned hypersensitive resistance to Races 3 and 4, and as has been reported previously was completely linked to the *I* gene which conditions hypersensitive resistance to BCMNV on linkage group B2. QTL conditioning resistance to halo blight strains HB16 and HB83-Sc2A were previously mapped in this population (Ariyaranthe et al., 1999) to the same locations as *Pse-1*, *Pse-3*, and *Pse-4*, suggesting these race-specific genes confer quantitative resistance to other halo blight races. This is the first report for genomic position of the *Pse-1* and *Pse-4* genes on linkage group B4 and that a moderate linkage exists between them. Further research is being conducted to verify presence of the *Pse-1 – Pse-7 – Pse-9* gene cluster. Currently, individual RILs recombinant for resistance to Races 1, 7, and 9 will be single-plant selected and challenged again by multiple races of the pathogen.

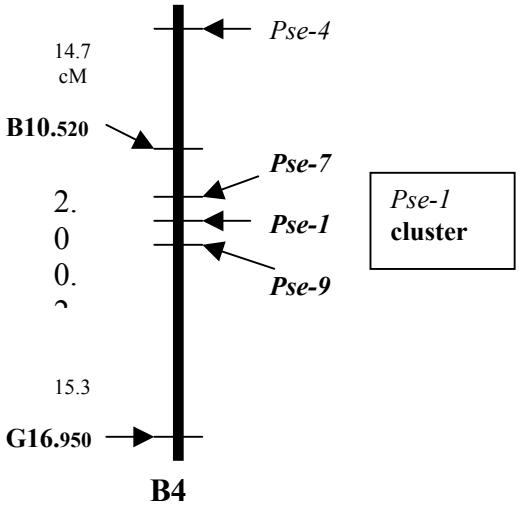

Figure 1.

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RESISTANCE TO COMMON BACTERIAL BLIGHT AND HALO BLIGHT AMONG SPANISH COMMON BEAN LANDRACES

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INTRODUCTION

Halo blight (HB) caused by *Pseudomonas syringae* pv. *phaseolicola* (*Psp*) and common bacterial blight (CBB) caused by *Xanthomonas campestris* pv. *phaseoli* (*Xcp)* and its variant *fuscans (Xcpf*) are among the major constraints of common bean production in the North Central part of Spain.

In this region, the main commercial cultivar corresponds to the international market class "white kidney". This variety is highly susceptible to CBB and HB. Resistant genotypes used at the bean breeding program developed at ITA, come from international programs and are significantly distant from the varietal type mentioned above. Usually, these genotypes do not show adequate levels of adaptation to local environments and the desirable commercial traits (large, white seeds and determinate growth habit).

OBJECTIVE

The objective of this work was to identify, among 185 traditional local varieties (landraces), resistant genotypes with morphological traits similar to the "white kidney" type and better local adaptation than the foreign parents used at present by the bean breeding program.

MATERIAL AND METHODS

Germplasm was provided by the National Plant Genetic Resources Center (CRF), Spain. Plant material was chosen from this collection giving preference to accessions with large white seeds and determinate growth habit (Table 1). All of them corresponded to landraces and came, mainly, from the central region of Spain.

Accessions were characterised in field, in two independent assays, for its resistance to both pathogens: *Xcp* and *Psp*, races 6 and 7, as they are the two predominant races in the region (3). Two unreplicated rows per genotype were sown. Plants were inoculated by aspersion according to the method described by (4). Symptoms, for both leaves and pods, were visually evaluated using the 1 to 9 scale described by (5).

Accessions initially selected for showing resistance to at least one of the two pathogens, were sown again to confirm this resistance by means of a second inoculation. Pod resistance to both pathogens was also confirmed by inoculation with multiple needles (1).

RESULTS AND DISCUSSION

Only, 7,5% (14 accessions) of the material evaluated showed some degree of CCB and/or HB resistance (Table 2). Five accessions were completely resistant to *Psp,* either in leaves or pods, whereas 9 accessions showed an intermediate level of HB resistance. No accession was immune to *Xcp,* nor in leaves or pods and only three accessions showed moderate CCB resistance. Our results seem to confirm the difficulty reported by other authors (4, 6) to find adequate levels of CBB resistance among *Phaseolus vulgaris* germplasm.

Although, the germplasm characterised had predominantly determinate upright growth habit (Table 1), the majority of the material finally selected showed prostrate or semiclimbing growth habit.

Those accessions with CBB resistance showed also HB resistance. Other authors (2) have pointed out before the possibility of an association between these two characters.

The four accessions with the best potential as resistance donors (ZJ-1215, ZJ-1217, ZJ-1220 and ZJ-1223) (Table 2), were poorly adapted to local conditions and corresponded to minority types, which did not display the expected commercial traits ("white kidney" type). These results would indicate the difficulties existing in finding common bean germplasm resistant to bacteriosis, which combine adequate local adaptation and morphological characteristics, as large white seeds and determinate growth habit.

TABLE 1. Growth habit and seed colour and size of the common bean accessions evaluated.

CHARACTERISTIC		%
GROWTH HABIT [a]	I	65.0
	II	7.0
	III	27.0
	IV	5.0
SEED SIZE	Large (> 40 g)	84.0
(weight of 100 seeds)	Medium (25-40 g)	10.3
	Small (<25 g)	5.4
SEED COLOUR	White	62.0
	Bi-color	12.5
	Yellow	3.2
	Other	22.3

[a] IV= climbing, III= indeterminate, II= indeterminate upright, I= determinate upright.

TABLE 2. Adaptation, growth habit, seed shape, size and colour and CCB and HB reaction in leaves and pods of 14 accessions selected for showing some degree of CCB/HB resistance.

Accession	Adaptation [a]	Growth habit [b]	Seed			Reaction [d]			
			Shape	Size [c]	Colour	HB		CBB	
						L	P	L	P
ZJ-1215	8	I	Round	Large	Yellow	R	S	I	S
ZJ-1217	5	III	Ovate	Medium	Yellow	R	R	S	S
ZJ-1220	7	II	Cylindrical	Medium	Bi-color	I	S	S	I
ZJ-1223	7	I	Cylindrical	Large	Yellow	I	I	I	S
ZJ-1212	7	III	Ovate	Small	White	S	I	S	S
ZJ-1213	7	III	Kidney-shaped	Large	Cream	S	R	S	S
ZJ-1214	8	III	Kidney-shaped	Large	White	S	I	S	S
ZJ-1216	8	III	Ovate	Large	White	I	S	S	S
ZJ-1218	9	I	Cylindrical	Large	White	S	I	S	S
ZJ-1219	9	III	Kidney-shaped	Large	White	S	I	S	S
ZJ-1221	8	III	Cylindrical	Large	White	I	I	S	S
ZJ-1222	6	III	Kidney-shaped	Large	White	I	R	S	S
ZJ-1224	8	III	Kidney-shaped	Large	Tri-color	S	I	S	S
ZJ-1225	7	I	Cuboid	Large	Bi-color	S	R	S	S

[a] Adaptation 1: excellent; 3: good; 5: intermediate; 7: poor; 9: very poor (Schoonhoven and Pastor-Corrales, 1987).
[b] III= indeterminate, II= indeterminate upright, I= determinate upright.
[c] Seed size: small (<25 g/100 seeds); medium (25-40 g/100 seeds); large (>40 g/100 seeds).
[d] Mean bacterial blight score for each pathogen; R: resistant (1 - 3,9); I: intermediate (4 - 6,9); S: susceptible (7 – 9) (Schoonhoven and Pastor-Corrales, 1987).

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Use of Marker-Assisted Selection to Breed for Resistance to Common Bacterial Blight in Dry Bean

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INTRODUCTION

Common bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *phaseoli* (Smith) Dye *(Xap*), is responsible for extensive yield losses worldwide. Additionally, the presence of this disease causes problems in an otherwise lucrative seed production industry. The traditional use of phenotypic selection has limitations in the selection of genotypes with adequate levels of stable resistance. The combination of molecular-marker technology with traditional phenotypic selection could greatly facilitate the selection of common bean lines resistant to CBB. To examine the application of this approach in the MSU breeding program, we developed black and navy bean populations using the CBB resistance source, VAX 5. These populations were screened for the presence of the SCAR marker, SU91, which is linked to a resistance QTL located on B8 of the integrated common bean linkage map. Molecular marker data was compared to visual ratings for CBB incidence in a field experiment. The efficiency of this marker in the identification of resistant lines is presented.

MATERIALS AND METHODS

*Marker-assisted Selection (MAS) – Genomic DNA was isolated from greenhouse-grown plants. This was used as the template DNA in PCR with the SCAR primer SU91 (1). Presence or absence of the resulting 700 bp fragment was determined using agarose gel electrophoresis. Black and navy bean populations were grown in the field as 1-row plots in the F5 generation (in 2002). Selections were made in these populations based on marker data and desirable agronomic traits. In total, 15 SU91-positive and 15 SU91-negative lines were chosen to compare the efficiency of using SU91 to select for CBB resistance.

*Bacterial Inoculum Preparation – Infected seeds of the navy bean variety 'Midland' were used as a source of primary inoculum in field experiments, randomly distributed within each replicate. In addition, 2-day old cultures of *Xap* (cultured on YDC media) were used to prepare inoculum with a concentration of 10^6 cells/ml, which was applied twice to the test plants (2).

*Field Experiments – Field plots were established on May 28, 2003. The experiment consisted of 42 entries (2-row plots), with 3 replications. Entries included the 30 F6 lines (+/- marker) plus 12 check and parental lines. All plots were inoculated with a bacterial suspension on July 16 and July 24, 2003. A power sprayer was used to deliver the inoculum at approximately 150 PSI. Plots were evaluated, as appropriate, for the following traits: time to flowering, CBB leaf infection, CBB pod infection, height, lodging, maturity, and overall desirability. CBB infection was evaluated using a 1-9 scale, described by Schoonhoven and Pastor-Corrales (3).

*Statistical Analysis – The data was analyzed using the PROC GLM function of SAS. Comparisons were made using Fischer's Protected LSD (a=0.05). These results are summarized in Table 1.

Table 1. Statistical Analysis of Molecular Marker and Field Data

Genotype[a]	CBB Leaf Rating 1 (08/06/03)[b]	CBB Leaf Rating 2 (08/13/03)[b]	Maturity[c]
SU91+	3.37	3.48	95.3
SU91-	4.26	4.71	94.6
$LSD_{0.05}$ (*p*-value)	0.4038 (0.0001)	0.3833 (0.0001)	0.5951 (0.0001)

Notes: a= The genotype category is a composite of all genotypes included in the study that carried (SU91 +) or lacked (SU91 -) the SU91 SCAR marker. b= The leaf ratings (taken as described in Materials and Methods) are the mean rating for all entries in the genotypic class (SU91 + or SU91 -). c= Recorded as days after planting.

RESULTS AND DISCUSSION

Presence of SU91 was significantly associated with leaf resistance (p=0.0001) in this experiment. The marker was not, however, associated with pod resistance. These results are not unexpected, as it is widely recognized that leaf and pod resistance to CBB are under separate genetic control. Higher levels of leaf resistance may, however, result in decreased pod infection, due to decreased levels of inoculum in infected fields. The presence of SU91 was also significantly associated with later maturity (p=0.0151) although the difference is less than one day. These results are summarized in Table 1.

To reach a conclusive decision regarding the feasibility of using SU91 for MAS of CBB in dry bean, the 30 lines will be evaluated across multiple years, locations, and environments. Greenhouse experiments are currently underway to verify the level of CBB resistance of these lines. In addition, a multiplex PCR program is being utilized to pyramid various QTL conferring resistance to CBB and anthracnose (the other major seed-borne pathogen of dry bean). Selections made based on marker data will be evaluated for disease reaction, and agronomic traits in replicated field and greenhouse experiments in 2004.

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DISEASE FORECAST MODELING of *Uromyces appendiculatus* in the HIGH PLAINS BEAN PRODUCTION REGION

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Rust, caused by *Uromyces appendiculatus* (Pers.:Pers.) Unger, is a sporadic but serious disease which affects dry beans throughout the Central High Plains production region of Colorado, Nebraska, Wyoming and Kansas. Its annual occurrence and severity are influenced by many factors including the proportion of disease resistant cultivars planted, pathogen race diversity, and environmental conditions. Infection of susceptible cultivars can be severe if it occurs before early pod development (late July to early August). Analysis of weather data since 1992 has revealed associations between rust outbreaks and temperature and rainfall patterns. Efforts are underway to relate rust reports to environmental patterns that could be associated with disease outbreaks, and to develop a forecast model that would help crop consultants and growers predict future rust problems, fine-tune scouting calendars, and improve the overall effectiveness of integrated pest management programs for rust-susceptible cultivars affected by this pathogen.

Materials and Methods

Key weather stations located within 25 – 50 km of bean areas with a history of rust were selected to generate temperature and rainfall patterns. Disease surveys were conducted annually during June to August, with emphasis upon northeastern Colorado and southwestern Nebraska. We focused upon dry bean production areas and sites where rust was reported the previous year or during the current season by cooperators such as extension agents, crop consultants and growers. Initial rust infection dates were noted on symptomatic volunteer (sexual stages) and new crop (asexual stages) bean plants.

Multiple logistic regression models were developed to quantify the probability of sexual and asexual stages of bean rust in relation to environmental conditions. Monthly and cumulative seasonal rainfall, monthly mean daily high, low and overall mean temperature, rust the previous year (present or absent), number of days with > 2.5 mm rainfall, and continuous days with > 2.5 mm daily rainfall were chosen as predictor variables of disease appearance. Models were initially constructed with forward (entry α=0.05), backwards (removal α=0.05), and stepwise selection (entry α=0.15). Final models were selected based upon predictive ability (number of years correctly classified by cross-validation), number of predictor variables, Akaike's information criterion (a measure of model inaccuracy and complexity), and biological factors.

Results and Discussion

From 1992 to 2003, the sexual and asexual stages of bean rust were observed in 17 and 28 of 60 location-years, respectively. Two stepwise regression models were selected that accurately predicted sexual and asexual stages of bean rust occurrence in the Central High Plains region.

Sexual stage occurrence was predicted by: $\mathbf{E(Y/x) = -62.2101 - (0.5653 * P_{July}) + (0.6437 * RD_{0.1August}) + (0.3663 * T_{aveApril}) + (0.5986 * T_{aveJuly})}$

Asexual stage occurrence was predicted by: $\mathbf{E(Y/x) = -73.7589 + (1.0809 * P_{April}) + (0.2612 * T_{maxJune}) + (0.5335 * T_{aveApril}) + (0.3358 * T_{aveAugust})}$

In these models, the predictor coefficients are $\log_e$ of the odds ratio when the other predictors are held constant. The probability of disease developed was determined by $p=1/[1+e^{E(Y/x)}]$. Sexual stage occurrence of *U. appendiculatus* was predicted by moderate daily mean temperatures in April and July, cumulative rainfall in July, and days with greater than 0.1 inch (2.5 mm) of rain in August. Asexual stages were favored by moderate daily mean temperatures in April and August, mean daily high temperatures in June, and cumulative April rainfall.

A probability of 0.5 was selected as the cutoff for classification of a year as an outbreak year. Reducing the probability to 0.3 increased the sensitivity of the model (sexual stage 4.6% and asexual stage 9.4%), but reduced model specificity and increased the occurrence of false negatives. However, we suggest a higher sensitivity is more important than a high specificity in the Central High Plains because it is better to predict disease and have it not occur than to not predict a bean rust outbreak and have one occur.

The relation between asexual and sexual stage development was also quantified by logistic and simple linear regression of predicted probabilities. The occurrence of an outbreak of the asexual stage of *U. appendiculatus* was correctly classified in 65% of years and locations using the prior occurrence of the sexual stage in that region as the only predictor (P=0.023). The predicted probability of asexual stage occurrence explained 31.9% ($R^2 = 0.319$, $P<0.0001$) of the variability in asexual stage predicted probability given by $y = 0.594x + 0.31$, where y is the dependent response variable.

Additional work is underway to validate these bean rust forecast models and incorporate them within effective and economical integrated pest management strategies to minimize losses from this important pathogen in the Central High Plains region of the United States.

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Analysis of Rust Resistance in the Dry Bean CNC

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Introduction

Compuesto Negro Chimaltenango (CNC) is part of the differential set of dry bean (*Phaseolus vulgaris*) genotypes used to detect races of *Uromyces appendiculatus*, the bean rust fungus. CNC is resistant to all races of *U. appendiuclatus* found in the northern Great Plains. Because of this, the resistance in CNC may be useful for breeding programs. However, little is known of the genetics of rust resistance in CNC.

The eventual objectives of this research are to understand the genetics of bean rust in CNC and to use the resistance for breeding purposes. Toward that goal we have used CNC as a parent to develop a dry bean population that segregates for rust resistance, determined reaction of progeny to different races of the fungus used to detect specific resistance genes, and developed AFLP markers linked to gene(s) of interest.

Methods and Materials

Population development. CNC was crossed to the rust-susceptible 'Othello'. An $F_{2:4}$ population of 100 recombinant inbred (RI) lines was developed in the greenhouse using the single seed descent method.

Disease evaluations. The 100 F_2 individuals used for population development were inoculated with races 49 and 73 of the bean rust fungus (Stavely 1983). F_3 families and select F_4 RI lines were inoculated with race 49. Disease reaction was evaluated 12 to 14 days post-inoculation as described (Stavely 1984).

AFLP markers. PstI/MseI markers were generated according to Vos et al. (1995). Bulked segregant analysis (Michelmore et al. (1991) was used to facilitate identification of markers linked to resistance. DNA from F_4 bean lines homozygous resistant and homozygous susceptible to race 49 were used to form the bulks.

Results and Discussion

Disease reactions. Othello pinto bean was susceptible and CNC was resistant to races 49 and 73 of the bean rust fungus. The 100 F_2 individuals analyzed from Othello/CNC segregated 3:1 (resistant:susceptible) to both races 49 and 73 (Table 1). All combinations of susceptibility and resistance to the two races were found in the F_2 population (Table 2). This suggests the segregation of at least two rust resistance genes in the population, one effective against race 49 and another against race 73. However, this conclusion is tentative. Disease reactions to race 73 have been determined only one time and only with the F_2 population. By comparison, F_3

families segregated 29:45:26 (homozygous resistant:heterozygous:homozygous susceptible) to race 49. This confirms the segregation of a single resistance gene effective against this race. (P^2 = 1.18).

Table 1. Segregation ratios of 100 F_2 progeny to races 49 and 73. The expected ratio for a single dominant gene for resistance to each race was 75:25 (resistant:susceptible).

	Disease Reaction		
Race	Resistant	Susceptible	P^2
49	74	26	0.053
73	76	24	0.053

Table 2. Reaction of parents and select F2 individuals to races 49 and 73.

	Disease Reaction[#]	
F_2 individual.	Race 49	Race 73
2	S	S
3	R	R
7	R	S
8	S	R
9	R	R
10	R	S
11	S	R
39	S	S

[#]S = susceptible, R = resistant

AFLP markers. Select lines were analyzed at the F_3 and F_4 generations to determine reaction to race 49. Homozygous susceptible and homozygous resistant lines were identified and DNA was isolated and pooled for bulked segregant analysis. Several polymorphic AFLP bands have been identified associated with resistance (data not shown).

Future work. The population is being advanced to the F_6 generation. Future plans include replicated testing of population against multiple races, confirming the segregation of two independent rust resistance loci in the population, identifying the loci in question, and mapping and tagging the loci with molecular markers.

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Pathotype Variation and Sources of Resistance to the Common Bean Rust Pathogen in Southern Mozambique

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Introduction

Mozambique is a country in which more than 80% of the population practices subsistence agriculture. Most farms are 0.5 to 4.0 hectares where diverse crops are grown using traditional farming methods. Dry bean is cultivated both in monoculture and more often associated with corn during the rainfed season in the north and central regions, and during the cool season with irrigation in the south. Snap beans are planted in succession during the year in the south. The area of bean production is ~370,000 ha (includes other bean species) and the yield is ~750 kg/ha. Both Andean and Middle American origin beans are cultivated, but the majority are Andean types.

One of the major constraints for snap and dry bean production in southern Mozambique is rust caused by *Uromyces appendiculatus* (Pers.) Unger. The consecutive cultivation of beans makes the inoculum of the rust fungus available throughout the year, resulting in high incidence and severity of rust. High pathogenic variability in bean rust has been reported (2). Strategies for bean rust management include fungicides, cultural practices and disease resistance. However, in Mozambique, fungicides are expensive or not available and cultural practice modifications do not fit the cropping systems used. Disease resistance is the most effective and least expensive strategy for the farmers. For developing effective and durable rust resistance, the pathogenic variability of the pathogen needs to be studied. The objectives of this study were to identify pathogenic variability and sources of resistance to bean rust in southern Mozambique.

Materials and Methods

Rust infected bean leaves were collected in 11 bean fields in Chókwe, Chibuto, Maputo, Boane, Namaacha and Moamba regions during the middle growing season (July-August) in 2002. The samples were processed in the greenhouse, in Lincoln, Nebraska during fall-spring, 2002-2003. Field collections of urediniospores were increased on a nearly universal susceptible cultivar Pinto U.I. 114 (P114) and Early Gallatin. Twelve new standard bean differentials were inoculated with each field collection to isolate the single uredinia (pustules). Each single pustule culture was increased on the differential plant from which it was isolated. To avoid pathotype contamination, isolation of the single pustule was done before rupture of the leaf epidermis. If a plant had a mixed rust reaction, the single pustule was reisolated.

Rust inoculum was prepared by suspending 2.5 mg urediniospores in 30 ml of tween 20 solution (40 ul/1000 ml distillate water). Primary leaves of 7-day-old bean plant differentials were uniformly inoculated with each single pustule culture using a hand sprayer. Inoculated plants were put in a mist chamber at 100% RH and 21 ± 1^{o}C for ~16 hours before placing in the greenhouse at 22 ± 2^{o}C. The inoculation process was repeated twice. Disease reaction (urediniun size measured with a hand lens) was recorded using 1-6 standard grading scale 14 days after inoculation. The scale was converted to 1.1 - 6.1 quantitative disease score (1) and then assigned resistant, intermediate and susceptible reactions (Table 1).

Results and Discussion

A total of 69 pathotypes of *U. appendiculatus* were identified on the 12 bean differential lines/cultivars and the susceptible P114. Reaction on P114 was included because the virulence of isolates from the same field collection on this cultivar often differed from the reaction on the 12 differentials. Most of the Andean lines with resistance genes were susceptible to these isolates, and Montcalm showed a susceptible reaction to all isolates. However, Redlands Pioneer showed a resistant reaction to 34 isolates (49%) (Table 1). Most of the varieties cultivated in southern Mozambique are susceptible and are Andean origin beans. Genes from Middle American origin were resistant to most of the isolates (Table 1). The Ur-11 gene was resistant (no sporulation) to all 69 isolates. Ur-3, Ur-5 and Ur-11 rust resistance genes were also reported to be useful sources of resistance to rust pathogen populations from South Africa (2).

Conclusion

The Middle American beans provide resistance genes for rust from southern Mozambique. For variety development, one or more of the Middle American Ur-3, Ur-5, Ur-11 genes and the unknown gene of CNC should be incorporated into adapted germplasm as sources of resistance to the common bean rust pathogen.

Table 1. Reaction of bean differential cultivars to 69 rust isolates from southern Mozambique

Bean cultivar/ line	Gene pool *	Resistance gene	% resistant reactions	** Reaction to isolates		
				Resistant	Intermediate	Susceptible
1.Early Gallatin	A	Ur-4	6	4	0	65
2.Red-Pioneer	A	Unknown	49	34	24	11
3.Montcalm	A	Unknown	0	0	0	69
4.PC 50	A	Ur-9, Ur-12	13	9	48	12
5.GGW	A	Ur-6	29	20	22	27
6.PI 260418	A	Unknown	3	2	52	15
7.GN 1140	MA	Ur-7	29	20	22	27
8.Aurora	MA	Ur-3	90	62	2	5
9.Mex 309	MA	Ur-5	93	64	3	2
10.Mex 235	MA	Ur-3+	94	65	2	2
11.CNC	MA	Unknown	93	64	4	1
12.PI 181996	MA	Ur-11	100	69	0	0

* Gene pool: A=Andean, MA=Middle American. GGW=Golden Gate Wax; CNC=Compuesto Negro Chimaltenango.
** Grading scale: Resistant, grade 1.1 - 3.1; Intermediate, grade 3.4 - 4.1; Susceptible, grade 4.4 - 6.1.

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PHENOTYPIC AND GENOTYPIC VARIATION IN *UROMYCES APPENDICULATUS* FROM REGIONS OF COMMERCIAL PRODUCTION AND CENTERS OF COMMON BEAN DOMESTICATION

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INTRODUCTION

Common bean (*Phaseolus vulgaris*) was domesticated in two main centers of the Americas, the Andean and Middle American regions. It is thought that isolates of the bean pathogen *Uromyces appendiculatus* co-evolved within these regions with its only hosts, *Phaseolus* spp. The genetic diversity present in *U. appendiculatus* collected from wild (*Phaseolus vulgaris aborigineus*), weedy, landraces and breeder developed (commercial) beans from different regions in Honduras and Andean Argentina isolates from wild and commercial beans may give new insights into the co-adaptation of this pathogen with its host. In this study Argentinean *U. appendiculatus* isolates were compared with the Honduran isolates to determine if there are similarities or differences in phenotype and genotype related to host ecology.

MATERIAL AND METHODS

Rust infected leaves were collected from wild *P. vulgaris*, landraces, and breeder developed (commercial) lines in Honduras and Argentina. Single uredinium isolates were increased on a selective susceptible host and inoculated into the primary leaves of the new standard 12 bean rust differentials. Disease reaction was determined using a 1-6 grading scale (Mmbaga et al., 1996). Molecular characterization of the isolates was performed by extracting DNA of germinated uredinospores using the PEX method described by Linde et al. (1990). BOX- Rep PCR (Rademarker and DE Bruijn, 1997) was conducted for DNA amplification. The molecular data was analyzed using polymorphic bands scored using a binary system (1 and 0) and the simple matching co-efficient (SM) in NTSYS pc version 2.0. Dendrograms were constructed using hierarchical clustering by SAHN, unweighted pair grouping by UPGMA methods and the tree program in NTSYS.

RESULTS AND DISCUSSION

The virulence analysis of *U. appendiculatus* isolates on common bean differential lines showed that in general isolates collected from commercial varieties were more virulent than isolates collected from wild beans (Table1). Honduran pathotypes collected from wild beans were more virulent than pathotypes from Argentinean wild beans. The Honduran pathotypes from wild beans produced an average of 65% susceptible reactions on the 12 sources of resistance from both host gene pools, while the Argentinean pathotypes from wild beans produced only 46% susceptible reactions. The higher virulence and lack of gene pool specificity for host resistance observed in Honduran rust pathotypes collected from wild beans may be the result of the proximity of wild beans, landraces and commercial beans. Conversely the spatial isolation between wild and commercial beans in Argentina may contribute to the lower virulence of Argentinean pathotypes on the bean differential representing MA gene pool and to a higher specificity of Argentinean isolates for Andean resistance genes.

		Andean sources of resistance						Middle Amreican sources of resistance					
	Resistance genes	Ur-4			Ur-9	Ur-6		Ur-7	Ur-3	Ur-5	Ur-3+		Ur-11
Host	Differential host / Isolates	EG*	RP*	MO*	PC50	GGW*	PI26041	GN1140	AU*	MEX309	MEX235	CNC	PI181996
Commercial	ARG96-2-16	S**	S	S	S	S	R	S	S	R	R	R	R
Commercial	ARG96-2-12	R	S	S	R	S	S	S	R	S	R	S	S
Commercial	ARG96-2-13	S	R	S	S	S	S	R	S	S	S	S	R
Commercial	ARG96-2-P114	S	R	S	S	S	S	R	R	R	R	R	R
Wild	ARG96-1-13	S	R	S	S	S	S	R	R	S	R	R	R
Wild	ARG96-9-1	S	S	S	S	S	R	R	S	R	R	R	R
Wild	ARG96-9-6	R	R	S	R	S	R	S	R	R	R	R	R
Wild	ARG96-9-6	R	R	S	S	S	S	S	S	R	R	R	R
Commercial	HON02A-12-P114	S	R	S	R	S	S	S	R	S	R	R	S
Commercial	HON00-6-8	S	S	S	S	S	R	R	S	R	S	R	S
Commercial	HON02A-18-P114	R	S	R	R	S	S	S	R	S	R	S	S
Landrace	HON02A-48-P114	S	S	S	S	S	S	R	S	S	R	R	R
Landrace	HON00-2-19	R	S	S	R	S	S	S	S	S	S	S	R
Landrace	HON00-2-3	R	S	S	R	S	S	S	S	S	S	S	R
Landrace	HON02A-49-2	S	R	S	R	R	S	R	R	R	R	R	R
Wild	HON00-3-13	S	S	S	S	S	R	S	S	S	S	S	R
Wild	HON01-14-4	R	R	S	R	S	R	S	R	R	R	R	R
Wild	HON01-15-13	S	S	S	S	S	R	S	R	R	R	R	R
Wild	HON01-24-4	R	R	S	R	S	R	S	R	S	S	R	R
Wild	HON02-4-1	S	R	S	R	S	S	S	R	R	R	R	R

Table 1. Reaction of 12 bean rust differentials to *U appendiculatus* isolates collected on wild, landrace and commercial beans from Honduras and Argentina**.** *EG= Early Gallatin, RP= Red Pioneer, MO= Montcalm, GGW= Golden Gate Wax, AU= Aurora. **R=grade 1-3 (resistance), S= grade 4-6 (susceptibility).

Molecular analysis using Box-Rep-PCR grouped isolates collected from Honduras and Argentina based on geographic regions. These results agree with a previous study using RAPD-PCR and Box-Rep-PCR where *U. appendiculatus* molecular analysis grouped the isolates by geographic regions, suggesting that host ecology may play a role. In the present study neither virulence analysis nor Rep-PCR analysis found differences that discriminate between pathotypes from wild, landrace or commercial bean. Recently Rep-PCR was used to characterize molecular markers associated with pathotypes of *U. appendiculatus* (with avirulence or virulence to the *Ur-6* rust resistance gene) from two populations from Colorado and Nebraska. This study resulted in the grouping of the isolates by geographic regions using amplified bands generated by ATA-2. Sequence analysis of the bands showed a similarity to *Ada*-like transcription proteins, which are associated with gene activation in plant, animals, fungal and bacterial species.

CONCLUSIONS

- Rep–PCR in conjunction with phenotypic virulence can be used to characterize *U. appendiculatus* populations and assist in developing a database that can be used for resistance gene deployment in different geographic areas.
- More information about the resistance present in wild beans is needed to increase resistance gene sources available in beans and improve disease management of bean rust.

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Survey of RAPD and SCAR Markers Linked to the *Ur-6* Gene in Middle American and Andean Beans

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Park et al. (2003a) reported that six RAPD markers were detected in a coupling phase linkage with the *Ur-6* gene in an F_2 population from the Middle American (MA) common bean cross Olathe x Nebr. #1 sel. 27. The Andean gene was flanked by two coupling-phase markers OBC06.300 and OAG15.300 at distances of 1.3 cM and 2.0 cM. The coupling-phase RAPD marker OBC06.300 tightly linked to the *Ur-6* gene was converted into a SCAR marker based on the specific forward (GAAGGCGAGAAGAAAAAGAAAAAT) and reverse (GAAGGCGAGAGCACCTAGCTGAAG') 24-mer primer pair (Park et al., 2003b). The marker SOBC06.308, the name of the SCAR marker amplified with the specific primer pair, showed no recombination with the RAPD marker OBC06.300 in the F_2 population, and thus, the SCAR and RAPD markers were observed at the same locus on the linkage group. The SCAR marker SOBC06.308 was also closely linked to the *Ur-6* gene at 1.3 cM. Park et al. (2003a) also reported that among five repulsion-phase markers identified, OAY15.200 was the most closely linked to the *Ur-6* gene at 7.7 cM.

Our objective was to determine the presence or absence of the two RAPD markers OBC06.300 and OAG15.300, and the SCAR marker SOBC06.308 in 13 Andean beans as well as 24 MA Great Northern (GN), black, navy, and other bean cultivars and breeding lines with or without the *Ur-6* gene.

Rust resistant lines GN BelNeb, GN BelMiNeb, and navy BelMiDak carrying at least two different rust resistance genes were developed (Stavely et al., 1989, 1994). Particularly, the *Ur-6* gene was incorporated into BelNeb-RR-1 and BelMiNeb-RMR-4. Five BelNeb, BelMiNeb, and BelMiDak lines were noted resistant to race 51. The marker fragments were present in GN BelMiNeb-RMR-4 (Table 1), while, due to recombinations of the gene with the markers, they were absent in GN BelNeb-RR-1. Other MA beans without the gene, mostly susceptible to race 51, lacked the marker fragments.

Golden Gate Wax, resistant to race 51, possessed the coupling-phase RAPD and SCAR markers tightly linked to the *Ur-6* gene (Table 1). This result is consistent with the finding of Stavely et al. (1983), who reported that the wax bean cultivar also possessed the *Ur*-6 gene. However, the three markers were also present in six Andean cultivars without the *Ur-6* gene. The marker fragments were absent in other six susceptible Andean beans lacking the gene. This would be expected because the gene is originally from the Andean gene pool. Miklas et al. (1993) and Haley et al (1993) reported similar results that Andean beans lacking the *Ur-4* gene had a marker linked to the Andean gene, and MA beans lacking the *Ur-5* gene possessed a marker linked to the MA gene.

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Table 1. Presence (+) or absence (!) of two coupling-phase RAPD markers OBC06.300 and OAG15.300, and the SCAR marker SOBC06.308 tightly linked to the *Ur-6* gene in Middle American (MA) or Andean (A) bean cultivars and breeding lines resistant (R) or susceptible (S) to rust race 51.

Cultivar/breeding line		Resistance	Race	Coupling-phase markers		
Entry	Origin	gene (*Ur*)	51	OBC06.300	OAG15.300	SOBC06.308
				GN GN1140	MA	7
GN Harris	MA		S	-	-	-
GN Marquis	MA		S	-	-	-
GN Valley	MA		S	-	-	-
GN Nebr.#1	MA		S	-	-	-
GN Nebr.#1sel. 27	MA		S	-	-	-
GN P#44	MA		S	-	-	-
GN P#59	MA		S	-	-	-
GN P#92	MA		S	-	-	-
GN BelNeb-RR-1	MA	5, 6 & 7	R	-	-	-
GN BelMiNeb-RR-1	MA	4 & 11	R	-	-	-
GN BelMiNeb-RMR-4	MA	4, 6 & 11	R	+	+	+
GN BelMiNeb-RMR-7	MA	3, 4 & 11	R	-	-	-
Black HT 7719	MA		S	-	-	-
Black A 55	MA		S	-	-	-
Navy Seafarer	MA		S	-	-	-
Navy Midland	MA		S	-	-	-
Navy BelMiDak-RR-8	MA	4 & 11	R	-	-	-
Aurora	MA	3	S	-	-	-
Ecuador 299	MA	3	S	-	-	-
Mex 309	MA	5	S	-	-	-
Viva	MA		S	-	-	-
BAC 6	MA	BAC 6	S	-	-	-
Chichara	MA		S	-	-	-
Golden Gate Wax	A	6	R	+	+	+
Early Gallatin	A	4	R	+	+	+
Brown Beauty	A	4	R	+	+	+
Redkloud	A		R	+	+	+
Miss Kelly	A		S	+	+	+
Montcalm	A		S	+	+	+
PC-50	A	9	R	+	+	+
US 3	A	8	S	-	-	-
Jose Beta	A		S	-	-	-
XAN-159	A		S	-	-	-
Pompadour Q1	A		S	-	-	-
Pompadour D	A		S	-	-	-
Pompadour U	A		S	-	-	-

Presence of Bean Common Mosaic Necrotic Virus in the Dominican Republic: A New Challenge for Dry Bean Breeders and Growers in the Caribbean Region

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Bean Common mosaic (BCM) is a serious and widespread disease of common bean (*Phaseolus vulgaris* L.). The BCM virus infects mainly *Phaseolus* spp. and *P. vulgaris* is the primary host. The virus spreads via seed, pollen and numerous aphid species. Bean common mosaic necrosis virus (BCMNV) is a new species with three strains (NL-3, -5 and -8) and is separate from bean common mosaic virus. BCMNV causes "black root" symptoms on beans with the *I* gene and mosaic symptoms on non-*I* gene varieties. Black root symptoms were observed on black seeded varieties such as Arroyo Loro Negro in 1999 in the San Juan Valley of the Dominican Republic. The objectives of this study were to determine the source of BCM/BCMN infection and to determine strain of the virus.

Materials and Methods

For commercial fields, leaves from bean plants showing mosaic symptoms were collected from grower fields in the valley. Leaves were macerated in phosphate buffer and applied to leaves of differential bean varieties with Carborundum powder. After 21 days at 25± 3ºC plants were evaluated for top necrosis, susceptible mosaic symptoms or resistance. ELISA tests were also conducted.

For foundation seed lots, one kg of seed was tested per lot. A randomly selected 1000 seeds were grown in the greenhouse at 25± 3ºC for 30 days and evaluated for mosaic symptoms. Leaves from symptomatic plants were evaluated by inoculation to the strain-separating differentials. ELISA tests were used to confirm inoculation tests.

Results and Discussion

BCMNV was identified in the San Juan Valley (southwest) for the first time in 1999 and San Rafael del Yuma (east) region in 2003 of the Dominican Republic. Symptomatic plants underwent multistage testing for strain determination: ELISA for general potyvirus was positive, specific ELISA to distinguish BCMV and BCMNV was positive for BCMNV, host differential subset for BCMNV was positive for NL-8 and molecular characterization using RT-PCR was positive for BCMNV. By 2003, the virus had spread throughout the valley (Table 1). When foundation seed was tested for BCMNV, percent infected seed ranged from 0.2 to 22.1 (Table 2) and 60% of the seed lots were infected with NL-8.

Foundation seed from farmer fields was mixed with off types that developed mosaic symptoms along with commercial varieties with the *I* gene that developed "black root". The contamination of foundation seed is the likely source of the increased incidence of the virus. All the red mottled varieties grown in the San Juan Valley are susceptible. Thus, the introduction of the protected *I* gene (*I* gene with *bc-3* or *bc-1*^{2}) into new red mottled and black seeded varieties is a priority for the Dominican Republic as well as Haiti, where BCMNV is also found.

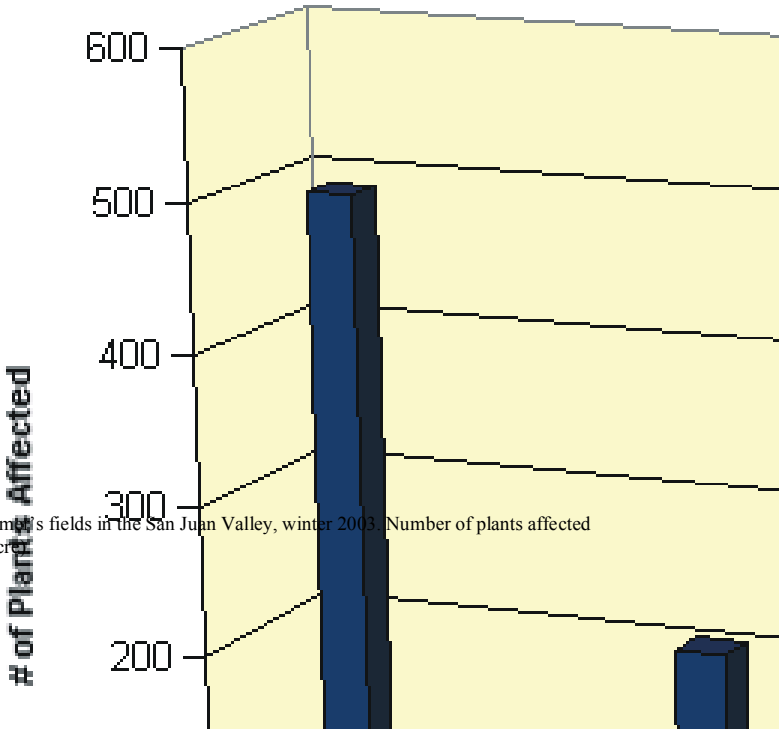

Table 1. BCMNV incidence in farmer's fields in the San Juan Valley, winter 2003. Number of plants affected (symptomatic) is per tarea, (0.16 acre).

Table 2. Foundation seed lot infection by BCMNV in the Dominican Republic in 2003

Seed Source Locality	Variety	% Infected Seed*
Chalona	Venezuela-44	10.3
Hato del Padre	Venezuela-44	9.1
Sabana Alta	Venezuela-44	9.7
Barranca	Venezuela-44	13.5
Chalona	Venezuela-44	10.3
Punta Cana	Venezuela-44	22.1
Barranca	Arroyo Loro Negro	0.2
Cuenda	Arroyo Loro Negro	4.8
Chalona	Arroyo Loro Negro	0.9
Solorin	Arroyo Loro Negro	2.6
Magueyar	Arroyo Loro Negro	5.9
Pedro Corto	Negro P.G.	0.5
Ginova	Negro Largo	0.6
Km.11	Blanco Commercial	5.0

% of 1000 seed.

EVIDENCE OF A GENOMIC RECOMBINANT BETWEEN BEAN COMMON MOSAIC VIRUS AND BEAN COMMON MOSAIC NECROSIS VIRUS

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Bean common mosaic virus (BCMV) and *Bean common mosaic necrosis virus* (BCMNV) belong to the genus *Potyvirus* (Family: *Potyviridae*) and can cause major losses to bean production worldwide. The common NL-3 D strain of BCMNV causes top necrosis on bean host differentials with resistance genes *I* and *I+bc-1*. Recently, a more virulent strain of NL-3 was discovered in Kimberly, Idaho, and designated NL-3 K. Strains NL-3 D and NL-3 K both reacted similarly across the host group differentials but responses were different across some *bc-3* and *I + bc-3* genotypes (Miklas et al, 2000; Strausbaugh et al, 2003). Characteristic*s* of NL-3 K include more severe symptoms with earlier onset. Tests by ELISA indicated that NL-3 K was not a mixed infection with any other strain of BCMV or BCMNV. Consistent with NL-3 D, results from ELISA assays using monoclonal antibodies placed NL-3 K in Pathogroup 6. The objective of this research was to locate significant differences in the genome of NL-3 K that may be responsible for changes in host response to the virus.

Materials and Methods

NL-3 K and NL-3 D were inoculated across a set of host group differentials as checks and for comparison of symptom response. The viruses were also inoculated across additional bean genotypes containing the *bc-3* or *I + bc-3* resistance genes. Lines USLK-1, USLK-2, USLK-3, USDK-4, USDK-5, USWK-6, USCR-7, USCR-9 each contained the resistance genes *I+ bc-3* while USCR-8 and P94231 contained only the *bc-3* gene (Miklas et al., 2000). For virus purification, NL-3 K was propagated in bean 'Sutter Pink' and harvested 12-14 days post inoculation. After two stages of differential centrifugation, the partially purified virus was separated by isopycnic banding in a cesium chloride gradient. cDNA was synthesized from the purified viral RNA using SuperScript II reverse transcriptase and oligo(dT_{24}) primer (3). The cDNA was cloned into pBluescript SK+ using the *EcoRV* cloning site. Plasmid inserts obtained from five selected overlapping clones were sequenced using the dye termination system.

Results and Discussion

Reactions of plants included in the host group differential set and germplasm containing *bc-3* or *I + bc-3* are detailed in Table 1. None of the *bc-3* or *I + bc-3* germplasm lines expressed symptoms after inoculation with NL-3 D, but symptoms caused by NL-3 K included necrotic local lesions, and severe vein necrosis. Notable exceptions were USWK-6, USCR-7 and USCR-9 as where NL-3 K also failed to elicit a host response (notated in Table 1 Host Genes as *I, bc*-3*a*). A recessive gene is responsible for the differential reaction of these genotypes to NL-3 K but it has not yet been determined if it is a new allele at *bc-3* or a gene at a different locus.

The entire genome sequence generated for NL-3 K was compared to that of NL-3 D. In addition the sequence was analyzed using BLAST (National Center for Biotechnology Information, Bethesda, MD). The first 109 deduced amino acids beginning at the 5' N terminal

of the P1 protein of NL-3 K resulted in 86% identity with the cowpea strain of BCMV (BCMV-cowpea). Five subsequent amino acids beginning at position 110 shared no identity with either BCMV-cowpea, NL-3 D, or any other known plant virus. The remaining 3186 amino acids of NL-3 K downstream shared 99% identity with NL-3 D. These data suggested that NL-3 K was a genomic recombinant of NL-3 D and BCMV-cowpea. Because the five amino acids shared no similarities with BCMV-cowpea or NL-3 K, they were considered the transition zone representing the recombination event between BCMV-C and NL-3 K. The genome length of NL-3 K was increased by 293 nucleotides located at the 5' end when compared to NL-3 D, resulting in a final genome size of 9905 bp. To further demonstrate that plants did not contain a mixed infection of NL-3 D and BCMV-cowpea, PCR primers were designed to flank the recombination event of NL-3 K. RT-PCR reactions on total nucleic acid preparations from infected bean plants produced amplification products from the NL-3 K strain but not from bean infected only with NL-3 D or BCMV-cowpea (Fig. 1). Similarly, primers specific to NL-3 D and BCMV-cowpea amplified products only from the plants infected with the respective viruses. Hence, NL-3 D and BCMV-cowpea can be differentiated by RT-PCR using strain-specific primers. The biological data, sequence analysis and RT-PCR reactions clearly demonstrate that NL-3 K is a naturally-occurring and stable genomic recombinant between potyviruses BCMNV strain NL-3 D and the cowpea strain of BCMV. This is the first report of genomic recombination between two distinct virus species within the family *Potyviridae*.

Table 1. Differentiation of NL-3 D and NL-3 K strains of BCMNV across a set of germplasm lines and a subset of host group differential cultivars.

Germplasm	Host Genes	NL-3 D	NL-3 K
USLK-1	*I, bc-3*	ns	NLL, SVN, Ch
USLK-2	*I, bc-3*	ns	NLL, SVN, Ch
USLK-3	*I, bc-3*	ns	NLL, SVN, Ch
USDK-4	*I, bc-3*	ns	NLL, SVN, Ch
USDK-5	*I, bc-3*	ns	NLL, SVN, Ch
USWK-6	*I, bc-3a*	ns	ns
USCR-7	*I, bc-3a*	ns	ns
USCR-8	*bc-3*	ns	ns; (ELISA +)
USCR-9	*I, bc-3a*	ns	ns
P94231	*bc-3*	ns	MM
Host Group Differentials			
Sutter Pink	none	sM, st	sM, st, pd
Black Turtle II	none	sM	sM, st, pd
UI-123	*bc-1*2**	mM	M
Red Kloud	*I, bc-1*2**	P=VN; S=ns	P=VN; S=VN
Jubila	*I, bc-1*	VN	NS
IVT 7233	I, bc-2*2*	LL	LL

Legend

Ch =chlorosis	sM =severe mosaic
mM =mild mosaic	st =stunting
NLL =necrotic local lesions	SVN =systemic vein necrosis
ns=no symptoms	
P=primary leaf	VN =vein necrosis
S=secondary leaves	pd =plant death

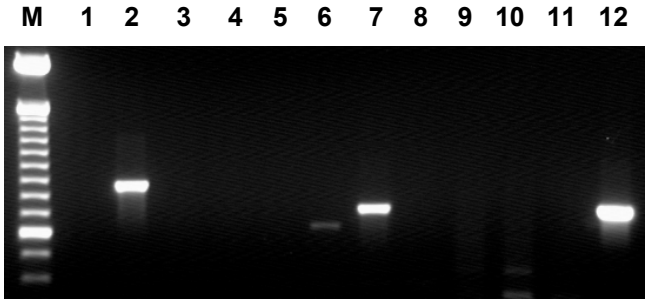

Figure 1. Agarose gel showing amplification products from RT-PCR of total nucleic acid extracts using virus-specific primers designed to detect NL-3 K (lanes 1-4), NL-3 D (lanes 5-8), and BCMV-cowpea strain (lanes 9-12) in bean. Lane 1: 100 bp marker; lanes 1, 5, 9: healthy bean; lanes 2, 6, 10: NL-3 K; lanes 3, 7, 11: NL-3 D; lanes 4, 8, 12: BCMV-cowpea strain.

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A PCR-based Assay for Differentiation of Clover Yellow Vein Virus and Bean Yellow Mosaic Virus in Common Bean

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Clover yellow vein virus (CYVV) and *Bean yellow mosaic virus* (BYMV) (Family: *Potyviridae*) are important viruses of snap and dry bean. Serological differentiation of the two viruses has typically been difficult because of the similarity of their coat proteins. In addition, symptoms on bean are highly variable and this often results in erroneous identification of field isolates. Symptoms vary greatly according to virus strain, cultivar and growth stage at time of infection. Typical symptoms include mild to severe mosaic or yellow mosaic, leaf malformation and stunting. Selected strains of both viruses are reported to cause vein necrosis and apical or top necrosis resulting in plant death. Pod distortion or malformation may occur on plants infected with either virus but is generally more severe on those infected with CYVV.

In the Great Lakes region during the 2000-2003 growing seasons, snap beans were observed showing extensive pod necrosis or "chocolate pod" in many fields (3). CYVV was suspected as the causal agent based on preliminary host range studies but identity of the pathogen was not conclusive. Monoclonal antibodies have been produced that distinguish between BYMV and CYVV (1, 2) in ELISA, but these antisera are not readily available. Hence, a reverse transcription polymerase chain reaction (RT-PCR) assay has been developed that can unambiguously distinguish between CYVV and BYMV in single and mixed infections of bean.

Field isolates and nucleic acid preparation. Bean samples exhibiting mosaic symptoms and pod necrosis were collected from fields in Washington State, Idaho, and Wisconsin during 2000-2003. A strain of CYVV originating in Oregon and a strain of BYMV from bean in Washington were used as positive controls. Their identity was verified by comparing the nucleic acid sequence of their respective viral coat proteins and 3-prime non-translated regions to known sequence data available in GenBank. Total nucleic acid was extracted from bean samples using a modified method of Dellaporte *et al.* (4).

Primer design. DNA primers for CYVV and BYMV were designed using available sequence in GenBank including sequence date from the isolate of BYMV from Washington and the isolate of CYVV from Oregon. Target areas for forward and reverse primers were identified in the 5' and 3' regions of the viral coat protein genes, respectively.

CYVV-F 5'-TTGATGACAGCCAGATG-3'

CYVV-R 5'-AATCGTGCTCCAGCAATG-3'

BYMV-F 5'-GCGCTCAAGCACCTATACACT-3'

BYMV-R 5'-CTCGCTCTACAAAGATCAG-3'

RT-PCR conditions. Reverse transcription reactions were carried out using Malone Murine Leukemia Virus reverse transcriptase with the respective reverse primers for each virus, and incubated at 42 C for one hr. Two microliters of the cDNA product were added to the PCR mixture and amplified with 25 cycles of 94 C for 1 min, 58 C for 1 min, 72 C for 1 min, and a final extension at 72 C for 10 min. Amplification products were resolved through 1.4% agarose gels in TAE buffer.

Results and Discussion

RT-PCR using DNA primers designed for the detection of CYVV produced amplicons of the expected size (844 bp) from bean samples infected with CYVV but it did not amplify BYMV. Similarly, the BYMV primers amplified products (1113 bp) from plant samples infected with the homologous virus.

Each primer pair produced the expected amplicon from extracts infected with both CYVV and BYMV. These primers could be used in a multiplex reaction with no interference.

Primers designed to detect CYVV were used to demonstrate that this virus was directly associated with pod necrosis or "chocolate pod" symptoms on snap bean collected from production fields in Wisconsin. BYMV was not detected in leaves or pods of these samples. Neither *Cucumber mosaic virus* nor *Alfalfa mosaic virus* were detected in the symptomatic pods when evaluated using ELISA, although mixed infections have been known to occur. Interestingly, leaf samples of many plants bearing pods infected with CYVV did not contain detectable levels of this virus. In addition, when selected bean varieties were mechanically inoculated with leaf extracts from "chocolate pod" samples from Wisconsin, all inoculated plants were negative for CYVV, but plants inoculated with extracts of symptomatic pods taken from the same plants were positive for the virus**.** Thus, CYYV could be predominantly localized in the pods of diseased plants, while the virus was absent or present in very low titer in the vegetative portion of the same plants. BYMV was not detected in leaves or pods of any of the samples from Wisconsin infected with CYVV.

This work has demonstrated that the RT-PCR assay was able to unambiguously differentiate between BYMV and CYVV in single or in mixed infections in bean, and can be easily conducted in a single day. Furthermore, all samples of pods exhibiting typical 'chocolate pod' symptoms were positive for CYVV. This suggests that CYVV should be considered an important virus associated with the "chocolate pod" symptom in snap beans.

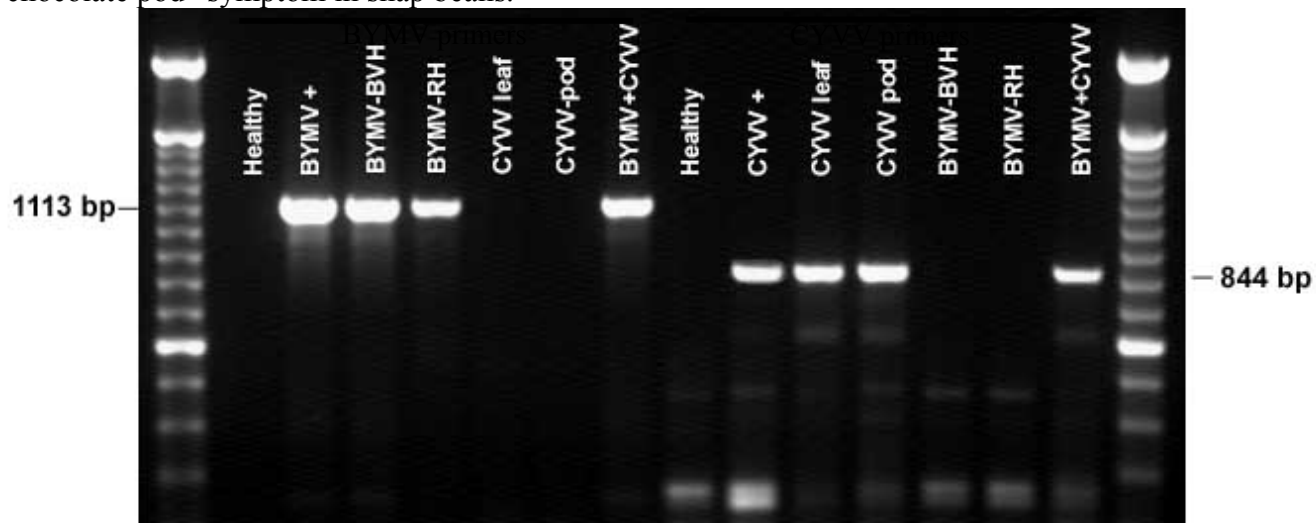

Figure. 1. Agarose gel showing amplicons of RT-PCR using primers specific to BYMV and CYVV. Total nucleic acid was extracted from bean tissue infected with BYMV (strains BVH and RH) or CYVV in naturally-infected snap bean from Wisconsin. BYMV+CYVV indicates samples with mixed infection.

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Integrating Planting Time and Seed Treatment to Control Bean Root Rot

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In central Minnesota, most dry beans are produced under irrigation on sandy soils. A complex of soilborne pathogens including *Fusarium solani, F. oxysporum* and *R. solani* are causing increasingly severe root rot in dry beans grown in this area. A variety of factors contribute to the increase in root rot severity. These include the unavailability of resistant varieties, crop sequences that include alternate hosts for *F. solani* and *R. solani*, shortened intervals between crops of dry beans, the use of high rates of nitrogen fertilization, low soil pH and the adoption of conservation tillage practices implemented to reduce soil erosion. An additional problem associated with soils in Minnesota is the presence of a well-developed Bt horizon that contains higher clay and silt content than layers above or below it (Wang, et. al. 2003). This horizon is characterized by increased bulk density, reduced hydraulic conductivity, and increased soil strength. Shallow tillage and irrigation tend to exacerbate these characteristics (Allmaras, et. al., 1988). The presence of this layer prolongs periods of soil saturation because it impairs drainage and contributes to poor root development due to mechanical impedance. In addition to these factors, recommended early planting dates may also contribute to increase root rot severity. Bean growers attempt to sow dry beans from early to mid-May. Soil minimum temperatures observed at 10 cm depth are normally less than 10 ^{o}C throughout May (Fig.1). Dry bean seed may remain in the ground for extended periods of time before emergence. Seed treatments applied to reduce root rot severity are frequently ineffective under these conditions. The purpose of this study was to determine if delayed planting to avoid low temperatures and saturated soils will be decreased root rot severity and increase grain yield and to determine if the effectiveness of seed treatment will improve as the period of time that seed remains un-germinated is reduced.

The study was conducted at the Central Lakes Agriculture Center, near Staples, MN. Cultivar 'Montcalm' was sown at 70 kg ha^{-1} and arranged in a split-split design with sowing time in the main plots and seed treatment with Apron/Maxx and inoculation with *Bacillus subtilis* and *Rhizobium* in the subplots. When dry beans were sown at four different planting dates in a field naturally infested with the root rot pathogens, disease severity was lower in the second and fourth times of planting (DS 3.5 and 3.4, respectively) and it was not influenced by seed treatment (Table 1). Plant emergence was higher at the first planting date and was not influenced by seed treatment (Table 2). Root dry weight was unaffected by both planting date and seed treatment. However, plant biomass was significantly less in the first, second and third planting dates. Seed treatment had little effect on plant biomass. Yields were significant higher at the fourth planting date than at the first, second and third planting dates. Seed treatment did not have any effect on yield. Yield was less in plots planted at the second date where plant dry weight and plant emergence was also the lowest. Traditionally bean producers in Minnesota sow dry beans by the third week of May. Early planting increases the likelihood of root rots because of cool soil temperatures and excess moisture. Results from this study indicate that dry bean planted when soil average temperatures are above 15^{o}C produced greater yields than dry beans planted at lower temperatures.

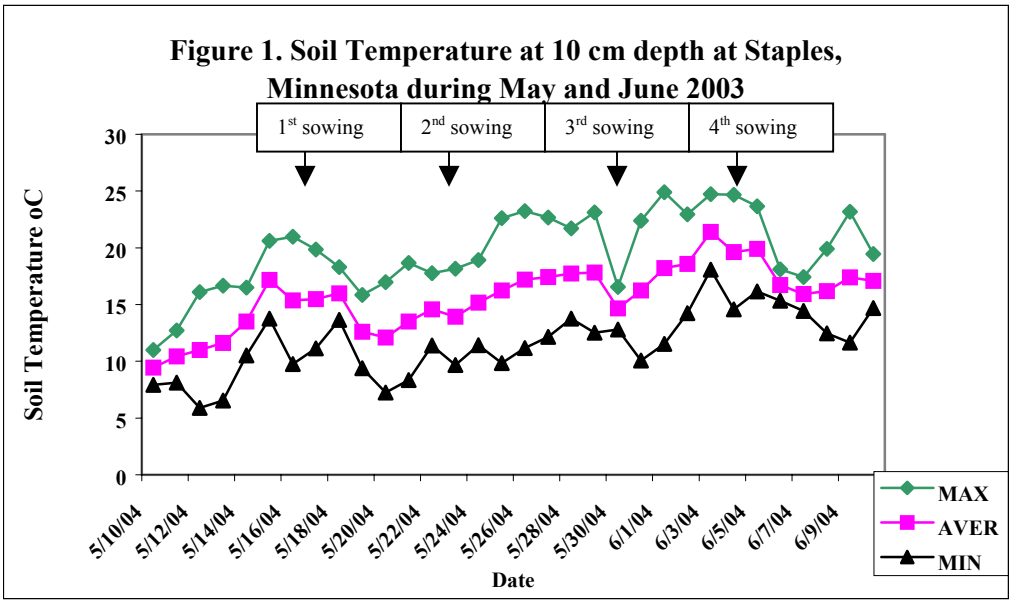

Figure 1. Soil Temperature at 10 cm depth at Staples, Minnesota during May and June 2003

Table 1. Influence of sowing time on disease severity, emergency, root and plant dry weight, and yield of dry bean.

		Date of sowing			
	May 16	**May 22**	**May 29**	**June 5**	**Average**
Disease severity (1-9)	5.0 a*	3.5 b	4.8 a	3.4 b	4.3
Emergence # of plants	118 a	91 b	92 b	95 b	99
Root dry weight (g/plant)	0.53 a	0.56 a	0.57 a	0.57 a	0.55
Plant dry weight (g/plant)	2.1 bc	1.9 c	2.5 b	3.0 a	2.3
Yield (Kg/ha)	521 b	490 b	537 b	693 a	

* Values with different letters in the same column are significantly different at $P = 0.05$.

Table 2. Influence of seed treatment on disease severity of dry bean.

		Date of sowing			
	May 16	**May 22**	**May 29**	**June 5**	**Average**
Untreated	5.0	3.5	4.8	4.4	4.6 a
Treated	3.4	3.3	4.8	4.6	4.0 a

- Values with different letters in the same column are significantly different at $P = 0.05$.

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Genetic Variability of New Subgroups of *Rhizoctonia solani*, Cause of Web Blight of Dry Beans: Implication for Resistance Breeding and Disease Management

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Web blight of dry beans is caused by *Thanatephorus cucumeris* (Frank) Donk [Anamorph: *Rhizoctonia solani* (Kuhn)]. This disease is endemic in the Central America and Caribbean region. Examples of economic losses include US$7.1 million in El Salvador in 1993, 19% of bean production acreage damaged in Honduras in 1993 and 30-60% yield loss plus 50% bean seed damage in three provinces in the Dominican Republic in 1994. Web blight management is limited to use of a fungicide which is costly and not always effective. No web blight resistance is available at present in commercial varieties. Use of mulch for management of web blight is only effective for specific members of pathogen groups and particular locations.

Rhizoctonia solani is a complex species composed of subgroups within Anastomosis Groups. Members of at least six subgroups cause symptoms of web blight. Variability among members of these subgroups comes from virulence, fungicide resistance, optimal growth temperature and epidemiology (disease development rate, fungal propagule type, dissemination and survival). The objective of this study was to determine phenotypic and genotypic variation in *R. solani* isolates from bean fields throughout the Americas.

Materials and Methods

New subgroups of *R. solani* were determined by PCR-RFLP of the internal transcribed spacer region of rDNA from 45 web blight pathogen isolates from South and Central America plus the Caribbean in comparison to data reported by Carling et al. (1). Amplified product sequence generated by specific primers were compared to all known *R. solani* sequences reported in GenBank. Virulence was determined by the detached leaf test (DTL) (2).

Results and Discussion

New subgroups AG-1-IE, AG-1-IF and AG-2-2 WB are reported. These subgroups are associated with distinct web blight symptoms on common bean and can be distinguished by primers (Fig. 1). These three subgroups also differ in virulence as seen on the DLT using 28 lines/varieties of bean with some levels of resistance to web blight (Fig. 2). Isolates of AG-2-2 WB from wild *Phaseolus* spp. and commercial varieties have sequence similarity and are similar in other characteristics. None of the primers amplified isolates from the *R. solani* root and stem rot group (AG-4).

Genetic variation of the web blight pathogen can affect disease management and should be considered for dry bean breeding programs attempting to incorporate web blight resistance and other disease management strategies such as use of mulch.

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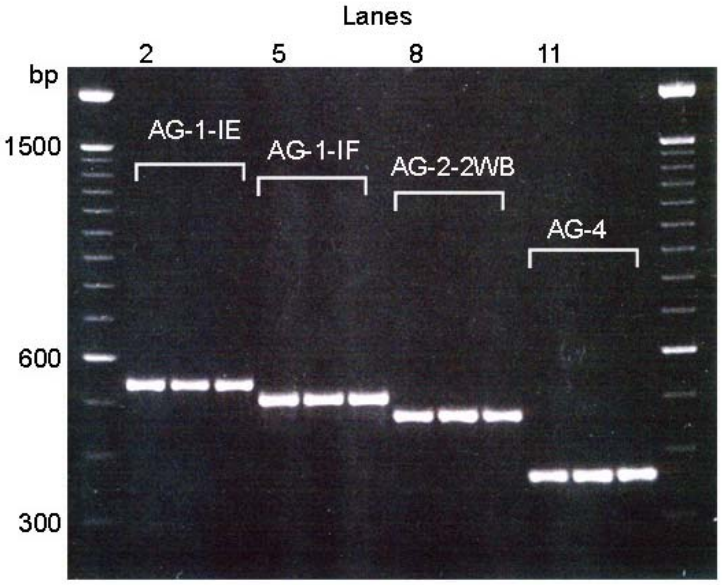
Figure 1. Amplifications with primers WB-A (lanes 2-4), WB-B (lanes 5-7), 2-2WB (lanes 8-10) and AG4 (lanes 11-13). WB isolates by lane-2: G7, 3: P53, 4: PR48, 5: BV3, 6: BV5, 7: BV7, 8: H2002-1, 9: DR-LV-1, 10: AL202. Stem rot isolates by lane-11: PC-50, 12: PC-50 DR63-1, 13: PC-50 DR633.



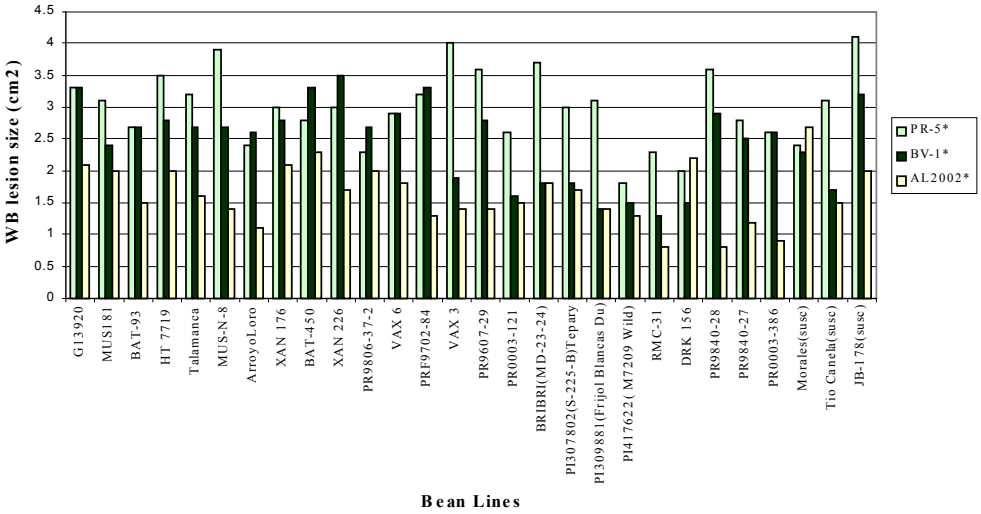


Figure 2. Virulence (lesion size) of three isolates representing the three subgroups on 28 cultivars/lines of bean with partial resistance to web blight. *PR-5 = AG-1-IE; BV-1 = AG-1; Al2002 = AG-2-2WB

Andean beans with Resistance to Angular Leaf Spot and Virulence Diversity of *Phaeoisariopsis griseola* in Southern and Eastern Africa

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Introduction

Andean beans are the preferred class of beans in several Southern and Eastern African countries. In this region, angular leaf spot (ALS), caused by *Phaeoisariopsis griseola*, is the most widespread and economically important disease of the common bean. Production losses attributed to ALS in Southern Africa are estimated at 93,500 tons (2). Bean cultivars with genetic resistance are needed to effectively control ALS in this region. However, the success of these resistant cultivars can be marred by the virulence diversity of *P. griseola*. This pathogen has many races that often vary from one location or year to another. To address the ALS problem, a project was initiated in Malawi to find Andean beans with ALS resistance and to explore the virulence diversity of the ALS pathogen in Southern and Eastern Africa.

Results and Discussion

CAL 143, an Andean bean line well adapted and with high yield potential in Africa, was free of ALS under field conditions at Bunda, Malawi, during the crop season of 1992-93 and at Bembeke, Malawi, during 1993-94, 1994-95 and 1995-96 (Table 1). Two other Andean bean lines, AND 277 and AND 279, were also ALS resistant under field conditions at Bembeke. CAL 143 was also ALS resistant under field conditions in South Africa, Tanzania, and Zambia, but it was susceptible in Uganda. This susceptibility was attributed to the presence of a race of the ALS pathogen in Uganda that was not present in the other countries.

The virulence diversity of 15 isolates of *P. griseola* collected in Southern and Eastern African countries was characterized by inoculating each isolate on a set of 12 differential cultivars: six Andean and six Middle American. These isolates were characterized as nine different races of *P. griseola* (Table 2). Five of six isolates from Malawi and two of seven from Uganda, all obtained from large-seeded Andean beans, were characterized as four different Andean races. These races typically were compatible only or mostly with Andean differential cultivars (Table 2). The other five isolates from Uganda, and one each from Malawi, Rwanda, and the Democratic Republic of Congo, obtained from small or medium-seeded Middle American beans, were characterized as five different Middle American races. These races were compatible with both Andean and Middle American differential cultivars. When inoculated under greenhouse conditions with each of these isolates, CAL 143 was resistant to all but one of these isolates (Data not shown). CAL 143 was susceptible only to Ugandan isolate PG2UGD, obtained from a medium-seeded bean that was characterized as race 63-21 (Table 2). It is plausible that an isolate of race 63-21, present in Uganda but not in the other countries, rendered CAL 143 susceptible to ALS under field conditions in Uganda.

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Table 1. Reaction of CAL 143 and other common bean cultivars to the angular leaf spot pathogen *Phaeoisariopsis griseola* under field conditions at Bembeke, Malawi, during the 1993 to 1996 crop seasons

Bean Cultivar	ALS Disease Score and Reaction Type[a]					
	Crop Seasons 1993-94	Reaction	1994-95	Reaction	1995-96	Reaction
1. Preliminary Bean Yield Trial (PBYT)						
Andean						
CAL 143	3	R	4	I	3	R
Nasaka	8	S	4	I	-	-
CAL 113	5	I	6	I	6	I
Middle-American						
A 286	2	R	-	-	-	-
EMP 308	-	-	-	-	3	-
2. Southern African Regional Bean Yield Trial (SARBYT)						
Andean						
CAL 143	2	R	2	R	3	R
Nasaka	7	S	-	-	-	-
Phalombe/Local	6	I	9	S	7	S
Middle American						
BAT 477	2	R	-	-	-	-
Nandi	-	-	4	I	4	I

[a] ALS disease evaluations using a 1 to 9 rating scale, where 1 = No visible symptoms of the disease and 9 = very severe symptoms; (1) Schoonhoven and Pastor-Corrales, 1987. ALS reaction type: 1-3 = Resistant (R); 4-6 = Intermediate (I); 7-9 = Susceptible.

Table 2. Identification and virulence phenotype of 15 isolates of *Phaeoisariopsis griseola* (PG) obtained in common bean-producing countries in Africa. Isolates from large-seeded beans and Andean races are highlighted

		Bean Differential Cultivars[b] /and their Binary Number Value[c]												
		Andean						Middle American						Virulence
PG Isolate	Origin	A	B	C	D	E	F	G	H	I	J	K	L	Phenotype
ID[a]	Seed size	1	2	4	8	16	32	1	2	4	8	16	32	(Race)
PG4MWI	L		b	c	d	e								30-0
PG5MWI	L		b	c	d	e								30-0
PG2MWI	L	a	b	c	d	e								31-0
PG3MWI	L	a	b	c	d	e								31-0
PG6MWI	L		b	c	d	e		g		i				30-5
PG3UGD	L	a	b	c	d	e	f	g	h	i				63-7
PG4UGD	L	a	b	c	d	e	f	g	h	i				63-7
PG2UGD	M	a	b	c	d	e	f	g		i		k		63-21
PG1UGD	S	a	b	c	d			g	h	i			l	15-39
PG1MWI	S	a	b	c	d	e		g	h	i			l	31-39
PG5UGD	M	a	b	c	d	e		g	h	i			l	31-39
PG6UGD	S	a	b	c	d	e		g	h	i			l	31-39
PG7UGD	S	a	b	c	d	e		g	h	i			l	31-39
PG1RUA		a	b	c	d	e	f	g	h	i			l	63-39
PG1ZAR		a	b	c				g	h	i		k	l	7-55

[a] Country of origin of isolate: MLW = Malawi, UGD = Uganda, RUA = Rwanda, ZAR = Democratic Republic of Congo (Formerly Zaire).
[b] Andean differential cultivars: A =Don Timoteo, B = G11796, C = Bolón Bayo, D = Montcalm, E = Amendoin, F = G5686;
Middle American differential Cultivars: G = PAN 72, H = G 2858, I = Flor de Mayo, J = Mexico 54, K = BAT 332, L = Cornell 49242.
Lower case letters a to i indicate a compatible host pathogen interaction. [c] Binary values for the differential cultivars are: A and G = 1, B and H = 2, C and I = 4, D and J = 8, E and K = 16, and F and L = 32. The sum of the values of the susceptible cultivars will give the binary number of that specific race. A hyphen is used to separate the sum of the Andean and Middle American cultivars; e.g., Race 30-5 = virulent on Andean cultivars B, C, D, and E and on Middle American Cultivars G and I.

Resistance to Multiple Races of *Fusarium* Wilt in Common Bean

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Fusarium wilt of dry bean caused by *Fusarium oxysporum* Schl.:Fr. f. sp. *phaseoli* Kendrick & Snyder (Fop) causes rapid yellowing of foliage, defoliation, and can ultimately cause severe yield loss (4). Woo et al. (7) characterized five races of Fop based upon RAPDs, RFLPs and vegetative compatibility groups. Ribeiro and Hagedorn (3) reported that resistance in Andean germplasm was controlled by one or two major genes, depending on race, while Cross et al. (1) and Fall et al. (2) reported both mono- and polygenic forms of inheritance in Middle American germplasm. Most sources of resistance show resistance to one or two races of Fop, however, broader forms of resistance are needed to protect against multiple races of the pathogen. The objectives of this research were to determine and compare the genetic control of resistance to Fop that controls races 1, 4, and 5 found in LEF-2RB, and resistance to race 4 found in the cultivar Sierra (6).

Materials and Methods

Genetic studies: F_2 progeny and a recombinant inbred line population (RIL) developed at Michigan State University (5) were used to determine the inheritance of resistance found in LEF-2RB and Sierra. The F_2 progeny were screened with race 4 Fop to develop a hypothesis for genetic control in each parent. The RIL population was screened with races 4 and 5 that allowed us to determine if the genes from Sierra and LEF-2RB were allelic and to test the hypotheses developed from F_2 data. **Inoculation procedure:** All progeny and RIL populations were screened for reaction to races of Fop using a modified root-dip inoculation procedure (4). Plants were evaluated for reaction to Fop 21 days after inoculation using the CIAT severity scale and root discoloration, where 1= no disease symptoms, 3=10% leaf surface showing disease symptoms, 5=25% of leaf showing disease symptoms, 7 = disease symptoms on 50% of leaves, and 9= plant death. Based on these scores, progeny were classified as either resistant (CIAT rating 1 to 3), intermediate (4 to 6), or susceptible (7 to 9).

Results and Conclusions

F_2 progeny from crosses LEF-2RB X Viva and Sierra X Viva were initially screened with race 4 Fop (Table 1). Both progeny segregated approximately 3:1 (R:S), suggesting a single dominant gene controls resistance in each resistant parent. Progeny from these crosses had been tested previously and also segregated 3:1 (data not shown). To determine if the resistance genes from Sierra and LEF-2RB were allelic, F_2 progeny from the cross LEF-2RB X Sierra were tested with race 4. The resultant progeny segregated 15:1, which suggested independent genes in resistant parents. To test the hypothesis of independent genes in Sierra and LEF-2RB, a recombinant inbred population derived from this cross was also screened for races 4 and 5 independently. Because previous studies had shown that LEF-2RB was resistant and Sierra susceptible to race 5, the RIL population was expected to segregate 1:1 (R:S) for reaction to race 5. The results from segregation among RILs confirmed this segregation, and suggested that a single dominant gene in LEF-2RB conferred resistance to race 5 and that Sierra was susceptible to race 5. The RIL population was also screened for race 4. Based on our hypothesis of independent genes in LEF-2RB and Sierra that control resistance, the entire RIL population should segregate 3:1 (R:S), and

lines susceptible to race 5 (based on the previous screening), should segregate 1:1 (R:S) for reaction to race 4. This result would also support the hypothesis that independent dominant genes in both parents control resistance to race 4 Fop. Although the number of RIL lines was low, these results and the results from the F_2 progeny confirmed that independent single dominant genes occur in Sierra and LEF-2RB, with the Sierra gene providing resistance only to race 4 and Lef-2RB possessing a gene which confers resistance to both races 4 and 5.

Table 1: Segregation, ratios tested, observed and expected values, and P-values for Chi-square tests among F_2 and RIL populations.

Progeny/ RIL Population Race Fop	Hypothesis Tested	Ratio Tested (R:S)	Observed		Expected		P-value
			Res.	Sus	Res.	Sus.	
F2 progeny/ Lef-2RB x Viva Race 4	Single Dominant gene in Lef-2RB	3:1	73	32	78.8	26.2	0.195
F2 progeny/ Sierra x Viva Race 4	Single Dominant gene in Sierra	3:1	101	21	91.5	30.5	0.047
F2 progeny/ Lef-2RB x Sierra Race 4	Genes in Lef-2RB and Sierra are not allelic	15:1	29	2	29.1	1.9	0.963
RIL Population/ Lef-2RB x Sierra Race 5	Segregation for Resistance to race 5 in RIL	1:1	26	21	23.5	23.5	0.466
RIL Population/ Lef-2RB x Sierra Race 4	Segregating for resistance to race 4 in lines sus. to race 5	1:1	11	6	8.5	8.5	0.225

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Identification of Anthracnose Resistance Genes in Dry Bean Cultivars Grown in Western Canada.

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Introduction

Throughout the world, anthracnose is regarded as a major constraint to increased dry bean (*Phaseolus vulgaris* L.) production. Many molecular markers that are closely linked to anthracnose resistance genes in common bean have been described offering new opportunities for identification and developing bean lines with complex and durable resistance through marker-assisted selection (MAS).

Material and Methods

Twenty one dry bean cultivars commonly grown in western Canada and all the anthracnose race differential lines were included in the combined analysis of five molecular markers (RAPD markers, $OF10_{530r}$ and $B355_{1000c}$ for *Co-1* and *Co-2*, respectively; SCAR markers, $SAS13_{950c}$ for *Co-4*, $SAreoli_{1300/1000}$ for *Co-2* and $SAB3_{400c}$ for *Co-5*) and resistance tests against the four anthracnose races, 23 (delta), 73, 89 (alpha Brazil) and 1096 of *C. lindemuthianum* under controlled environmental conditions, to identify specific anthracnose resistance genes.

Results and Discussion

The combined analysis of genotypes estimated by markers and race reactions showed that most cultivars possess only one resistance gene from either the Andean (*Co-1*, formerly gene *A*) or the Meso-American (*Co-2*, formerly gene *Are* and the Michelite gene) gene pools, only three cultivars combine the genes from both gene pools, and two cultivars carry two genes (*Co-2* and the Michelite gene), but no resistance genes were detected in the three susceptible cultivars.

Two cultivars 'Envoy' and 'Morden 003' pyramided three (*Co-1, Co-2* and *Co-4*) and two (*Co-1* and *Co-4*) resistance genes from both gene pools, respectively, and were resistant to anthracnose races 23, 73, 89 and 1096. This combination of resistance genes would afford resistance to all the known anthracnose races in Northern America (Kelly et al. 1994). However, 'AC Mariner' (the combine of gene *Co-2* and Widusa gene from Andean) seems to be an exception to the theory.

Molecular marker analysis showed that five cultivars contain gene *Co-4*, but none possess gene *Co-5*. However, the race reaction only confirmed two of the five, suggesting that the SCAR marker $SAS13_{950c}$ is not specific for the gene *Co-4*[2], and doesn't work in all bean backgrounds, but appears to identify the *Co-4* locus regardless of the type of allele (James D. Kelly, personal communication). Fine mapping of *Co-4* locus (Melotto, M. and J. D. Kelly, 2001) suggests development of SNPs (single nucleotide polymorphisms) markers inside the gene families to allow the clear identification of the different alleles of *Co-4*.

Gene *Co-1* from the Andean gene pool still provides useful resistance to races of anthracnose in the cultivars ‘Chardonnay’, ‘ROG 802’, ‘ROG 912’ and ‘CDC Nordic’, which was detected by the marker $OF10_{530r}$ and their race reactions. Gene *Co-2* was commonly detected by marker analysis among cultivars used in this study, but only seven cultivars were confirmed by the resistance tests. Race reactions to race 1096 suggested that seven cultivars may carry the Michelite gene, and it seems as important as gene *Co-2* among the cultivars grown in western Canada.

MAS can be efficacious to guide and identify the pyramiding of resistance genes in bean lines, but the results should always be confirmed with resistance tests because of the possibility of gene mutations, background effects, recombinants and adverse interactions among resistance genes during dry bean breeding program.

RAPD Marker linked to *Co-1*^{5} Anthracnose Resistance Gene in Widusa

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Introduction

Random amplified polymorphic DNA (RAPD) markers have the potential to be a useful breeding tool in common bean, where monogenic disease resistance genes have been tagged. Young and Kelly (1997) found one marker in repulsion phase with *Co-1* locus, while Mendoza et al. (2001) had reported one AFLP marker that is tightly linked at *Co-1*, in repulsion phase. The primary goal of this study was to identify random amplified polymorphic DNA (RAPD) tightly linked to *Co-1*^{5} gene, present in Widusa cultivar that confers resistance to race 73.

Material and Methods

The common bean cultivar Widusa was crossed with Cornell 49-242, and 10-day-old seedlings at the primary leaf stage of the parents, F_1, F_2 , and $F_{2:3}$ families were tested for their disease reactions to the race 73 of *Colletotrichum lindemuthianum*. Prior inoculation with *C. lindemuthianum*, one foliole tissue was collected from young primary leaves, approximately six days post-emergence, from greenhouse-grown plants. The plants were spray-inoculated with a spore suspension (1.2×10^6 spores/ml^{-1}) of the race. Ninety-one F_2 individual plants and their respective $F_{2:3}$ families, each, were used to confirm putative linkages between a RAPD marker and *Co-1*^{5} in Widusa. Two contrasting bulks were formed with DNA from 6 F_2 homozygous resistant and 6 F_2 homozygous susceptible individual plants derived from mapping population.

DNA extraction method was conducted according to Edwards et al. (1991). An uniform concentration of 10 ng.µl^{-1} of extracted DNA with DNA fluorometry was used to standard.

Amplification reactions were performed similarly to that described by Young and Kelly (1996). The phenotypic segregation was analyzed by the Chi-square test on the F_2 population, and $F_{2:3}$ families from the cross Widusa x Cornell 49-242. Ten plants of each $F_{2:3}$ family were inoculated, and based on the number of susceptible plants observed. The family was classified as homozygous resistant, heterozygous resistant, or homozygous susceptible and used to confirm the F_2 genotype. Linkage analyses were performed using the program Mapmaker (Lander et al., 1987). The Kosambi's function by the Linkage-1 computer program was used to determine the expression, in centimorgans (cM), of the linkage estimates between loci.

Results and Discussion

The inheritance supported an expected 3:1 ratio of resistant to susceptible individuals in the F_2 population of the cross Widusa x Cornell 49-242. According to Figure 1, the RAPD marker $OA18_{1500}$ (generated by 5'-AGGTGACCGT-3' decamer primer) was linked in repulsion-phase with the *Co-1*^{5} gene at distance of 1.2 cM. This marker co-segregated with the resistant gene in 91 individuals in F_2 population derived from the cross Widusa x Cornell 49-242, when this population was inoculated with the race 73, and one recombinant was observed. Similarly Young and Kelly (1997) found one marker in repulsion phase with *Co-1* locus. Mendoza et al.

(2001) had reported one AFLP marker that is tightly linked at 2.7cM from *Co-1*, in repulsion phase. According to Haley et al. (1994), selection based on a repulsion–phase RAPD yields a greater proportion of homozygous resistant selections than selection based on a coupling-phase RAPD even at greater recombination frequencies between marker and resistance loci.

This marker was linked in repulsion-phase with the dominant *Co-1*^{5} gene, which has proven to be effective on providing broad resistance to anthracnose. It would be useful in marker-assisted selection for the introgression of *Co-1*^{5} into susceptible germplasm.

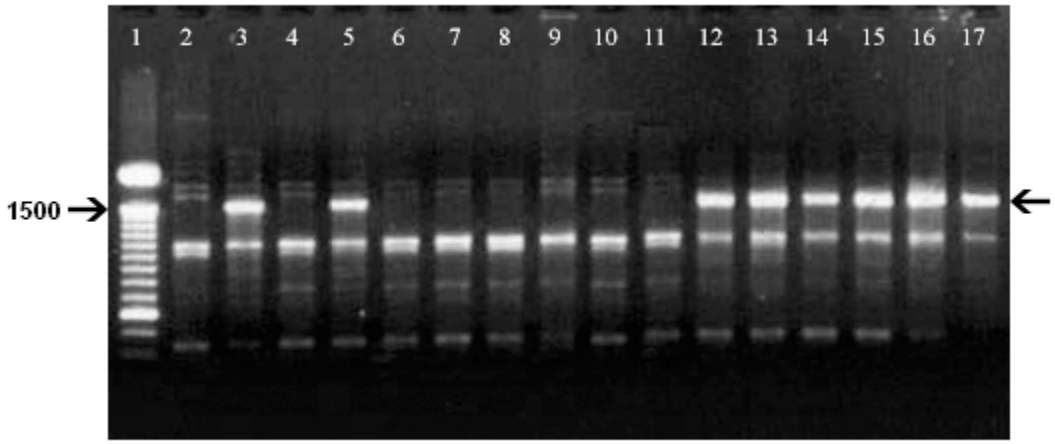

Figure 1 – Electrophoretic analysis of amplification products obtained with $OA18_{1500}$ RAPD marker. Lanes are as follows: 1, molecular weight marker (100bp ladder); 2, Widusa (resistant); 3, Cornell 49-242 (susceptible); 4, resistant bulk; 5, susceptible bulk; 6-11, F_2 plants resistant to race 73; 12-17, F_2 plants susceptible to race 73. The arrow indicates a DNA band of 1500 bp linked in repulsion-phase to the resistance gene.

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EVALUATION OF SELECTION TRAITS AND SEED YIELD IN COMMON BEAN

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Introduction

A uniform objective of all bean breeding programs is to develop genotypes with high yield and good agronomic traits (Brick and Grafton, 1999). However, genetic improvement of seed yield in bean (*Phaseolus vulgaris* L.) has been low compared with other crops (Nienhuis and Singh, 1988; Kelly et al., 1998). García et al. (1997) found that correlations among morphological and agronomic traits were lower in domesticated bean than in a wild population, suggesting the domestication process has changed the genetic structure of the species. Several breeding strategies have been proposed and tested, with varying degrees of success in increasing seed yield (Kelly et al., 1998; Singh et al., 1999). Efforts also have been made to find quantitative trait loci (QTL) associated with yield and other agronomic traits in bean (Tar'an et al., 2002). The objective of this research was to evaluate the selection traits used in dry bean breeding programs and to determine their correlation with seed yield.

Materials and Methods

Thirty-seven advanced pinto bean genotypes and five cultivars used as checks were evaluated at Erie and Hatton, North Dakota in 2001 using a randomized complete block design (RCBD) with three replicates. Maturity was measured as number of days from planting to harvest. Plant height (cm) was measured in each plot from soil level to tip of central axis. Lodging was measured using a scale of 1 (no lodging) to 9 (prostrate). Architecture was based on growth habit (I=bushy determinate; II=indeterminate, short vine; III=prostrate indeterminate; IV=climbing indeterminate). Leaf retention was measured at, or close to, harvest using a scale of 0 (no leaf) to 5 (mature pods with green canopy remaining). Desirability was based on the general appearance of the plots using a scale of 1 (less desirable) to 7 (most desirable). Common bacterial blight and white mold were evaluated using a scale of 0 (no symptoms) to 9 (severe symptoms). Yield was calculated as kg ha^{-1}, and seed weight was measured in g for 100 sound seeds. The analysis of variance was performed for each location with SAP (Hammond, 1992), using CWT (100 lb/ac) for seed yield. The correlation analysis was performed with SAS (SAS Institute, 1988).

Results and Discussions

Seed yield, as measured by cwt/A, varied from 13.8 to 32.0. Seed weight varied from 29.3 to 51.6 g for 100 sound seeds. Plant height varied from 32.7 to 55.3 cm. Maturity was between 84 and 98 days. Architecture was of Types II and III. Lodging varied from 1 to 7.7. Leaf retention varied from 0 to 5, and desirability from 1.7 to 5. For common bacterial blight, the scores varied from 2.7 to 8. White mold was important only at Hatton, with scores between 1.3 and 4. There was good variability for all traits, with a coefficient of variation between 4.1 for seed weight and 60.8 for leaf retention. Significant correlation coefficients ($P<.05$) among traits are presented in Table 1. The highest coefficients were found between maturity and leaf retention, with 0.66 at Erie and 0.94 at Hatton. Seed weight, maturity, lodging, leaf retention and desirability were positively correlated with yield. Common bacterial blight and white mold were negatively correlated with yield, seed weight, plant height and desirability. Nienhuis and Singh (1988) reported that direct selection for yield would be more efficient that indirect selection even in cases where there is a positive correlation between seed yield and selection traits; but, as noted by Kelly et al. (1998), if selection is practiced only for yield, growth habit will change towards Type IV, which may not be acceptable for commercial production in North America.

Conclusion

There was significant variability for all traits evaluated. There was more variability in lodging, leaf retention, desirability, common bacterial blight and white mold scores than in yield, seed weight,

maturity and plant height. The nature and strength of the correlation among traits can explain why progress has been modest in the genetic improvement of seed yield in common bean. Broadening the genetic base of the species through wide crosses and/or appropriate breeding strategies may help increase genetic gain for seed yield.

Table 1. Pearson's correlation coefficients ($P<.05$) among selection traits* for advanced pinto bean genotypes evaluated at Erie and Hatton, ND in 2001

		CWT	SDWT	MAT	PHT	ARC	LR	DS
SDWT	Erie							
	Hatton	.35						
DTM	Erie							
	Hatton	.57						
LDG	Erie			.43	-.49	.46		
	Hatton	.38			-.39	.36		
LR	Erie			.66				
	Hatton	.49		.94				
DS	Erie	.34		-.51		-.32	-.58	
	Hatton		.33					
CBB	Erie							
	Hatton	-.33	-.63					-.63
WM	Erie							
	Hatton				-.32			-.36

* CWT= 100 lb/A, SDWT=weight of 100 seeds (g), PHT=plant height (cm), DTM=days to maturity, ARC=architecture, LDG=lodging (0-9), LR=leaf retention (0-5), DS=desirability (1-7), CBB=common bacterial blight (0-9), WM=white mold (0-9).

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DEVELOPMENT OF AN EARLY GENERATION TEST FOR PREDICTING COLOR LOSS OF BLACK BEANS

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Retention of color in black seeded bean from the dry condition to the canned or cooked product (thermally processed) is an important consumer and processor expectation. Many black bean cultivars and breeding lines leach during thermal processing giving an end product that is gray to reddish brown. Color loss of processed black bean is unappealing and often results in severe consumer dissatisfaction. Prior study has indicated that color retention after cooking is heritable and may be altered through plant breeding. Typically the selection for cooked bean color- an important canning quality trait- is not made until the F5 or F6 generation when the large quantities of seed needed for the tests are available. The development of tests for predicting color retention of black beans after thermal processing, which are rapid, relatively inexpensive, and amenable to small samples, would allow testing in early generations after hybridization; thus saving time and fiscal resources.

A recombinant inbred population of black bean was used to develop a test predictive of color retention on small samples (less than 25g of beans). The essentials of the test were to determine whether the amounts of water imbibition and color loss due to soaking were related and ascertain the effect of seed coat shininess on color loss.

The test population consisting of 98 lines plus the two parents, 'Shiny Crow' and 'Black Magic', was grown near Christchurch, New Zealand during the winter of 2002-2003. Of the 98 lines, 36 were still segregating for shiny or opaque seed coats and were omitted from the experiment. The remaining 62 (F3:7) lines plus the two parents were used in the water uptake experiments.

Two samples of ten beans from each line were selected, weighed and placed into a 25 ml vial with 10 mls of distilled water that had been adjusted to 100 mg·ml Ca^{+2} with CaCl. The vials were placed into a water bath at 83° C for ten minutes to simulate the high temperature blanch used by many canners in the commercial processing industry. The beakers were then removed from the water bath and placed in the dark for the remainder of the experiment. Twenty minutes following removal from the water bath and at 30 minute intervals thereafter for a total experiment time of 120 minutes, the seed was removed from each beaker, blotted dry and weighed. The amount of water absorbed by the seeds through imbibition was determined and expressed as the increase (% w/w) of the ten seeds.

At the conclusion of the water uptake experiment, the beans were removed from the soak water and the water from each beaker was then transferred into a clean 10 ml vial. The water color was rated on a 5-point scale that was developed using a Munsell Color Chart. Exposure of the vials to light will rapidly change the color of the soak water, thus color scale ratings were done immediately. The 5 point scale was developed to correspond to the color of the water, from essentially colorless (scale = 1) to dark black (scale =5).

To ascertain the linearity of the color scale, the means of the number of RILs that fell into each color category were plotted against percent water uptake. This correlation was r=0.99 (data not shown). The shiny lines fell into categories 1 and 2 while the opaque lines fell mostly into categories 3, 4 and 5. Category 2 of the color scale was the only category where both shiny and

opaque RILs overlapped in score with the opaque RILs accounting for only 1 of the 11 RILs that fell into this category (Figure 1).

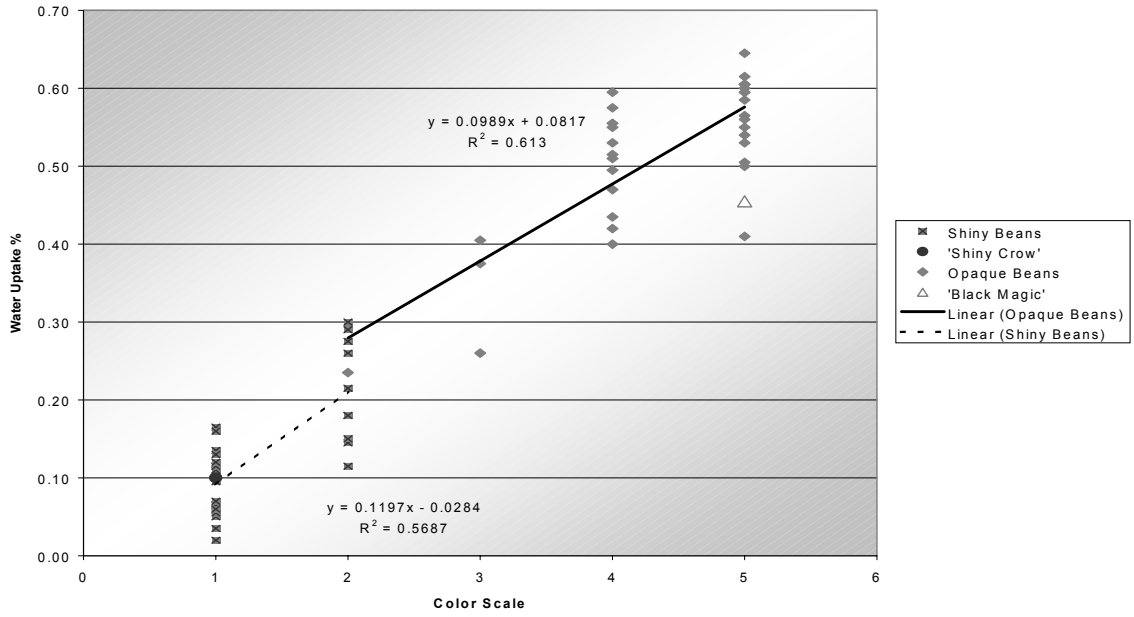
There were significant correlations between the color of the soak water and the amount of water imbibed after 120 minute soaking (r=0.96), seed shininess and the color of the soak water (r=0.93) and seed shininess and the amount of water imbibed (r=0.91) (Table 1). Overall, RILs with opaque seed coats imbibed more water over time, 24% to 65%, than the RILs with shiny seed coats, 2% to 30% (Figure 1).

The results of the current experiments indicate that color retention in black bean after thermal processing can be predicted by soaking beans for 120 minutes and comparing the soak water color. This test is rapid, inexpensive, fairly accurate and can be performed on small seed samples- this feature makes it useful to screen bean genotypes as early as the F3 generation. It must be pointed out though that the current results are only valid for this RIL population. Further testing in other populations is necessary before generalizations regarding color loss and water absorption of soaked beans can be extended to all black bean lines. However, experience in this laboratory with soaking and the amount of color loss noted appears to be a general phenomenon in black beans.

Table 1. Pearson Correlation Coefficients Comparing Percent Water Uptake, Shininess, and Color of the Soak Water Following a 120 Minute Soak.

	Percent Water Uptake	Color	Shininess
Percent Water Uptake	1.00	0.96	0.91
Color	0.96	1.00	0.93
Shininess	0.91	0.93	1.00

Figure 1. Mean Percent Water Uptake for 64 Black Bean Lines



WHEN IS A PINTO A PINTO?

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Introduction

As the seed coats of pintos darken, it creates a significant problem for producers and merchants. Consumers are reluctant to buy pinto beans with darkened seed coats as it suggests that the beans are old, will take longer to cook, and will have higher levels of anti-nutritional compounds (Jacinto-Hernandez et al., 2001 and Martin-Cabrejas et al., 1997). A Crop Development Centre (CDC) pinto bean breeding line, SC11743-3, darkens more slowly than convention pinto beans. We investigated the genetic control of this phenomenon.

Materials and Methods

CDC Altiro and CDC Pintium are commercial varieties available in western Canada. Seed coats of these two varieties darken over time with exposure to heat, light and humidity. SC11743-3 and 1533a-15 are pinto breeding lines that exhibit slow-darkening characteristics.

CDC Altiro and SC11743-3 were crossed and selfed to produce a F_2 population. The F_1 of CDC Altiro and SC11743-3 was backcrossed to SC11743-3 to create a BC_1 population. CDC Pintium was crossed with both CDC Altiro and 1533a-15 and F_2 populations were produced.

Seed coats of the parents, the F_1, the F_2, and the BC_1 generations were artificially darkened by placing the beans in open petri dishes in a seed germination cabinet set at 30ºC, 80% RH and full light for three weeks. The color of the beans was then measured using a Hunter Lab colorimeter, which measures reflectance on three coordinates labeled L (0 dark to 100 light), a (- green to + red), and b (- blue to + yellow).

Results and Discussion

The F_2 seed coats of CDC Pintium x 1533a-15 segregated 65 normal darkening to 21 slow-darkening. This indicated that the slow-darkening phenotype is controlled by a single recessive gene ($\chi^2 = 0.02$, $p = 0.90$). When the Lab values were graphed, the normal darkening phenotypes formed a cluster separate from the slow-darkening phenotypes (Fig 1). All F_2 seed coats of CDC Altiro x CDC Pintium showed normal darkening as expected. Seed coats of seeds with a pinto phenotype in the BC_1 population from CDC Altiro x SC11743-3 darkened slowly but when compared to the parents, the BC_1 pinto seed coats were less dark than those of CDC Altiro but darker than those of SC11743-3.

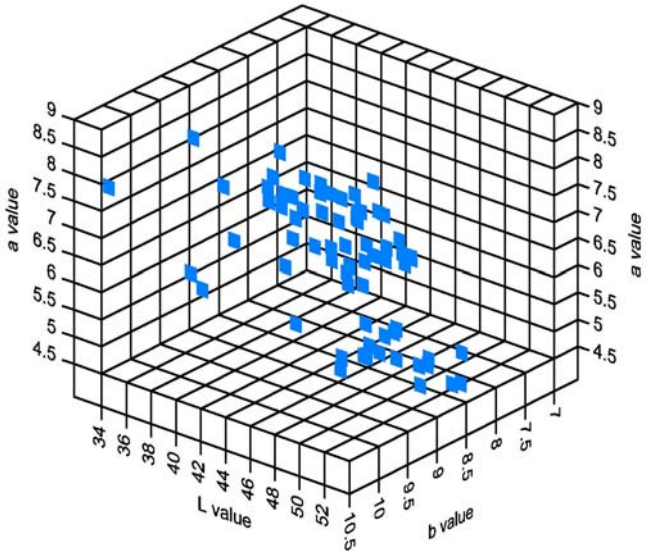

Figure 1. The Lab values from the Hunter Lab Colorimeter for the F_2 population of the cross CDC Pintium by 1533a-15.

All parents were true breeding for pinto seed coat. The F_1 seed coat of CDC Altiro x SC11743-3 was brown with no pattern and the F_2 seed coats segregated for both colour (brown, buff, red, and pink) and pattern (no pattern and pinto pattern). The BC_1 segregated 15 brown with no pattern to 19 normal pinto. All progeny of CDC Pintium x 1533a-15 and CDC Pintium x CDC Altiro had pinto type seed coats. A possible genetic hypothesis that we are investigating to explain this phenomenon is that there are two loci interacting causing two different pinto genotypes and the non-pinto phenotypes.

Currently work is being conducted to determine if crosses between SC11743-3, CDC Altiro and other pinto varieties result in non-pinto phenotypes. The transfer of the slow-darkening trait from SC11743-3 and 1533a-15 into other market classes that are predisposed to darkening during storage is also being investigated.

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MOLECULAR MARKER ASSISTED DIFFERENTIATION OF NEW BEAN BREEDING LINES

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Introduction

In recent years, various bean breeding lines with similar morphological traits were developed in the North of Spain by SERIDA (Servicio Regional de Investigacion y Desarrollo Alimentario). These lines carry different anthracnose resistance genes and exhibit different growth habits. Initially two new breeding lines were developed that carried the anthracnose resistance genes *Co-2* and *Co-9*, originating from the parental donors S34 and A493 respectively. These new breeding lines were phenotypically identical to Andecha, the most important commercial bean in the North of Spain. This is an important advantage in our country were 90% of the cultivated bean have Type IV growth habit type and required a trellis for support or corn to climb. We also developed a new line with determinate growth habit, named Xana that is very similar to Andecha. The breeding programs were combined to develop two additional breeding lines with determinate growth habit that carry the anthracnose resistance genes *Co-2* and *Co-9*.

The combined breeding program described has developed 5 new breeding lines that are phenotypically identical to Andecha, the most commercial bean in the North of Spain. All these materials with different genetic combinations cannot be easily differentiated, therefore molecular markers provide a useful tool for a quick differentiation instead of morphological differentiation. We have chosen molecular markers based in "Polymerase Chain Reaction" as RAPD, SCAR or CAP, because of their easy use (Ortiz et al, 2000). The molecular markers, linked to specific genes, have also the important advantage that most are already mapped. There are already reported quite many molecular markers linked to specific mapped genes that can be used with a wide spectrum of bean materials. The objective of this work was to examine the utility of some molecular markers linked to the known genes *Co-2*, *Co-9* and *Fin,* for the differentiation of 6 materials morphologically identical.

Results

At the beginning of the work we collected and tested many molecular markers linked to the selected genes (Table 1). Some of the markers were previously developed by our group some years ago, and others come from literature. From a total number of 22 molecular markers analyzed, only 7 were monomorphic in our material. The other 15 displayed polymorphism, and the most useful markers that differentiated our lines are shown on Table 2. Two markers linked to *Co-2* gene and one molecular marker linked to *Co-9*, that worked in our material were chosen.

Table 1. Molecular markers analyzed for this study.

Marker	Size (bp)	Type	Gene	Phase	Reference
OQ04	600	RAPD	*Co-2*	Repulsion	Méndez de Vigo, 2001
OQ04	1440	RAPD	*Co-2*	Coupling	Young & Kelly, 1996
B355	1000	RAPD	*Co-2*	Coupling	Young & Kelly, 1996
SCAreoli	500	SCAR	*Co-2*	Coupling	Méndez de Vigo, 2001
SCAreoli	1000	CAP	*Co-2*	Coupling	Geffroy et al , 1998
SB12	350	SCAR	*Co-9*	Coupling	Méndez de Vigo et al 2002
OI19	500	RAPD	*Co-9*	Coupling	Méndez de Vigo, 2001
OZ10	800	RAPD	*Fin*	Coupling	Pañeda, 2001
OA04	1100	RAPD	*Fin*	Repulsion	Pañeda, 2001
OD08	1150	RAPD	*Fin*	Repulsion	Unpublished results
OI19	375	RAPD	*Fin*	Repulsion	Pañeda, 2001
OQ03	450	RAPD	*Fin*	Coupling	Park et al, 1999
OF16	1400	RAPD	*Fin*	Coupling	Park et al, 1999
OA17	600	RAPD	*Fin*	Coupling	Park et al, 1999
OA17	950	RAPD	*Fin*	Coupling	Park et al, 1999
OU12	450	RAPD	*Fin*	Coupling	Park et al, 1999
OU19	350	RAPD	*Fin*	Coupling	Park et al, 1999
OU19	450	RAPD	*Fin*	Coupling	Park et al, 1999
ON12	800	RAPD	*Fin*	Coupling	Park et al, 1999
OV10	250	RAPD	*Fin*	Coupling	Park et al, 1999
OK19	450	RAPD	*Fin*	Coupling	Park et al, 1999
OT14	800	RAPD	*Fin*	Coupling	Park et al, 1999

All the molecular markers linked to *Fin* gene (Park et al, 1999) were monomorphic in our material. This is a frequent problem in common bean, because of the big differences between the genetics pools, therefore we initiated a search for new molecular markers linked to this gene. The F2 population proceeded from a cross between Andecha (Fin) and BRB130 (fin), and we characterized all the F2:3 families to detect homozygous and heterozygous individuals. The screening was conducted using BSA method (Michelmore, 1991) with around six hundred random Operon primers and sixteen were found to be significantly associated with the *Fin* gene (Pañeda, 2001). For the present work we have select some RAPD markers that proved useful in our material (Table 2).

Table 2. Results for the molecular markers that were polymorphic in our materials

Marker	Gene	Andecha	Xana	S34	A493	A1183	A1220	X1358	X1319
$OQ04^{600}$	*Co-2*	+	+	-	+	-	+	-	+
$OQ04^{1400}$	*Co-2*	-	-	+	+	+	-	+	-
$SCAeroli^{500}$	*Co-2*	+	+	-	-	-	+	-	+
$SCAeroli^{1000}$	*Co-2*	-	-	+	-	+	-	+	-
$OI19^{500}$	*Co-9*	+	+	-	-	+	-	+	-
$SB12^{350}$	*Co-9*	-	-	-	+	-	+	-	+
$OI19^{375}$	*Fin*	-	+	-	-	-	-	+	+
$OZ10^{800}$	*Fin*	+	-	+	+	+	+	-	-
$OA04^{1100}$	*Fin*	-	+	-	-	-	-	+	-
$OD08^{1150}$	*Fin*	-	+	-	-	-	-	+	+

We are also working with another architectural trait because plant architecture is an important trait in breeding programs for common bean. There are some commercial lines (Cimera) in the North of Spain that are morphological similar to Andecha and we need to develop new molecular markers to differentiate between future breeding lines. The main feature that differentiates these two commercial lines is the pod distribution. Cimera is a line with Type IVa growth habit, with the pods uniformly distributed along the plant, whereas Andecha has Type IVb growth habit, with pods mainly in the upper part of the plant (Debouck and Hidalgo, 1985). We studied the genetic of this trait, and we found evidence for a new locus involved in the genetic control of the pod distribution in the indeterminate climbing habits (p= 0,68 for the single dominant locus IVa).

The patterns for the polymorphic useful markers are show in Table 2, and from all of them we selected: $OQ04^{600}$ and $OQ04^{1400}$ linked to the anthracnose resistance gene *Co-2*, $OI19^{500}$ linked *Co-9* gene, and $OI19^{375}$, $OD08^{1150}$ and $OZ10^{800}$ linked to *Fin* gene for further study. The results for the selected markers, confirm the presence of *Co-2* gene in A1183 and X1358 lines and *Co-9* gene in A1220 and X1319 lines. We can conclude with the present results that molecular markers can be used to differentiate without mistake genetically similar breeding lines.

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ALLELIC RELATIONSHIPS OF ANTHRACNOSE RESISTANCE GENE CLUSTER B4 IN COMMON BEAN.

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Anthracnose is one of the most widespread and economically important diseases of common bean (*Phaseolus vulgaris* L). It is especially harmful in tropical and subtropical areas but it also causes considerable yield losses in temperate areas such as Northern Spain where valuable dry bean landraces are grown. Andecha is a very large white seeded cultivar (proceeding from a selection of Asturian landraces) susceptible to races 6 and 38 and with moderate resistance to races 3, 102 and 787 of *Colletotrichum lindemuthianum*. These five races are the most commonly found in Northern Spain (Ferreira et al. 1998). Two parallel backcross breeding programs were developed using germplasm lines A321 and A493 as resistance donors and Andecha as the recurrent parent. After 6 backcross generations, the lines A1231 and A1220 were obtained, each one carrying one dominant resistance gene proceeding from A321 and A493, respectively. The introgressed gene(s) conferred resistance to races 6 and 38 and enhanced the resistance to races 3, 102 and 787 in both breeding lines. Genetic resistance has shown to be the most effective strategy for protecting crops. To date, up to ten genes that confer dominant resistance (except *co-8*) to different pathogenic races of anthracnose have been described. Genes *Co-1*, *Co-2*, *Co-4*, *Co-6* and *Co-9* are known to be located on the linkage groups of *Phaseolus vulgaris* L. map B1, B11, B8, B7 and B4, respectively. Molecular markers linked to these genes and to *Co-5* have also been identified (Kelly et al. 2003). Little is known about the resistance genes conditioning resistance in the differential cultivar Mexico 222.

In this work, we describe the identification of anthracnose resistance genes present in two breeding lines A1220 and A1231 derived from cultivar Andecha by means of allelism tests and molecular marker analysis.

The segregation ratios observed in the selection for resistance to anthracnose conducted in two breeding programs indicated that both lines A1220 and A1231 each carry a single dominant gene for resistance to race 38. No segregation was observed in the F2 population derived from the cross A1220 x A1231, suggesting that the dominant gene in both lines is located at the same locus. No segregation was observed in F2 populations derived from the crosses A1220 x Mexico 222, A1220 x PI 207262, A1220 x BAT 93, A1231 x Mexico 222, A1231 x PI 207262 and A1231 x BAT 93, indicating that the dominant resistance gene present in lines A1220 and A1231 is located at the same locus as the resistance genes in Mexico 222, PI 207262 and BAT 93. The 3:1 ratio of F2 families derived from the crosses (S x R) Andecha x Mexico 222, Andecha x PI 207262 and Andecha x BAT 93 and the lack of segregation in the cross PI 207262 x Mexico 222 support the theory that the same locus is responsible for resistance to race 38 in all these genotypes (Table 1).

Table 1. Allelism tests for genetic characterization of the resistance to race 38 in lines A1220 and 1231

	Observed frequencies		Expected frequencies				
F_2 Population	Resistant	Susceptible	ratio	Resistant	Susceptible	χ^2	Probability
A1220 x A1231	171	0	15:1	160.3	10.7	11.400	0.001
A1220 x Mexico 222	115	0	15:1	107.8	7.2	7.66	0.006
A1220 x PI 207262	108	0	15:1	101.3	6.8	7.20	0.007
A1220 x BAT 93	213	0	15:1	199.7	13.3	14.200	0.000
A1220 x Cornell 49242	98	6	15:1	97.5	6.5	0.04	0.839
A1220 x AB 136	105	7	15:1	105.0	7.0	0.00	1.000
A1220 x TU	116	16	15:1	123.8	8.3	7.76	0.005
A1231 x Mexico 222	246	0	15:1	230.6	15.4	16.400	0.000
A1231 x PI 207262	222	0	15:1	208.1	13.9	14.800	0.000
A1231 x BAT 93	95	0	15:1	89.1	5.9	6.33	0.012
A1231 x Cornell 49242	208	9	15:1	203.4	13.6	1.63	0.201
A1231 x TO	102	11	15:1	105.9	7.1	2.34	0.126
A1231 x AB 136	236	13	15:1	233.4	15.6	0.45	0.502
A1231 x TU	102	14	15:1	108.8	7.3	6.70	0.010
Andecha x Mexico 222	76	20	3:1	72.0	24.0	0.88	0.346
Andecha x PI 207262	197	54	3:1	188.3	62.8	1.62	0.202
Andecha x BAT 93	80	22	3:1	76.5	25.5	0.64	0.424
PI 207262 x Mexico 222	159	0	15:1	149.1	9.9	10.600	0.001

To find molecular markers linked to the resistance gene present in breeding lines A1220 and A1231 the amplification patterns of 374 decamer primers along with twenty different SCAR markers were compared in lines Andecha, A493, A321, A1220 and A1231. Both breeding lines conserved from their corresponding donor parents RAPD markers $OB12_{350}$, $OAH18_{1100}$, $OI19_{460}$ and $OY17_{1100}$ and SCAR markers SI19 (Melotto and Kelly, 1998) and SW12 (Miklas et al. 2000). The SCAR SB12, coming from RAPD $OB12_{350}$ (Méndez de Vigo et al. 2002) amplified a single band only in the two breeding lines, in the resistance donors, in BAT 93 and in PI 207262. The $OY17_{1100}$ RAPD band was also present in genotypes A321, A493, A1231, A1220, BAT 93 and PI 207262 although a weaker amplification was observed in G 2333 and SEL 1308. The SAH18 amplification band (coming from the RAPD $OAH18_{1100}$,) was present in a larger number of genotypes. The SW12 SCAR primers produced 6 different amplification phenotypes in the 22 genotypes analyzed: no amplification (Michelite, MDRK, Widusa, AB136, Catrachita and Jalo EEP558); one single band of 700 bp (Cornell 49242, Kaboon, TO, SEL 1308, A321 and A1231); two bands of 700 and 600 bp (Perry Marrow, TU and Andecha); two bands of 700 and 575 bp (G 2333 and SEL 1360); two bands of 700 and 475 bp (PI 207262, BAT 93, A493 and A1220); and two bands of 700 and 450 bp (Mexico 222).

The F2 population derived from the cross Andecha x A493 was used to test the linkage of these markers and the resistance gene (Table 2). Markers SI19 and $OY17_{1100}$, previously described linked to the rust gene *Ur-5* located on B4 linkage group (Melotto and Kelly, 1998; Miklas et al. 2002) were present. The SCAR marker SW12 linked to a major QTL conferring resistance to bean golden mosaic virus (BGMV) located on B4 (Miklas et al. 2000) was confirmed.

Table 2. Chi-square (χ^2) and linkage analysis of markers $OB12_{350}$, $OAH18_{1100}$, $SI19_{460,}$ $OY17_{1100}$ and $SW12_{475}$ and the resistance to race 38 of *C. lindemuthianum.*

Marker	Expected ratio*	Observed frequency	χ^2	p	cM **
$OB12_{350}$	3:6:3:1:2:1	20:27:0:0:4:18	52.96	0.00	3.4
$OAH18_{1100}$	3:6:3:1:2:1	20:29:1:0:3:16	50.66	0.00	4.7
$SI19_{460}$	3:6:3:1:2:1	4:30:17:16:3:1	37.04	0.00	12.6
$OY17_{1100}$	3:6:3:1:2:1	20:29:0:0:2:18	59.91	0.00	1.6
$SW12_{475/575}$	1:2:1:2:4:2:1:2:1	17:1:0:2:30:0:0:1:17	114.13	0.00	2.2

*The expected ratios are based on 1:2:1 genotypic segregation ratio for the resistance gene and marker $SW12_{475/575}$ (codominant inheritance), and 3:1 ratio for the remaining markers (dominant inheritance)

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

Geffroy et al (1999) proposed the symbol *Co-9* for the anthracnose resistance gene present in the breeding line BAT 93 and the names Co-y/Co-z for the resistance present in Jalo EEP558 that also mapped to B4. Due to the origin of the resistant donor A493 (proceeding from BAT 93, derived from a 4-way cross that included the differential cultivar PI 207262) and to the information provided by molecular markers in this work, we conclude that the resistance gene present in line A1220 is in fact the *Co-9* gene. The lack of segregation for resistance to race 38 in the F2 derived from crosses with lines A1220, A1231, BAT 93, PI 207262 and Mexico 222 (Table 1), suggests the presence in all five genotypes of the same locus on the B4 linkage group, conferring resistance to race 38.

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Agronomic Potential Value of Great Northern Recombinant Lines
and Breeding Implications in Common Bean

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INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) was introduced into the Iberian Peninsula (Spain and Portugal), mainly from Central America around 1506 and from the southern Andes after 1532, through sailors and traders, which brought the nicely colored, easily transportable seeds with them as a curiosity. The principal cultivated bean types in this area are cultivars of Andean origin and belonging to the white kidney, Canellini, marrow, "Favada", large cranberry, cranberry, red pinto and "Canela" market classes, and cultivars of Mesoamerican origin and corresponding to the great northern and pinto market classes. The level of genetic variation has not eroded since the introduction of common bean from the American centers of domestication to the Iberian Peninsula. Instead, obvious signs of introgression between the two gene pools were observed, mainly among white-seeded genotypes (Santalla et al., 2002). A preliminary study of the productivity potential and breeding value of great northern recombinant genotypes is presented in this work.

MATERIAL AND METHODS

Fifteen landraces belonging to the large great northern market class (>40 g/100 seeds), that have been collected in areas from the Iberian Peninsula where traditional farming methods have encouraged the presence of old varieties, were included in this study. This genetic material is maintained in the germplasm collection at the MBG-CSIC (Ron et al., 1997). Allozyme and phaseolin analysis were carried out per each landrace. Seventy-five plants were sown for landraces, which had trellis support because all of them have a climbing growth habit. Morphological and agronomical data were recorded per plant. One hundred and fifteen inbred lines were derived from single plants within landraces. The inbred lines were planted in one-row plot, each 3.8 m long, in a randomized complete-block design with 2 replications. Distance between rows was 0.80 m and plants were spaced 0.25 m apart in the row. The field experiments were carried out in northwest Spain (42º 24' N, 8º 38' W, 40 masl, 14 ºC mean temperature, average annual rainfall 1600 mm) in 2002.

RESULTS AND DISCUSSION

Some landraces exhibited $Skdh^{100}$, Me^{100}, $Rbcs^{100}$ and $Diap\text{-}1^{95}$ or $Skdh^{103}$, Me^{100}, $Rbcs^{100}$ and $Diap\text{-}1^{100}$ allozyme profiles and they were considered as putative hybrids (Table 1). These Iberian recombinants had morphological traits that did not correspond with the characterization of Singh et al. (1991). Thus, there is a considerable overlap in seed size between the Mesoamerican and Andean groups.

Table 1. Average of the distribution of allozyme variants and phaseolin pattern in great northern landraces from the Iberian Peninsula.

Phaseolin pattern		*Skdh*		*Me*			*Rbc*			*Diap-1*		*Mdh-1*		*Mdh-2*	
S	B	103	100	102	100	98	102	100	98	100	95	103	100	102	100
0.82	0.18	0.74	0.26	0.09	0.81	0.10	0.06	0.84	0.10	0.07	0.93	0.23	0.76	0.02	0.98

Significant variation was observed among great northern landraces for flowering aspects such as days to first flowering and first dry pod, seed size and yield (Table 2).

Table 2. Analysis of variance of agronomic traits of great northern landraces from the Iberian Peninsula.

Source of variation	D.f.	Mean squares					
		First flowering	First dry pod	Seeds per pod	Pods per plant	Seed yield	100-seed weight
		days				g/plant	g
Block	1	437	1.92	2.86 *	3149.8 **	17873.2 **	132.7
Landrace	14	538.97 **	488.34 **	19.65 **	723.3 **	3414.7 **	2085.2 **
Genotype (L)	99	15.78 **	29.83 **	0.99 **	195.9	1133.8	77.4 **
Error	109	8.71	13.98	0.46	204.4	1209.3	39.1

*, ** null hypothesis rejected at the 0.05 and 0.01 levels respectively.

In addition, a wide variation was found among genotypes within landraces for important agronomic traits. Thus, some genotypes had values of seed yield of approximately 100 g/plant and a seed size of 90 g/100 seeds. This new genetic material could serve as bridging germplasm to transfer genetic diversity between the Andean and Mesoamerican gene pools that could not be achieved by direct crosses. Productivity potential of these recombinant genotypes is confirmed.

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The Selection of Dry Bean Cultivars by Potato Leafhoppers Based on Visual Cues

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Introduction

The potato leafhopper (PLH) is a serious pest of dry beans in Ontario, reducing yield by up to 60% (OMAF 2001). PLH preferentially select some cultivars over others (Wylde 1999). Recent findings suggest that foraging PLH primarily discriminate between hosts using visual stimuli (Todd et al. 1990), with wavelength-specific colour preference and reflectance intensity of host leaves having the greatest influence (Lapis and Borden 1995). PLH are preferentially attracted to green and yellow, with spectral reflectance values ranging from 520 to 580 nm, typical of the abaxial surface of host leaves (Chu et al. 2000).

Materials and Methods

Laboratory studies were conducted at Ridgetown College on three dry bean cultivars, to determine the preferential selection of the following cultivars by adult PLH based on leaf colour.
1)Berna brown - medium size brown seed, very attractive to PLH but little commercial value
2) Stingray white - small, white seeded, moderately attractive to PLH, a commercial cultivar
3) EMP 419 – small, white seeded, an experimental PLH tolerant cultivar
Intact leaves of each cultivar were placed under circular holes cut in black bristol board, to provide uniform sized leaflets. A black card was used as a control. A Plexiglas® chamber was placed over this arrangement, and 50 adult PLH were released through a sleeved hole in the top corner of the chamber, to ensure they selected leaves from an aerial position. A choice was recorded when an adult landed on a leaflet. Video equipment was assembled to observe the experiment at a distance, and to record the events.
In a second experiment, a spectroradiometer was used to quantify the wavelength reflectance values of 20 leaflets of each cultivar. For each measurement, the apparatus was referenced to a standard, and then re-configured for the sample. A percent reflectance value was obtained as a ratio (sample/ reference) at 2 nm increments from 400 to 800 nm.

Results and Discussion

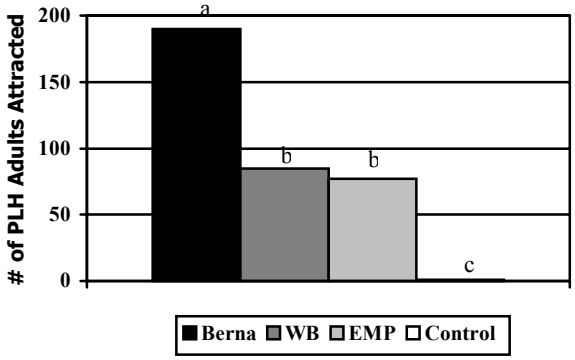
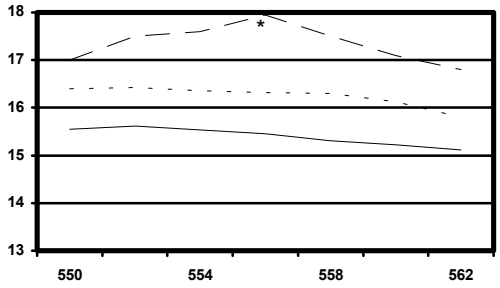


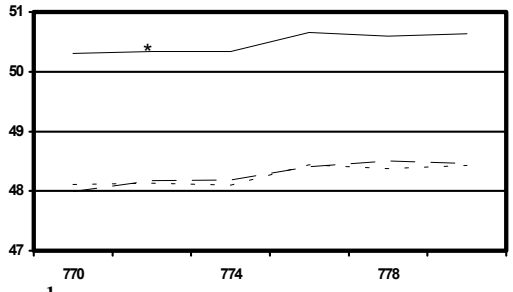
Fig. 1. Leaf choices by adult PLH in a Plexiglas® chamber. Bars with the same letter are not significantly different (P > 0.05, Tukey's HSD).

1) Adult PLH preferred Berna leaves two to one, compared to Stingray and EMP 419 leaves under controlled lab feeding studies (see Fig.1). The PLH adults were not attracted to the control. Once a choice was made, all PLH remained on the selected leaf for the duration of a 2 hour trial.

2) The greatest differences between cultivars in wavelength reflectance from 400 to 800 nm occurred at 446, 488, 546, 556, 648, 676, 748 and 772 nm.
3) Berna leaves had significantly higher reflectance values at 556 nm, in the true green region of the spectrum. This agrees with other studies (Chu et al., 2000) where colour card traps were used.
4) Analysis in the red region of the spectrum showed that EMP 419 had the highest reflectance values, which may explain part of its tolerance to PLH feeding.
5) Leaf reflectance may hold some promise to screen for PLH resistance in edible beans, as this analysis can be done nondestructively in the field. In order for this to be truly effective, a more intense examination, using several cultivars at each level of resistance is necessary.
6) Dry bean cultivars that are preferred by adult PLH, based on leaf colour, may be utilized as a trap-crop, if they provide sufficient attraction during the period of pest colonization, and these cultivars have commercial appeal



a



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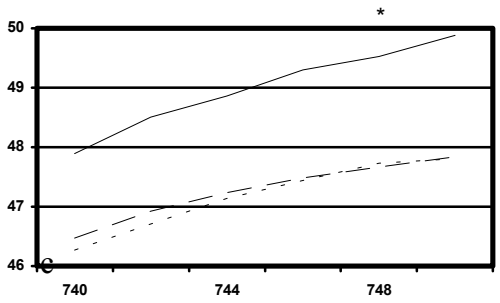

c

Fig. 5a-c Reflectance curves for three dry bean cultivars (Berna - - - -, Stingray ——— and EMP419 .....) analyzed using a spectroradiometer between 550 and 560 nm, 740 and 750 nm and 770 and 780 nm. *Peak at which percent reflectance values differ ($P < 0.05$, Tukey's HSD).

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RAPD FRAGMENT SEQUENCE ANALYSIS IN COMMON BEAN

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INTRODUCTION

Phaseolus vulgaris is one of the smallest genomes in legumes (0'66pg/haploid genome) with approximately 637Mbp (Arumuganathan & Earle, 1991). Little information was available about the *P. vulgaris* genetic map until the development of new molecular markers in the 90´s that allowed an increase in the number of markers and maps. In recent years, the more useful tools for breeding programs are SCAR markers, due to their reproducibility and specificity. So the objectives of this study were to obtain a new core map based on SCARs markers and determine if there is any specific association of the polymorphic RAPD sequences with cloned genes or intergenic sequences.

MATERIAL AND METHODS

DNA sequences from polymorphic RAPD markers uniformly located in the 11 bean linkage groups were analyzed. This selection was based in a map developed by our group (Méndez de Vigo, 2001). The general molecular characteristic of the markers selected are present in Table 1: name of the Operon RAPD, map location, amplification pattern for the parents of our F2 mapping population, and the molecular weight estimated at first by comparison with a ladder and then by sequencing.

The selected fragments were cloned and the nucleotide sequence was determined on both strains. The PCR fragments were analysed with a ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) and the homology with Gen Bank sequences was performed using BLAST analysis. It is worth mentioning that the Gen Bank contains only 1,495 nucleotide entries and 760 protein entries for *Phaseolus vulgaris*, so most of the homologies will be related with other species**.** In recent years, the number of legumes being sequencing is increasing so we expect that the number of sequences and species analyzed will increase and the homologies studied should be better. We have just concentrated in plant sequences, so any other homologies are not shown.

Table 1. Summary table of the RAPD markers analyzed

		Amplification pattern			
RAPD	**LG**	**A25**	**A252**	**MW_1**	**MW_2**
OAS15	4B	0	1	250	264
OD13	2D	0	1	625	633
OE02	6G	1	0	490	496
OE04	8F	0	1	700	724
OE15	9K	0	1	525	551
OF04	2D	1	0	750	735
OF10	1H	0	1	500	479
OG14	10I	1	0	700	671
OG16	3C	1	0	375	374
OH05	7A	0	1	750	745
OH08	10I	1	0	950	968
OH18	5E	1	0	1000	*
OY08	11J	0	1	350	361

LG: linkage group, MW1: Molecular weight estimated by comparison with molecular weight marker, MW2: Molecular weight estimated by sequencing, *no estimation

RESULTS

Present work displays the study of 13 sequences, from which only 5 exhibit an E value under or equal to 10^{-13}, a significant value determined by other homology studies. There are also 8 sequences that exhibit E values bigger than 0,05**.**

One of the best values for E was obtained for the $OE02^{490}$ RAPD sequence, with an arcelin protein from *Phaseolus vulgaris*. This protein is part of the lectin family, which is coded by a genetic family mapped to the B4 linkage group (Freyre *et al*., 1998). There are other loci for these genetic families, that have not been mapped yet (Sales *et al*., 2000; Kelly *et al*., 2003).

Our marker is located in B6 linkage group in our map (Méndez de Vigo, 2001), which suggest that one of the other loci for the lectin family is B6 linkage group.

The $OF10^{500}$ fragment was described by Young *et al* in 1997, as a molecular marker linked from *Co-1* at 1.4-1.9 cM (anthracnose resistance gene). Our group has already mapped it in B1 linkage group (Méndez de Vigo, 2001) and has developed the SCAR marker that would be very useful in all the breeding programs involving this gene. Nevertheless, we found that the sequence structure (Figure 1) has 22 repeats of a 13 bp sequence in which the primer is included (5'CCAAGCTTCCATA 3'). A shorter sequence of 197 bp without any repeats was selected to design the new 24 bp SCAR-primers that were specific for one band. No significant homology was found for this fragment, although it is supposed to be related with anthracnose resistance genes.

Figure 1. Complete sequence of $OF10^{500}$ fragment. It is indicated with bold letters the RAPD primers and with italic letters the 22 sequence repeats

5'YGGAAGCTTGGGGAACACGAGCTCGAAATGAATAGGCTTGTTGTCCAAGAAAGTGAGGAAAAACA
CAACAAGGGCATAACACTCTAAGCTTGCAATCACAAACAACAACAAGACTCAAGTGGAAGTGATGAA
GACACTATGAGTTTGTTGTCAAGAAAATTCAACAAGTTCTTGAAGAAGAAAAG*CCAAGCTTCC*ATA*CCA*
*AGCTTCC*ATA*CCAAGCTTCC*ATA*CCAAGCTTCC*ATA*CCAAGCTTCC*ATA*CCAAGCTTCC*ATA*CCAAGCTTCC*
ATA*CCAAGCTTCC*ATA*CCAAGCTTCC*ATA*CCAAGCTTCC*ATA*CCAAGCTTCC*ATA*CCAAGCTTCC*ATA*CCA*
*AGCTTCC*ATA*CCAAGCTTCC*ATA*CCAAGCTTCC*ATA*CCAAGCTTCC*ATA*CCAAGCTTCC*ATA*CCAAGCTTCC*
ATA*CCAAGCTTCC*ATA*CCAAGCTTCC*ATA*CCAAGCTTCC*ATA*CCAAGCTTCC*ATA**CCAAGCTCCY3'**

The $OD13^{625}$ sequence presented a good homology with the endopolygalacturonase inhibitor protein gene (PGIP) from *P. vulgaris* with a very significant E value. The location of this marker (Freyre *et al*., 1998) with the design of a RFLP from the gene sequence (Toubart *et al*., 1992) is also in chromosome B2 where our $OD13^{625}$ sequence is mapped (Méndez de Vigo, 2001). Therefore we can assume that this sequence is part of the gene that codify for the PGIPs.

Another good homology (E=2x10-88) was observed for the $OG14^{700}$ sequence. In this case results of BLAST showed that the homology is not for the complete sequence of mARN C subunit vacuolar H+-ATPase, because there is a 350 bp fragment in the $OG14^{700}$ that is not present in other plant species. This small fragment could correspond to an intron sequence, and therefore is not present in the processed mARN of other species.
Only most relevant results for homology are present here, but there are quite a number of other sequences that have good homology with retroelements. These mobile genetic elements are present in plant genomic DNA in a high percentage (>50% corn genome and around 90% wheat genome) and it is also considered that these elements have significantly contributed to the evolution of structure and genome function (McCarthy *et al*., 2002). About the question: Could it make sense to relate polymorphic sequences with intergenic or conserved sequences and monomorphic with transcription sequences? We are unable to concluded any theory with these few sequences, but at least we can see that about 50% of them are sequences related with retroelements, 30% code transcriptional genes and finally a small percentage is not characterized.

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WHITE LEAF SPOT, A NEW THREAT FOR DRY BEAN PRODUCTION IN NORTH AMERICA

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INTRODUCTION

White leaf spot is caused by the fungus *Pseudocercosporella albida* (Matta & Belliard) Deighton (1). The disease, not previously reported in the US, was identified affecting plants at three fields near the town of Staples, MN in August 2002 (2). In 2003 two of these fields were planted to kidney bean cv. Red Hawk. Replicated plots established in one of such fields were used to monitor the disease progress and to estimate its impact on yield.

MATERIALS AND METHODS

A randomized complete block design was used to establish 8 experimental plots in a field planted to kidney bean 'Red Hawk', at the University of Minnesota Central Lakes Agricultural Center in Staples, MN. Each plot consisted of four rows 6.7 m long and spaced every 76 cm. Four plots were sprayed once with pyraclostrobin (Headline, BASF) at a rate of 147 g a.i./ha (8 fl oz commercial product/A) 45 days after planting, when the plants were entering the R-1 stage. The sprayer was calibrated to deliver 63 l/ha at 241 kPa. The other four plots were left untreated. Disease incidence and severity were recorded weekly by examining twenty plants arbitrarily selected in the middle of the central two rows of each plot. Yields were measured by harvesting all plants from the two central rows at each plot. The weight of 100 seeds was measured also. Analysis of variance for yield and seed weight was conducted using the GLM procedure of SAS (SAS Institute, Cary, NC).

RESULTS

Disease incidence and severity were monitored starting 45 days after planting. At that time 100% of the plants showed typical symptoms of the disease with an estimated severity of 10% leaf coverage. After the fungicide application, the disease progressed following a linear growth model in the unprotected plots, reaching maximum severity 72 days after planting (Figure 1). Symptomatic leaves senesced faster than non-symptomatic ones. Fungicide-protected plots yielded 871 kg/ha on average, while the unprotected plots yielded 15% less (Figure 2A). Seed size was also affected. The average weight of 100 seeds produced by fungicide-protected plants was significantly higher than that of unprotected plants (Figure 2B).

DISCUSSION

Despite its yield-reducing potential, very little is known about white leaf spot. The disease was identified in dry bean fields near Staples, MN in the fall of 2002 (2). However, empirical observations indicate that the pathogen may have been present there for much longer. How and when was it introduced to the region is not known; but it may only be a matter of time before it spreads into other areas. The previous northern most record of its presence was located in the highlands of Dominican Republic (3).

In the 2003 growing season, white leaf spot reduced yield of 'Red Hawk' by about 15% and seed weight by about 6%; however, yield reductions could have been greater. Generalized root rot incidence across the field resulted in poor canopy development and drier environment. As a consequence, the severity of the epidemic was only of moderate level. In the highlands of Colombia, where this disease is endemic, yield losses could be as high as 47% (4).

The potential impact of white leaf spot on dry bean production in the region warrants research efforts that address its epidemiology and management. Studies that evaluate the role of seeds on long-range movement of the pathogen, the reaction of cultivars of other bean market classes to this disease, within-field spread of the pathogen, and the impact of crop rotations on disease incidence are underway.

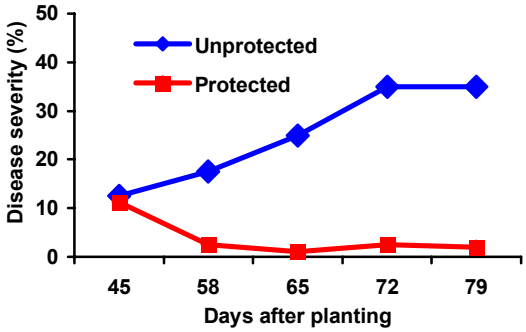

Figure 1. White leaf spot severity progress in plots protected with one application of pyraclostrobin at 147 g a.i./ha 45 days after planting and unprotected plots.

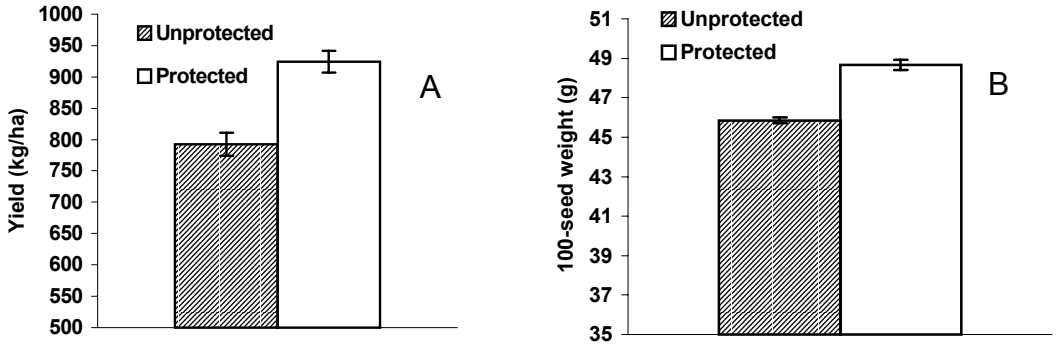

Figure 2. Impact of white leaf spot on (A) yield, and (B) 100-seed weight of kidney bean cv Red Hawk. Protected plots were sprayed once with pyraclostrobin at 147 g a.i./ha 45 days after planting.

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The Enola and NuZa Bean Patents in the Context of Intellectual Property Rights for Plant Cultivars

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It is fair to say that the news that two patents awarded by the U.S. Patent and Trademark Office for beans have been met with incomprehension, if not downright consternation by the bean research community. The two patents are for the yellow seed coat as shown by the common bean cultivar Enola (Proctor 1999; Patent no. 5,894,079, 1999) and for popping (nuZa or kopuru) beans adapted to temperate (U.S.) conditions (Ehlers and Sterner 2000; Patent no. 6,040,503, 2000). In addition, a Plant Variety Protection (PVP) certificate was also awarded for the Enola cultivar. The surprise caused by the awards of these IPRs (Intellectual Property Rights) is directly related to their perceived lack of novelty. This overview will address a number of topics, namely a brief historic overview of the introduction of IPRs on living organisms, the type of IPRs applied to crop cultivars, the specific cases of the Enola and nuZa patents, and a discussion about some issues related to biodiversity and crop cultivar IPRs.

Once upon a time, crop genetic resources were considered to be the "common heritage of humankind" (Herdt 1999). These resources were exchanged freely and at no cost among colleagues, in the public, and across borders. This was only 25 years ago. In this time span, a complete sea change has taken place, which has led to the current situation in which genetic resources are now a commodity subject to market prices, intellectual property rights, and national sovereignty. How did this change come about? The signature event was probably a 1980 U.S. Supreme Court decision (the so-called Chakrabarty v. Diamond decision: U.S. Supreme Court 1980) that instated a patent for a Pseudomonas bacteria capable of degrading hydrocarbons or "crude oil," presumably to be used in clean-up operations after a spill. This capability was unknown among naturally occurring bacterium. The U.S. Patent Office had refused claims of the patent application pertaining to the bacterium itself, but the Supreme Court decided to uphold the application, arguing that patentable subject matter is "anything under the sun that is made by man." This included living organisms.

This Supreme Court decision was further clarified in 1985 by the *Ex Parte* Hibberd decision of the Board of Patent Appeals and Interferences (BPAI), specifically regarding crop cultivars. Plant breeders could now obtain a "utility patent" for their cultivar. It should be noted here that since 1930, plant breeders could patent vegetatively propagated cultivars, principally potato and horticultural species (the so-called "plant patents"). Plant patents did not cover, however, non-vegetatively propagated species. The impact of the 1980 Chakrabarty decision is not to be underestimated because it was on of the stimuli for the development of the biotechnology industry, including the migration of breeding programs from public institutions (in many cases, land-grant universities) to private companies, especially for field crops, such as maize, cotton, and soybean.

The plant and utility patents are not the only legal instruments to protect cultivars. Breeders can also obtain a Plant Variety Protection certificate from the USDA Plant Variety

Protection Office. The PVP system is the practical consequence of the UPOV convention (UPOV being a French acronym, which stands for the Union for the Protection of Plant Varieties). The treaty was first established in 1961, with additional revisions in 1978 and 1991. Most of the countries that have subscribed to UPOV are developed countries. An exception is Kenya. Increasingly, cultivars are protected by both patents and PVP.

What are the similarities and differences between patents (U.S. House of Representatives 2002) and PVP (U.S. House of Representatives 2003) with regard to crop cultivars? Both patents and PVP represent a compromise between society (represented by the government) and inventors. On the one hand, an inventor makes public his or her invention, including the way of manufacturing the invention ("enablement"). On the other hand, the government, in exchange for this invention, grants the inventor a temporary monopoly allowing the inventor to control his invention, by preventing unauthorized use, allowing him or her to award licenses and charge royalties, etc. The duration of this monopoly is 20 years for patents and PVPs for seed crops. For perennial crop PVPs, the duration is 25 years. For patents, the main criteria of patentability are Novelty, Utility, Non-obviousness (or inventiveness), and Enablement. For PVP, the main criteria are Distinctness, Uniformity, Stability, and Non-essential derivation. The latter criterion was added in the 1991 iteration of UPOV to explicitly state that changes to a cultivar such as introduction of a gene by backcrossing or genetic engineering do
not qualify as major changes and, therefore, do not justify a change in ownership. Rather, the owner of the original cultivar also remains the owner of the "improved" cultivars with the minor changes. There is a grey area as to when a minor change becomes a major one, in which case a new PVP action would be warranted.

There are two important distinctions between patents and PVP. Unlike patents, PVP includes a farmer's exemption and a breeder's exemption. A farmer is allowed to harvest the seed and use it for free for further planting on his or her holdings. A breeder can use for free a PVP cultivar as a progenitor in crosses to generate the next generation of improved cultivars. Neither exemption exists for patented cultivars. In addition, courts have markedly reduced any research exemption associated with patents. The absence of the farmer's and breeder's exemption explains why patents have become increasingly popular as an IPR tool for cultivars the U.S. In Europe, only PVP can be used to protect cultivars. However, basic processes applicable to plants in general remain patentable.

Given this general background, what are the specific concerns associated with the Enola and nuZa bean patents? For any patent, one needs to read carefully the specific "claims" described in the text of the patent because these determine the overall scope of the patent. For the Enola patent (Proctor 1999), the key claims are as follows:

"*1. A Phaseolus vulgaris field bean seed designated Enola as deposited with the American Type Culture Collection under accession number 209549.*
4. A field bean plant having all the physiological and morphological characteristics of the field bean plant of claim 2.
5, 6, 7: Also claims progenies of crosses …
8. A field bean variety of Phaseolus vulgaris that produces seed having a seed coat that is yellow in color, wherein the yellow color is from about 7.5 Y 8.5/4 to about 7.5 Y 8.5/6 in the Munsell Book of Color when viewed in natural light.
10. The Phaseolus vulgaris of claim 9 wherein the hilar ring has a color of rom about 2.5 Y 9/4 to about 2.5 Y 9/6 in the Munsell Book of Color when viewed in natural light.

Thus, the patent claims a specific genotype (of which a seed sample was deposited with the ATCC) and a (fairly narrow) range of shades of yellow seed coat color. According to the patents description, the genetic material was obtained in 1994 in Mexico in a bag of mixed bean seeds. The material was then grown out for three years in Colorado and underwent presumptive selection for uniformity and seed color, upon which both a patent and a PVP certicate were applied for in 1997 and awarded in 1999.

To investigate the origin and potential distinctness of the Enola genotype, a DNA fingerprinting was conducted. In this fingerprinting experiment, we considered three essential aspects: a) the plant sample; b) the marker type; and c) probability calculations. A sample of 56 domesticated bean genotypes was assembled. This sample included not only 24 yellow-seeded genotypes but also 32 non-yellow genotypes, which were included as controls, especially for the probability calculations. Among the yellow-seeded materials were Enola (obtained from the official sample at the American Type Culture Collection), three Peruano-type cultivars (Azufrado Pimono78, Azufrado Peruano 87, and Azufrado Regional 87), a few breeding lines of this market type, one representative each of the original yellow beans for Mexico and Peru, and Sulfur BN142 (a presumed representative of Sulphur, described by Hedrick, 1931). Voysest (2000) describes how Mexican bean breeders developed the new Peruano market class by crossing Mexican Azufrado and Peruvian Canario types and selecting for yellow seed color and growth habit.Among the non-yellow seeded materials were representatives of the six major races of domesticated beans.

The second aspect to consider in a fingerprinting experiment is the type of marker. *A priori* we thought that markers ought to obey the following conditions: a) highly polymorphic and/or high number of markers; b) reproducible; c) well-distributed throughout the genome; d) well-known pattern of genetic diversity in the gene pool of interest; and e) well-known and used in the research community. Given the current status of markers in beans, three types of markers could potentially qualify for this type of study: a) AFLPs; b) ISSRs; and c) Microsatellites. Of these, AFLPs come closest to fulfilling the requirements. They generate a large number of markers (which will prove essential in probability calculations), they are reproducible, and there is prior history of their use in common bean for the analysis of genetic diversity.

The third aspect is the calculation of the probability of a match between an Enola fingerprint and fingerprints of other bean cultivars, especially yellow-seeded cultivars. The general formula for calculating the probability of a match between two profiles is $\tilde{P} = \prod_i \tilde{p}_i^2$ (Weir 1996: p. 218), with $\tilde{p}_i 0$ being the probability of obtaining the *i*th fragment state observed for the Enola profile (either presence or absence of the fragment). (The squared frequency is used because the frequency of a match between two fingerprints is calculated instead of the frequency of a specific fingerprint.) This formula is valid only if AFLP fragments show independence among each other. We defined independent markers as those markers for which less than 10% of the Fisher exact tests for independence with all other markers were statistically significant ($P \leq 0.10$). This greatly decreased the number of markers available. From an original 133 markers, only 25 to 30 markers are generally independent from each other. The lack of independence of the other markers is due in part to linkage and in part to common ancestry and population structures (gene pools, market classes, etc.). The actual probabilities ($\tilde{p}_i 0$) depend on the breeding scenarios envisioned for the development of Enola. Four major scenarios were considered, based in part on the known history of the Peruano-type marker class

in Mexico (Voysest 2000) (scenarios 1-3) and the patent description (scenario 4): 1) cross between any Andean and Mesoamerican genotype, regardless of seed color; 2) cross between an Andean and a Mesoamerican yellow-seeded genotype; 3) cross between any yellow-seeded type, principally Peruano types; and 4) selection within an existing yellow-seed cultivar. Further details are provided in an upcoming manuscript by Pallottini et al. (2004).

The main conclusions of this fingerprinting experiment were as follows (Pallottini et al. 2004). AFLPs were very useful in identifying differences or similarities even among closely related genotypes. AFLPs based on *Pst*I/*Mse*I primers revealed a three-fold larger number of polymorphic markers than those based on *Eco*RI/*Mse*I primers. AFLPs classified bean cultivars according to previously known relationships such as the split between Andean and Middle American cultivars (Fig. 1).

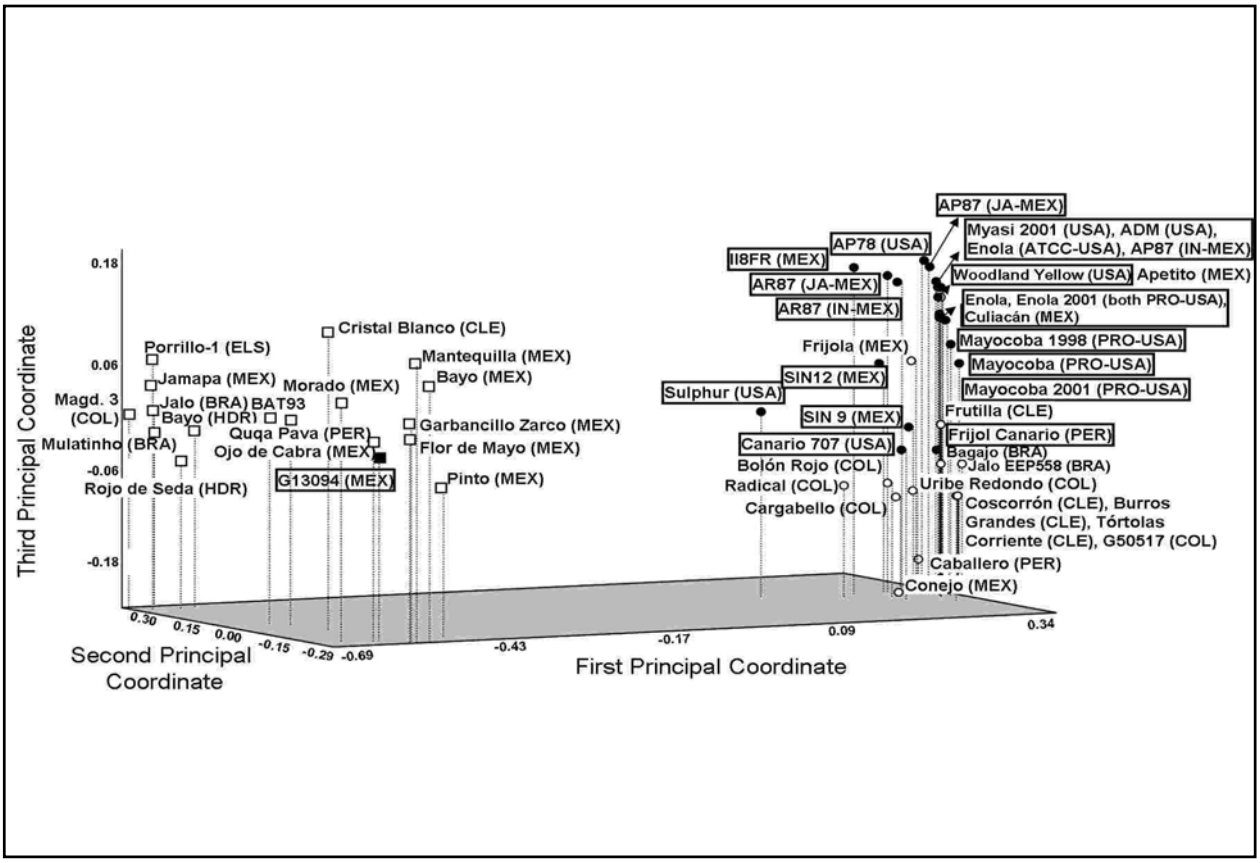

Fig. 1. Principal coordinate analysis of AFLP diversity in a sample of 56 common bean cultivars. Square symbols: Middle American gene pool; circles: Andean gene pool. Boxed entries and filled symbols: yellow seed coat entries. AP78: Azufrado Peruano 78; AP87: Azufrado Peruano 87; AR: Azufrado Regional 87. The eigenvalues of the three axes are 58%, 7%, and 5% (Pallottini et al. 2004).

They did not distinguish race Jalisco from race Durango cultivars in the Mesoamerican gene pool. No racial separation was observed in the native Andean cultivars as observed earlier (Singh et al. 1991). The Peruano group of cultivars fell within the Andean gene pool although it was distinct from the "native" Andean cultivars. Enola is part of the Peruano class of cultivars

and is most closely related to Azufrado Peruano 87. In fact the probability of generating independently the same fingerprinting ranged from 1 x 10^{-18} (scenario 1) to 3 x 10^{-5} (scenario 3) to 3 x 10^{-1} (scenario 4: selection within Azufrado Peruano 87).

The PVP certificate cites the cultivar Azufrado Pimono 78 as the most closely related cultivar to Enola and mentions leaf color as a distinguish factor between the two cultivars. A replicated greenhouse experiment was conducted to compare leaf color among yellow-seeded cultivars, including Enola, Azufrado Pimono 78, and Azufrado Peruano 87 with a Minolta Chroma Meter CR-200 (Minolta, Ramsey, NJ), a tristimulus colorimeter. Of the three color variable measures L, Hue, and Chroma, only Chroma showed significant differences among means. Enola had lighter leaf color than one sample of Azufrado Peruano 87 but not the other sample. Thus, there is heterogeneity within the Azufrado Peruano 87 cultivar. The differences in leaf color between Enola and Azufrado Pimono 78 were not significant in this experiment. In any case, leaf color is a secondary character in the discussion surrounding Enola.

Our conclusion is that, from a genetic fingerprinting standpoint, Enola is not different from the pre-existing Mexican yellow-seeded cultivars. Furthermore, Bassett et al. (2002) have shown that the genetic combination controlling the yellow seed color in Enola (*C;J;g;b;vlae;Rk;gy*) is also present in the obsolete cultivar Wagenaar. These conclusions raise questions about the rationale for providing a utility patent or a PVP certificate to the Enola cultivar. Although we are not legal scholars, the data suggest that Enola does not satisfy the novelty and non-obviousness statutory requirements of the patent legislation. It may not satisfy the distinctness and non-essential derivation requirements of the PVP legislation.

So far, CIAT (Cali, Colombia) has challenged the award of the Enola patent by requesting a re-examination (introduced on Dec. 20, 2000). This was followed by a re-issue request on the part of the patentee on Jan. 31, 2001. The Patent Office has not yet ruled on these requests. Nobody has challenged the PVP certificate. Time is running out to do so because it can only be done within five years of the award, which took place on May 27, 1999.

Why were these intellectual property rights awarded at all, especially the utility patent? There has been an overall trend towards easier and broader award of patents. For example, as stated by Demaine and Fellmeth (2003), "*subtly and without fanfare, the prohibition on patenting products of nature has fallen into desuetude*." In the case of beans, there are at least two other patents that seem to be questionable in terms of novelty. For the nuZa patent (Ehlers and Sterner 2000), the main claims are as follows:

" .... 9. A bean seed produced by a cross of a nuZa accession and a Phaseolus vulgaris cultivar exhibiting the characteristics of early maturity, bush type growth habit, synchronous fruiting, and photoperiod insensitivity, wherein said bean pops at a moisture of about 5 to 12 percent.

10. A bean seed of claim 9, wherein said nuZa accession is selected from the group consisting of accession numbers W6 4296, W6 4297, W6 4298, PI 298820, PI 298822, PI 298824, PI 316013, PI 316014, PI 316016, PI 316017, PI 316018, PI 316019, PI 316020, PI 316021, PI 316022, PI 316023, PI 316024, PI 316025, PI 316029, PI 316030, PI 316031, PI 316032, PI 390771, PI 390775, PI 511763, PI 511767, PI 531862, PI 577677, PI 577678, PI 577679, PI 577680, PI 577682, and PI 608402.

11. A bean seed of claim 9, wherein said Phaseolus vulgaris cultivar is selected from the group consisting of small white, small red, navy, dark red kidney, light red kidney, black or black turtle, pink, pinto, cranberry, and canario. ...." Neither of these claims is novel, nor would the combination of claims because it amounts to making crosses to introduce a trait from exotic germplasm into an adapted background. Furthermore, experiments have been conducted

although perhaps not published that have attempted to introduce the popping trait. What is intriguing is that the Patent Office allows the patentees to claim accessions that are part of an official USDA germplasm bank. Even more intriguing is that the patentees could claim market classes, including the canario (yellow) seed type, thus raising the spectrum of infringement of the Enola patent.

The second case is a recent patent describing a method to decrease flatulence in legumes , in general, and in beans, in particular (Bush et al. 2002). The main claim is "*soaking a cleaned legume in a water bath having stagnant, sprayed or flowing water at a first temperature* [note: 90-130 ºF] *which is above ambient temperature but less than the critical rehydration temperature of the legume and under conditions effective to rehydrate the legume to at least 50% by weight of that of a fully hydrated legume; ....*" This procedure is very similar to the one used in households around the world to pre-soak beans in lukewarm water as a first step to cook beans with reduced flatulence.

In addition to the lack of novelty, the Enola and nuña patents also raise the issue of ownership of foreign genetic resources. In addition to the yellow and nuña bean patents, other controversial patents involving foreign genetic resources include the neem tree oil (Roland and Blouin, 1996), maca (DeLuca et al., 2000; Zheng et al., 2001, 2002), turmeric (Das and Cohly 1995), ayahuasca (Miller 1986), and basmati rice (Sarreal et al., 1997). Their existence suggest that more stringent criteria should be developed for such awards, especially in light of the recent trend in international law assigning national sovereignty for biodiversity to individual countries (Anonymous, 1992; Commission on Genetic Resources for Food and Agriculture, 2001).

An additional issue is the type of scientific data required to document an invention. Color in the case of the Enola patent was documented by a Munsell Color chart. There are now more modern, accurate, and reproducible ways of documenting color. Likewise, molecular markers provide opportunities to more accurately document differences or similarities (depending on whether one seeks to document ownership or infringement!).

Finally, there is increasing reliance on utility patents to claim ownership over a new cultivar. The U.S. is the only country, with Japan and Australia, in allowing patents for cultivars. Other countries only provide PVP protection based on the UPOV convention. Because PVP offers a breeder's exemption, breeders can use a PVP cultivar as a parent in crosses to develop the next generation of improved cultivars. Utility patents offer no such exemption. This situation raises questions whether the absence of breeder's exemption is going to limit germplasm exchange and progress from breeding will be slowed down as a consequence. Given the 7-10 year time frame, the answer to these questions is not immediately forthcoming. However, in our opinion there has been almost no discussion in the breeding community in general about this issue.

From a broader, international perspective, the increased emphasis on intellectual property rights over crop cultivars, in particular, and biodiversity, in general, raises a number of questions (Gepts 2004), including whether living organisms and any of their constituting parts (including genes) be subject matter of IPRs; whether reliance on IPRs will assure efficient conservation and utilization of biodiversity; whether the non-utilitarian functions of biodiversity, such as ecosystem health and function as well as its esthetic role, well served by a IPR regime; and whether legal and economic frameworks can be instituted that address the conservation of both biological and cultural diversity? Given that the new era of IPR for biodiversity only started some 25 years ago, much needs to be discussed still. Biologists, in general, and breeders, in particular, should be involved.

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A BIOCHEMICAL TRAIT HELPS TO RECOGNIZE *PHASEOLUS PARVIFOLIUS* FREYTAG IN THE GENE POOL OF TEPARY BEAN

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Introduction

The section of *Phaseolus* currently including the tepary bean, i.e. the *Acutifolii*, consists of two species: *Phaseolus acutifolius* A. Gray (with three varieties: var. *acutifolius*, var. *latifolius* and var. *tenuifolius*) and *P. parvifolius* Freytag (Freytag & Debouck 2002). Schinkel & Gepts (1989) could not separate these varieties by nine allozyme assays. Garvin & Weeden (1994a) reported limited polymorphism for aconitase, apparently with no relationship with foliar attributes. Jaaska (1996) found a unique electromorph for three out of six accessions of var. *tenuifolius*, now classified in CIAT as '*parvifolius*'. In a study of 91 accessions with ten enzyme systems, Florez (1996) found that the allele *Aat-2*^{95} uniquely separates the twelve '*parvifolius*' materials from the rest of wild teparies. Zink & Nagl (1998) reported a minor difference in banding pattern of microsatellites between *P. parvifolius* and accessions of *P. acutifolius*. Muñoz et al. (2002) found in a diversity study with help of AFLPs that *P. parvifolius* forms a group separating from other wild teparies at the level of separation of common bean genepools. The purpose of this study was to find a biochemical marker ("diagnostic isoenzyme") for the recognition of either one of the varieties of tepary bean.

Materials and Methods

We analyzed 100 accessions (26 cultivated, 72 wild and 2 "escaped") of *P. acutifolius* from the world collection held at CIAT. These accessions represent the geographic, ecological, and morphoagronomic variability, as well as the variation of seed proteins found in tepary bean. Ten enzyme systems assayed by means of polyacrylamide and starch gel electrophoresis from different tissues were evaluated. The methodology for isozyme extraction, running and staining was the one reported by Ramirez et al. (1987). Globulin patterns (seed storage proteins) were analyzed by SDS-PAGE as in Gepts et al. (1986). For each allozyme, loci and alleles were designated as described by Koenig & Gepts (1989).

Results and Discussion

Out of all enzymatic complexes analyzed, the aspartate aminotransferase (AAT; E. C. 2.6.1.1) system obtained from root tips and polyacrylamide gel electrophoresis displayed alleles in *P. parvifolius* that were absent in the other varieties (Figure 1). In agreement with genetics of Aat isozyme (Garvin & Weeden 1994b; Garvin et al. 1989), the Aat-2 locus has three alleles (93, 95 and 100), all of them homozygous in the accessions evaluated. The allele *Aat-2*^{95} is present exclusively in *P. parvifolius* (Table 1). Only three patterns (IX, X and XII) of globulins were found in *P. parvifolius*. The "XII" type is dominant (present in all accessions), whereas in the other botanical varieties it appears with low frequency (4,1 % in wild var. *acutifolius* and 9,3 % in wild var. *tenuifolius*) (Florez, 1996).

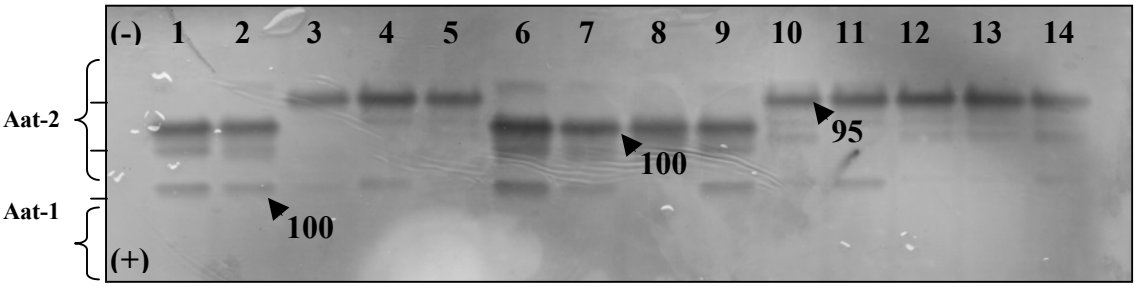

Fig. 1. Polyacrylamide gel phenotypes observed for aspartate aminotransferase (AAT). Individuals in lanes 1 and 2 are cultivated (var. *acutifolius*), individuals 6 and 7 are wild var. *acutifolius*, and individuals 8 and 9 are wild var. *tenuifolius*. The rest are classified as *P. parvifolius* (lane 3, 4, 5, 10, 11,12, 13, and 14).

Table 1. Distribution of electromorphs found for AAT isozyme[1] in varieties of *P. acutifolius* and *P. parvifolius*

Botanical variety	Biological Status	Loci/ alleles/ individuals				
		Aat-1		Aat-2		
		100/	n/n[2]	93/100	95/95	100/100
var. *acutifolius*	Cultivated	12	14	1	-	25
var. *acutifolius*	Wild	23	5	-	-	28
var. *tenuifolius*	Wild	21	3	1	-	23
P. parvifolius	Wild	20	-	-	20	-
Weedy forms	Intermediate	2	-	-	-	2

[1] The genetics of AAT isozyme has been reported by Garvin and Weeden (1994b), with three zones of migration observed. Nevertheless, we observed only two zones of migration (Florez, 1996).
[2] A null allele has been reported in tepary bean.

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BIOCHEMICAL EVIDENCE SUPPORTING THE EXISTENCE OF A WEEDY FORM IN TEPARY BEAN (*PHASEOLUS ACUTIFOLIUS* A. GRAY)

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Four forms of *Phaseolus acutifolius* A. Gray are known: three wild varieties and a domesticated form (Freytag and Debouck, 2002). However, until now an intermediate form between the wild and cultivated forms has not been identified (Pratt and Nabhan, 1988). The aconitase isozyme (ACO-2 allozyme) has been an informative evolutionary marker in teparies beans as to know the origin of its domestication. The slow ACO-2 allozyme was present in domesticated tepary, whereas the fast ACO-2 allozyme was present in wild tepary (Garvin and Weeden, 1994). Additionally, the seed storage proteins patterns (globulins) serve to separate wild from the domesticated form. 25 globulins types were found only in wild *P. acutifolius*, while in the domesticated accessions there were only two patterns, not having counterpart in the wild materials (Schinkel and Gepts, 1988; Florez, 1996). Both biochemical markers may therefore serve as an evolutionary marker to help identify wild and domesticated teparies, and by extension, in order to help identify a putative intermediate form. We analyzed a complex (wild-weedy-crop) from a wild population of teparies beans, from a biochemical (globulins and ACO-2 allozyme) and morphological viewpoint to find evidence that suggests the existence of a true intermediate form in tepary bean.

Materials and Methods

We studied a population (G40177) whose original seed were collected and classified as wild in Arizona. Later this seed was introduced as original seed in the *Phaseolus* germplasm bank maintained in CIAT. For the morphological analysis, we study seed size, color and pattern. In addition two accessions were chosen as comparison controls for the aconitase isozyme analysis: a cultivated (G40064) from Arizona (USA), and a wild (G40090) from the Mexican state of Durango. Using starch gel electrophoresis (Garvin and Weeden, 1994), we examined aconitase variation in 98 seeds of the population G40177, of which 87 were analyzed as "selfed materials" of aconitase isozyme type (nondestructive test of seed for isozyme extraction) and 11 were analyzed with destructive test of seed. In addition, we included a seed protein analysis (globulins) by ID-SDS-PAGE (Brown et al. 1981); using destructive test of seed.

Results and Discussion

The morphological analysis was done on seed multiplied in greenhouse. We observed segregation for seed size and colors indicating a possible wild-weedy-crop complex. Once stabilized in an advanced stage of increase, the materials were classified (98 in total) as cultivated [49 (55 %)], intermediate [41 (33 %)] and wild [10 (10 %)]. In addition, this complex shows a great diversity in seed size (from small to large) and color (Figure 1). The aconitase ACO-2 allozyme analysis shows two alleles in all phases of the complex, from typical wild seeds to fully domesticated forms. In addition, the weedy form displays a heterozygous allozyme (Fast/Slow). Only two patterns (IX and IV) of globulins were found also in all phases of the complex (Table 1). The "XI" type is present only in domesticated accessions, whereas the other globulin type (IV) is present exclusively in wild teparies (Schinkel and Gepts, 1988). These higher frequencies of intermediate materials between the wild and domesticated forms suggested by morphological data correlate well with the higher frequency contributed by the isozyme and globulin analysis. In contrast with reports of Pratt & Nabhan (1988), these evidences suggest the existence of a true intermediate form in tepary bean and confirm that natural hybridization happens in the zone (a natural vegetation located in the "Santa Rita Mountains", Pima County in Arizona, USA) where was collected this wild population.

Figure 1. Morphological variation in the wild-weedy-crop complex G40177 from a wild population.

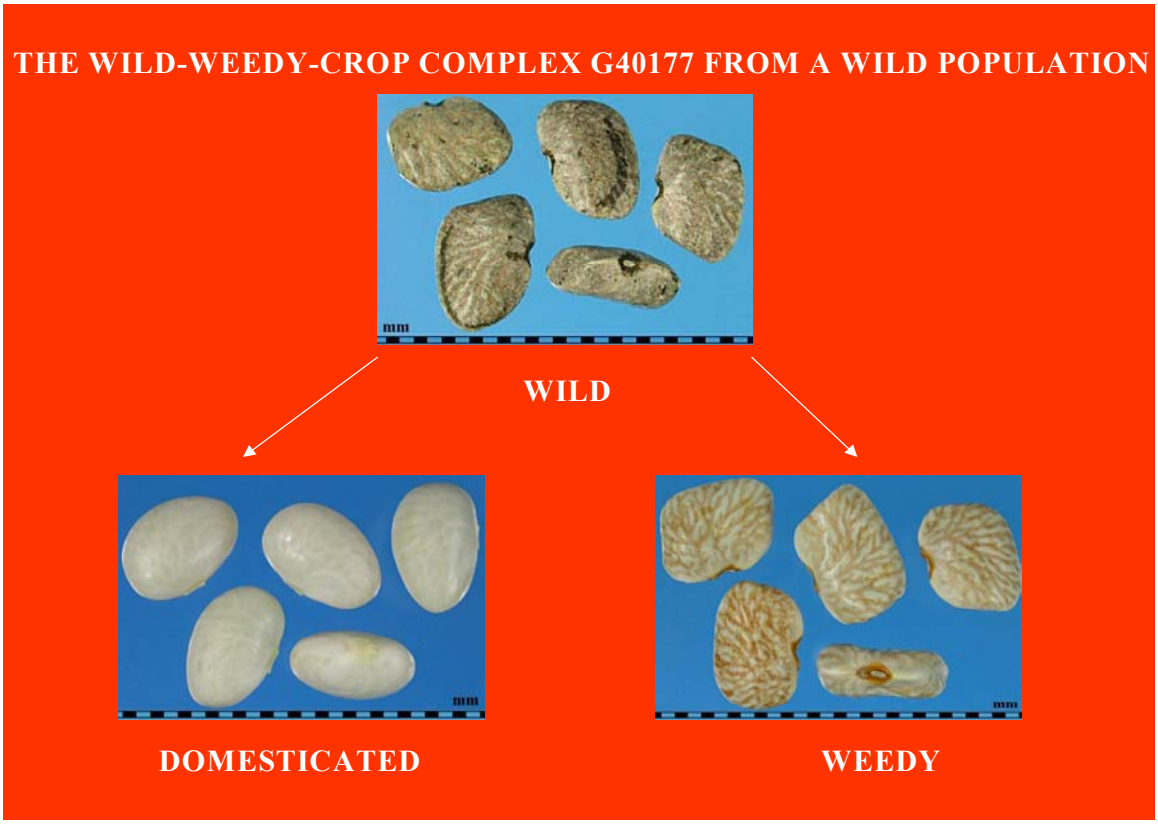

Table 1. Globulin types, ACO-2 allozyme constitution and seed size of the wild-weedy-crop complex G40177 from a wild population of teparies beans. The globulin types frequencies is in parenthesis.

Biological status	Analyzed Seeds		100 Seed weight (g)	Globulin type	ACO-2 Allozyme		
					Fast	Slow	Fast/Sslow
	"selfed materials"	Destructive test					
Cultivated	48		>10 g	XI (32) IV (16)	28	20	
Weedy	29		6-9 g	XI (20) IV (9)	19	10	
Wild	10		< 6 g	XI (4) IV (6)	8	2	
Weedy		11	6-9 g	IV (11)	5	3	3
Total	87	11	---- * ----	XI (56) IV (42)	60	35	3

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Additional Evidence on Gene Flow Events in *Phaseolus vulgaris* in Costa Rica

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We present here evidence on gene flow between wild and cultivated forms of common bean in Costa Rica in addition to our previous work (González-Torres et al. 2003).

Seeds were collected from natural populations in the Central Valley of Costa Rica as previously reported (González-Torres et al. 2003). We focus on 226 weedy materials selected initially on morpho-agronomic characteristics, which phenotype is inherited from possible hybridization between wild and cultivated materials. A similar procedure has been used by Papa & Gepts (2003). The analyses were conducted on: 1) morpho-agronomic evaluation; 2) biochemical analysis of phaseolin by SDS-PAGE (Gepts et al. 1986), and isozymes: Diaphorase (DIA) and Peroxidase (PRX) according to Ramírez et al. (1987), and 3) molecular marker analysis: eight microsatellite primers reported by Gaitán-Solis et al. (2002), and cpDNA polymorphisms by PCR- RFLPs (Chacón-Sánchez, 2001).

The wild populations showed mainly two phaseolin patterns, Simple-4 and S (Table 1). In cultivated materials, the phaseolins T, Sb and Simple-4 were also observed although in low frequency.

Table 1. Morphological, biochemical and molecular markers used and No. individuals analyzed for each parameter.

Biological status	Seed average weight (g)	Phaseolin type	Isozymes		Microsatellites		cpDNA haplotypes
			Pattern[1]	Allele[2]	Primer	Allele	
Wild	6 N=443	"Simple-4" "S" N=402	DIA –1 N=227	**PRX 100** **N=204**	BM140 BM172 BM175 BM183 BM187 BM188 BM189 BM205 N=134	160 ***80*** 162 110 163 146 137 122	G, H N=97
Weedy	13 N=226	"C" "CH" "H" ***"S"*** "X-7"[3] **"Simple-4"** N=191	DIA-1 DIA-2 DIA-4 N=170	**PRX 100** PRX 98 **N=170**	BM140 BM172 BM175 BM183 BM187 BM188 BM189 BM205 N=142	160, 177 ***80*** 162, 183 110, 106 163, 189 146, 150 137, 174 122, 135	G, H J, K, L N=100
Cultivated	23 N=188	**"S"** "X-7" "CH" N=186	DIA –2 DIA –4 N=150	PRX 98 N=150	BM140 BM172 BM175 BM183 BM187 BM188 BM189 BM205 N=35	177 ***80*** 183 106 189 150 174 135	J, K, L N=33

[1] According to Sprecher (1988); [2]according to Koenig & Gepts (1989); [3] Phaseolin pattern for further checking.

In Figure 1, the shortest bar represents mainly wild characteristics and the longest bar is a description of cultivated materials. The bars show exchange among individuals for the following markers: shared SSR alleles, change in cpDNA haplotypes, 100-seed weight, isozymes and phaseolin patterns. In individuals 1 and 2, all the evaluated parameters are "wild" and they have a hybrid SSR allele, which suggests a cross

of wild material with pollen of cultivated material. The seed size of the individual 3 could be a phenotypic consequence of more than one past event of gene flow from cultivated material to wild form, because all evaluated parameters are "wild" including hypocotyl color (purple), purple flower, 85 days to flowering and growth habit IV. Besides, its F2 displays a weight of 10.3 g, which suggests that it has acquired "wild" characteristics but conserves the "cultivated" seed size. Individual 8 has hybrid isozymes, "wild" microsatellite alleles and phaseolin, but it has a "cultivated" chloroplast haplotype. Individual 9 has the same characteristics as individual 8 but it has "wild" isozymes. These materials represent cases of repeated gene flow of cultivated materials crossed with wild forms. Individual 14 is hybrid (PRX enzyme and one SSR locus), meaning that it comes from recent flow of "wild" pollen to a cultivated form. The evaluation of these 22 cases from Costa Rica indicates that all materials are indeed products of hybridizations showing that the methodology implemented in the selection of the intermediate materials was the appropriate one. Papa et al. (2003) found that the contribution of cultivated parental population was significantly higher than the wild parental one in Mesoamerica, while the direction of gene flow in the evaluated individuals in our study was evidenced mainly from wild material to the cultivated type. The presence of gene flow events in the other direction was observed at lower frequency.

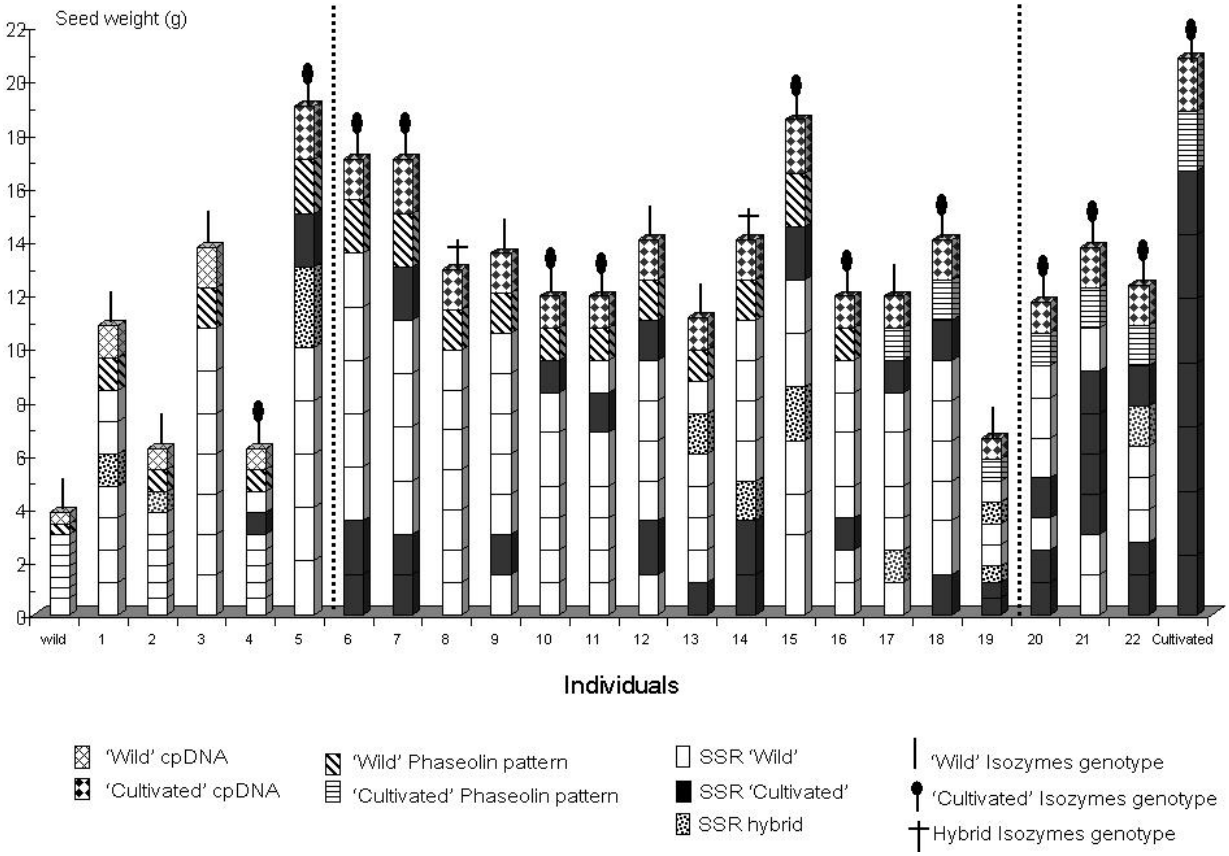

Figure 1. Graphical representation of individuals with their respective markers.

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DETERMINATION OF GENETIC DIVERSITY OF SNAP BEANS *Phaseolus vulgaris* L. CULTIVATED AT SECONDARY CENTERS OF DOMESTICATION, USING MORPHOLOGICAL AND BIOCHEMICAL DESCRIPTORS

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Since there are genetic differences for pod, flavor compounds, agronomic manegement among snap bean and dry bean therefore, it would be justifiable to establish separate gene pools for these, in spite that natural variability from the centers of domestication in the Americas may not be large (Singh, 1989). There is not accord about wideness of snap beans genetic pool. Information about its ancestors and genetic diversity remains diffuse (Myers and Baggett, 1999). The aim of this work is to verify if the results obtained from previous works on common beans are comparable to those obtained for snap beans germplasm.

Materials and Methods

Eighty-seven landraces of snap beans collected in Europe, Asia, Africa and America, and conserved at the Germplasm Bank of URG-CIAT, were compared using eighteen morphological (whole plant, pod and seed) traits. Two commercial varieties (Blue Lake, Milenio G51158) and two common beans genotypes [G4494 (andean), G5733 (mesoamerican)] were used as controls (Tofiño et al, 2004). Also, seed phaseolin patterns using SDS-PAGE (Gepts et al, 1986) and eight isozyme polymorphic systems [acid phophatase (ACP), peroxidase (PRX), diaphorase (DIA), shikimate dehydrogenase (SKDH), malic enzyme (ME), malic dehydrogenase (MDH), phosphogluco isomerase (PGI), and 6-phosphogluco dehydrogenase (6PGDH) from root tissues (Koenig & Gepts, 1989) were analyzed. The fitness degree of snap beans genotypic traits to the common beans ones was determinate following Singh et al (1991a). Correlation coefficient between phaseolin type and seed morphology was calculated according to Singh (1989). Genetic diversity indexes [Total diversity (Ht), intrapopulation diversity (Hs), interpopulation diversity (Gst) and genetic identity I)] were obtained using the molecular genetic analysis program POPGENE (Yeh & Boyle, 1999). Morphological and biochemical data were used to find the Dice simmilarity index and also the UPGMA dendrogram that describes genotypic relationships between accesions, using the SANH-clustering and tree subroutine of NTSYS pc v. 210 (Adams et al, 2000).

Results and discussion

Correlation coefficient (R=0.88) between morphological characteristics and type of phaseolin was highly significative. The best pod characteristics but the highest morphological variability were found in the mesoamerican genotypes. The low fitness degree observed between snap beans genotypes and the races description of Singh et al (1991a) (15%) can be explained probably by the genotypic biochemical variability. When we compared the grouping results to phaseolin types and biochemical characteristics no differences between mesoamerican and andean pools were observed, neither the high level of concordance between the representative alleles of each pool and the phaseolin type that have been observed in another works in common beans (Singh et al, 1991b; Table 1). Subgrouping differences can be explained by the gradient concordance of the combination of both phaseolin origin and the origin of the eight-isozyme systems studied. Such characteristics have been related to intermediate genotypes between common beans pools (Santalla et al, 2002). A high level of complexity in the studied sample of snap beans germplasm can be postulated after our results. The total diversity found for the analyzed isozyme systems in the snap beans sample is similar to that reported in common beans by other workers (Santalla et al, 2000). Nevertheless, we found contrasting values of genetic diversity for both into and between Andean and mesoamerican pools (Ht=0.3175; Hs=0.2549; Gst=0.0626; I=0.957).

Table1. Snap beans landraces cultivated at secondary centers of domestication grouped on isozyme characteristics, morphological traits and phaseolin type basis.

Cluster	Acc Nr.	Phaseolin	Growth habit	Isozyme characteristics	Morphological traits
I-A	32	S, CH, T, C	II,III,IV	DIAP- I:95>100, PRX:98, SKDH:103, MDH-I:100 103, ME:98-100	0.75 Mesoamerican- 0.25 Andean, Varied seed color and shape, low fiber content, low seed index
I-B	9	S, T	IV>III	DIAP-I:95>100, PRX:98, SKDH:103, MDH-I:100, ME: 100	0.88 Mesoamerican- 0.12 Andean Varied seed color, curved and flatted pod with hilum, medium fiber content, low seed index
I-C	5	S,Sb, CH, C	IV	DIAP-I:95, PRX:98, SKDH:103, MDH-I:100, ME:98-100	0.80 Mesoamerican- 0.2 Andean Creamy, brown seed color, elliptic pod cross section, slightly curved, commercial size, medium fiber content
I-D	5	T, C,H1, S	III, IV	DIAP-I:100, PRX:98-98/100 SKDH:103, MDH-I:100, ME: 98	0.60 Andean- 0.40 Mesoamerican, seed color creamy, white and rounded in shape. Varied pod shape, circular cross-section, low-medium fiber content, médium seed index
II-E	9	T, C, S	III, IV	DIAP-I:100>95, PRX:98>96 SKDH:100, ME:100 MDH-I: 100	0.67 Andean- 0.33 Mesoamerican, seed color creamy, white, brown. Varied pod characteristics
II-F	18	T, C, S	I, II, III, IV	DIAP:95>100, PRX:98, SKDH:100, ME: 98>100 MDH-I: 100	0.62 Andean- 0.38 Mesoamerican Varied seed color and pod characteristics.
III-G	8	C, T, S, CH	IV, III	DIAP-I:95-100; PRX: 98>96-98/100, SKDH:100-103, ME: 98>100-102, MDH-I: 100-103	0.55 Andean- 0.45 Mesoamerican. Sseed color creamy, brown, varied pod characteristics.

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ADDITIONAL EVIDENCE ABOUT WILD-WEED-CROP COMPLEXES OF COMMON BEAN IN DIFFERENT PARTS OF COLOMBIA

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Extensive wild-weedy-cultivated complexes were observed in common bean during collection expeditions in regions of Colombia where wild and cultivated beans are sympatric. Such interbreeding complexes may be important mechanisms for the generation of genetic variability in landraces (Beebe et al. 1997).

Here, we report the finding of new complexes (wild-weedy-cultivated) of common bean in Colombian regions where these have not been reported previously. Additionally, we analyzed these complexes from a biochemical (phaseolin and isozyme markers) and morphological viewpoint to estimate the variability as a contribution to their conservation and use.

Materials and Methods. Ten wild-weedy-crop complexes were selected after a geographic sampling in Colombia (Table 1). For the biochemical analysis, we only took the multiplied and conserved seed in the *Phaseolus* germplasm bank held in CIAT. In addition three accessions were chosen as controls: two cultivated P. vulgaris from the Andes and Mesoamerica (G4494 and G5773, respectively) and a Colombian wild (G24408). For the seed morphological analysis, we study seed size, color and pattern. The seed storage proteins were analyzed as "selfed materials" of phaseolin type found for each analyzed seed. This variation was first analyzed in ID-SDS-PAGE (Brown et al. 1981) and confirmed later in 2D-IEF-SDS-PAGE (O'Farrel, 1975). For the isozyme analysis only a complex was selected (G50849), being used for it thirty "selfed materials" of phaseolin type. We used only two polymorphic enzymatic complexes: peroxidase (PRX; 1.11.1.7) and diaphorase (DIA; 1.6.4.1). The selected isozyme loci carry alleles from both Mesoamerican and Andean gene pools: the Dia-1^{95}, PRX^{98} alleles are considered to be Mesoamerican and the Dia-1^{100}, PRX^{100} alleles are of Andean origin (Koenig and Gepts, 1989; Debouck et al. 1993). The methodology for isozyme analysis was the one reported by Ramirez et al. (1987).

Results and Discussion. The original seed of these populations was collected and classified as cultivated materials. However, during the initial seed increase, we observed segregation for seed size and colors indicating possible wild-weedy-crop complexes.

The materials (1,182 in total) were classified as cultivated [642 (54%)], intermediate [432 (37 %)] and wild [108 (9 %)] (Table 1). These segregating populations were considered to be complexes, since they involve wild and weedy stabilized forms. These complexes showed a great diversity in seed size (from small to large) and color. Additionally, a great diversity for phaseolin types was found within these complexes. The patterns were: five Andean and six Mesoamerican, with a frequency of 55% and 45%, respectively. In these complexes, the "S", "B", "C", "T", "C", and "Mu" phaseolins form a continuum across the full range of biological status (Table 1). For the isozyme analysis, both allozymes (Mesoamerican and Andean) are found in the analyzed complex (G50849). Nevertheless, only two allozymes were found in all phases of the complex: a "crossed" allozyme ($PRX^{98, 100}$) and an Andean allozyme (Dia-$1^{100, 100}$) (Table 2). The variability at the phaseolin and isozyme levels suggests an important genetic interchange in the study area in Colombia between Mesoamerican and Andean materials. These results are concordant with those obtained by Debouck et al. (1993); Paredes and Gepts (1995) and Beebe et al. (1997), using morphological and biochemical markers, and those obtained by Tohme et al. (1996), Chacón et al. (2002), and Ocampo et al. (2002), using molecular markers. However, we are reporting a extensive distribution of these introgressed complexes in Colombia, much more of the reported by Beebe et al. (1997). This distribution includes some departments where wild and cultivated beans are sympatric (Cundinamarca and Boyaca) or in departments where the common bean is an important crop (Antioquia, Caldas, Tolima and Cauca).

These results suggest a new map in Colombia for the distribution of these biological complexes of common bean and confirm that a considerable amount of natural hybridization occurs in the areas where these populations were collected.

Table 1. Description of the wild-weed-crop complexes from domesticated Colombian populations of common bean.

CIAT No.	Department	Generación Go (seed original)		Generation advanced (increased seed)	
		S. W.[1]	Gene pool	B. S.[2]	Phaseolin types (frecuency in parenthesis)
G50711	Antioquia	64.2 g.	Andean	Cultivated Weedy Wild	S (1), B (2), C (4), CAR (2) S (6), B (2), C (5), H_1 (1) S (5), C (3)
G50849	Antioquia	31.0 g.	Andean introgressed with M. P[3]	Cultivated Weedy Wild	S (37), C (41), H_1 (6), H_2 (3), T (4) S (15), C (6), H_1 (2), H_2 (1) S (6), C (3)
G50632	Antioquia	50.5 g.	Andean	Cultivated Weedy Wild	S (36) , CH (5), C (41), T (55), L (1) S (3), B (17), C (3), T (1) B (6), T (1)
G50646	Antioquia	64.8 g.	Andean	Cultivated Weedy Wild	S (14), B (2), CH (1), T (37), C (24), H_1 (1), H_2 (1) S (13), T (9), C (5) T (1), C (6)
G50785	Antioquia	60.6 g.	Andean	Cultivated Weedy Wild	S (16), B (3), CH (1), C (41), T (67), H_1 (8) S (19), B (12), CH (10), T (30), C (45), H_1 (2) S (4), B (4), CH (3), T (5), C (13)
G50879	Caldas	62.5 g.	Andean	Cultivated Weedy Wild	B (13), C (49), T (2), H_1 (22), H_2 (1) B (16), C (4), H_1 (4) B (2), C (1), H_1 (1)
G50983	Cundinamarca	21.0 g.	Andean introgressed with M. P[3]	Cultivated Weedy Wild	S (6), C (2), Mu (1) S (24), B (48), CH (13), C (9), H_2 (1), Mu (34) S (3), B (2), Mu (1)
G50988	Boyaca	35.4 g.	Andean introgressed with M. P[3]	Cultivated Weedy Wild	S (3), T (2), C (10), H_1 (5) S (10), C (4), H_1 (2) S (5), C (6), H_1 (1)
G50797	Tolima	61.0 g.	Andean	Cultivated Weedy Wild	S (1) S (6), C (3), H_1 (4) S (9), C (2), H_1 (4)
G50859	Cauca	33.0 g.	Andean introgressed with M. P[3]	Cultivated Weedy Wild	S(5),B (24),T(10),C(18),Ca_1(4),H_1(2),H_2(1),Car (7) B (36), C (6), H_1 (1) B (11)

S. W. : Is the seed weight derived from 100 seeds [2]B. S. : Biological Status; [3]M. P.: Mesoamerican Phenotype

Table 2. Allozyme constitution and seed size of the wild-weedy-crop complex G50849.

Biological material	Analyzed "selfed materials"	100 seed weight (g)	Isozyme loci	
			Prx	Dia-1
G50849 Cultivated	23	23.4-47.8	100 (5) 98 (14) 100/98 (4)	100 (17) 95 (6)
G50849 Weedy	4	10.0-24.0	100 (0) 98 (3) 100/98 (1)	100 (4) 95 (0)
G50849 Wild	3	5.3-7.2	100 (1) 98 (0) 100/98 (2)	100 (3) 95 (0)

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POLLINATION BIOLOGY OF COMMON BEANS

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Cross-Pollination of Male-Sterile Common Bean

A segregating population of a genetic male-sterile common bean in the background of 5-593 was obtained from Dr. Mark J. Bassett at the University of Florida. From this, a few homozygous recessive male sterile plants were selected and they were crossed to homozygous dominant male fertile plants, 5-593, to produce an F1 hybrid generation. This was selfed and the F2 generation was planted out on the Agricultural Experiment Station at the University of California, Riverside. A single row hand planter, with a bean plate, was used that dropped seed every 4 inches on average. There were two planting dates. The first, sown May 5th. 2003, flowered plants in mid June and July, when day temperatures ranged between 30 and 40 C. The 5-593 genotype is not well adapted to these conditions, and the plants grew poorly and even the male-fertile plants matured few pods. Very few pods were observed on the male-sterile plants. The second planting sown August 8th 2003, flowered in mid September when day-time maximum temperatures were less. Even so, the male-fertile plants tended to grow faster and smothered the male-sterile plants, which were smaller and weaker. The male-sterile plants are homozygous for the morphological marker, spindly branch, sb/sb, which is recognizable in the field and contrasts with Sb/Sb and Sb/sb phenotypes that have normal branches, and fertility. Two honeybee hives were placed in the field in mid September, when bean plants began to flower. Bees were seen to occasionally visit bean flowers, but they preferred to forage elsewhere. Cucumber beetles and flower thrips also lived among plants in the plot. Twelve, 30-feet rows were planted on each planting date, and the rows were 30 inches apart. Irrigation was by forrow, every other row, two days a week for eight hours.

The mean number of plants-per-row was 98. The mean number of male-sterile, spindly branch plants-per-row was 23, and the mean number of male-sterile plants that set at least one pod was 13 (56.5%) Therefore 43.5% of the male-sterile plants were not pollinated by any insect. For the male-sterile plants with at least one pod, the number of pods ranged from 1 to 12, with a total number of 415 and a mean of 2.78 pods per plant. Seeds per pod ranged from 1 to 7. A total of 1615 F1 hybrid seeds were obtained with a mean of 3.89 seeds per pod.

These field experiments demonstrated that an August planting was better than a May planting for pod and seed production with this Florida adapted, male-sterile material in southern California. Only about half of the male sterile plants produced a pod. Male-fertile plants were not rogued, but formed a source of fertile pollen for transfer to male-sterile plants by insects. Over 50 % of the male-sterile plants were pollinated by insects, possibly honeybees.

Alternating rows of homozygous male-fertile 5-593 plants with rows of segregating F2 plants, where the normal branched male-fertile plants are rogued to prevent the spindly-branched plants from being smothered by normal plants, might be a more efficient way to encourage pollen transfer to male-sterile plants. It should be possible to identify the spindly branch plants before flowering, and remove the normal branched plants. The male-sterile flowers form parthenocarpic pods, which confirm the male-sterile phenotype.

A search should be made for a more efficient pollen vector than the honeybee. In our previous field experiments we used bumblebees and carpenter bees. These were encouraged to visit common bean plants by locating the plots near *Mimulus* plots. *Salvia gregii*, or another plant attractive to bumble bees and carpenter bees might also encourage bee visits to common bean flowers.

A third way to increase the efficiency of hybrid seed production might be to transfer the spindly branch allele into a locally adapted cultivar that is more attractive to insect pollinators.

A Self-Pollinating White Kidney Line

Research by Tucker and Harding (1975) reported that common beans of various gene pools and classes were largely self-pollinated at UC Davis, CA, over two years. These results contrast with reports from northern California by Barrons (1939), and southern California (Wells et al. 1988; Ibarra-Perez et al. 1997) where outcrossing rates of 0.0 to 85.0% were recorded over one to two years. One explanation of these conflicting results may lie in the different methods used to sample segregating seed populations used by the various workers. Another may be the kind of pollinating insect present, honey bees are less attracted to common bean flowers than bumblebees or carpenter bees. Rarely in outcrossing experiments were the species of potential pollinators reported. All of the research on outcrossing rate in common bean implies that there should be cultivar or genotypic differences among accessions. Some cultivars may be almost 100% selfing in the presence of a specific pollinator, while others may show high rates of outcrossing. Moreover these differences should be inherited in inbred populations.

One culitvar that we have not been able to outcross over several years in the field in the presence of honeybees and other insects at Riverside, CA, is a ‘White Kidney’ line released by Smith in the 1940s. Morphological and genetic examination of the flower structure of this line may indicate why it is completely inbreeding under field conditions in southern California.

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I. EMS AND NEU MUTAGENIC EFICIENCY AND EFFECTIVENESS IN INDUCTION OF CHLOROPHYLL MUTATIONS IN PHASEOLUS VULGARIS L.

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INTRODUCTION

Chlorophyll mutations are used as markers in genetic, physiological and biochemical investigations, while chlorophyll formation in plants is the last result from long chine of biochemical processes where are included a lot of locuses. Chlorophyll mutations are used also as test systems for evaluation of genetic action of mutagenic factors (Gaul, 1964). They can be identified very easy in M_2 generation and can give quick information.

MATERIAL AND METHODS

Five years results with Bulgarian common bean varieties Dobroudjanski 7; Dobroudjanski 2; Plovdiv 11M and Plovdiv 10 were included as average in that study. Mutagenic factors were applied in the next concentrations: ***EMS*** $\Rightarrow$ ***2,5.10^{-2}; 1,25.10^{-2}; 6,2.10^{-3} M*** and ***NEU*** $\Rightarrow$ ***6,2.10^{-3}; 3,1.10^{-3}; 1,55.10^{-3} M.*** Buffers with pH 6,0 and 7,0 were used as controls.

Chlorophyll mutations were determined by classifications of Lamprecht (1960). As a criterion for mutagenic efficiency was used the coefficient of efficiency:

$$(\boldsymbol{C.E.} = \frac{\textit{frequency of surviving (\% M 1)}}{100} \; X \; \frac{\textit{frequency of mutations (\%)}}{100}),$$ (Krausse, 1968 by Mehandjiev et al., 1981), while mutagenic effectiveness was calculated on the basis of ratio ***Msd/L. Msd*** was the amount of mutations based on 100 M_2 plants and ***L*** was the lethality (Konzak et al., 1965 by Mehandjiev et al., 1981).

RESULTS AND DISCUSSION

It can be seen, from Table 1, that number of induced chlorophyll mutations and their frequency increased with increasing of mutagenic concentrations of EMS and NEU.

Mutagenic efficiency (C.E. – Table 1) was the highest in treatments with middle of lethality concentrations from the two applied mutagens ($3,1.10^{-3}$M NEU and $1,25.10^{-2}$M EMS – LD_{45-50}). In more of the cases, treatments with the lowest concentrations from the two applied mutagens ($1,55.10^{-3}$M NEU and $6,2.10^{-3}$M EMS – LD_{25-30}) showed higher mutagenic efficiency in induction of chlorophyll mutations, in comparison to application of the highest in lethality concentrations ($6,2.10^{-3}$M NEU and $2,5.10^{-2}$M EMS – LD_{85-90}).

Mutagenic effectiveness (Msd/L - Table 1) was in dependence of applied mutagens and their concentrations. There were also found varieties' peculiarities. In more of studied varieties, the highest mutagenic effectiveness was found when EMS and NEU were applied in middle of lethality concentrations ($3,1.10^{-3}$M NEU and $1,25.10^{-2}$M EMS – LD_{45-50}). Results for varieties Dobroudjanski 2 and Plovdiv 11M discover some exceptions, where treatment with the highest concentration of EMS – $2,5.10^{-2}$M (LD_{85-90}) showed a little higher effectiveness, in comparison to the treatment with middle of lethality concentration – $1,25.10^{-2}$M EMS (LD_{45-50}).

It can be concluded, from the conducted investigations, that mutagenic efficiency and effectiveness have to be studied in every case of mutagen treatment, application of different mutagenic concentrations and varieties. Mutagenic efficiency and effectiveness are not in dependence of mutations number or their spectrum. They are not also in dependence only from the mutagenic concentrations and applied mutagens. The two studied criteria (efficiency and effectiveness) characterized better the effects of applied mutagens in induction of chlorophyll mutations.

Table 1. EMS and NEU mutagenic effectiveness and efficiency in induction of chlorophyll mutations

Treatments	% lethality, in M_1 generation	Number of mutations	Mutation frequency, in %	***C.E.***	***Msd/L***	% lethality, in M_1 generation	Number of mutations	Mutation frequency, in %	***C.E.***	***Msd/L***
Variety Dobroudjanski 7						**Variety Plovdiv 11 M**				
Control pH 6,0	9,8	-	-	-	-	7,95	3	0,24 ± 0,14	0,0022	0,030
6,2·10^{-3} M HEK	81,73	50	4,76 ± 0,66	0,0087	0,058	81,13	91	7,42 ± 0,75	0,0140	0,091
3,1·10^{-3} M HEK	67,73	43	4,72 ± 0,70	0,0152	0,070	65,44	77	6,36 ± 0,70	0,0220	0,097
1,55·10^{-3} M HEK	51,34	25	1,99 ± 0,39	0,0097	0,039	49,51	54	4,27 ± 0,57	0,0216	0,086
Control pH 7,0	12,61	-	-	-	-	12,9	3	0,27 ± 0,15	0,0023	0,021
2,5·10^{-2} M EMC	78,8	46	3,54 ± 0,51	0,0075	0,045	75,9	74	6,13 ± 0,69	0,0148	0,081
1,25·10^{-2} M EMC	64,1	38	3,18 ± 0,51	0,0114	0,050	62,1	60	4,95 ± 0,62	0,0188	0,080
6,2·10^{-3} M EMC	50,7	34	1,52 ± 0,26	0,0075	0,030	49,2	38	3,10 ± 0,49	0,0157	0,063
Variety Dobroudjanski 2						**Variety Plovdiv 10**				
Control pH 6,0	8,7	2	0,19 ± 0,13	0,0017	0,022	8,56	1	0,09 ± 0,09	0,0008	0,010
6,2·10^{-3} M HEK	80,6	66	5,83 ± 0,70	0,0113	0,072	82,2	43	3,97 ± 0,59	0,0071	0,048
3,1·10^{-3} M HEK	65,8	63	4,97 ± 0,61	0,0170	0,075	65,4	48	4,12 ± 0,58	0,0143	0,063
1,55·10^{-3} M HEK	49,8	28	2,33 ± 0,43	0,0117	0,047	50,8	31	2,51 ± 0,44	0,0123	0,049
Control pH 7,0	13,3	3	0,23 ± 0,13	0,0020	0,017	13,3	-	-	-	-
2,5·10^{-2} M EMC	76,9	56	4,93 ± 0,64	0,0114	0,064	76,73	36	2,93 ± 0,48	0,0068	0,038
1,25·10^{-2} M EMC	62,1	49	3,84 ± 0,54	0,0146	0,062	62,9	29	2,52 ± 0,46	0,0093	0,040
6,2·10^{-3} M EMC	49,8	25	2,04 ± 0,40	0,0102	0,041	50,1	14	1,37 ± 0,36	0,0068	0,027

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II. EMS AND NEU MUTAGENIC EFICIENCY AND EFFECTIVENESS IN INDUCTION OF MORPHOLOGICAL MUTATIONS IN PHASEOLUS VULGARIS L.

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INTRODUCTION

In comparison to the chlorophyll mutations, morphological are more interesting for breeders because they can find, between them, forms representing specific breeding interest. Evaluation of mutability by frequency of phenotypic changes cannot be identical with induced breakings in cells. There are many biological barriers in fixation of mutations. Despite this fault, checking of visual mutations is widely used as enough objective method for evaluation of mutagenic efficiency and genotype mutability.

MATERIAL AND METHODS

Five years results with Bulgarian common bean varieties Dobroudjanski 7; Dobroudjanski 2; Plovdiv 11M and Plovdiv 10 were included as average in that study. Mutagenic factors were applied in the next concentrations: ***EMS*** $\Rightarrow$ $2{,}5\cdot10^{-2}$; $1{,}25\cdot10^{-2}$; $6{,}2\cdot10^{-3}$ ***M*** and ***NEU*** $\Rightarrow$ $6{,}2\cdot10^{-3}$; $3{,}1\cdot10^{-3}$; $1{,}55\cdot10^{-3}$ ***M.*** Buffers with pH 6,0 and 7,0 were used as controls.

Morphological mutations were determined by classification of Ivanov (1961). As a criterion for mutagenic efficiency was used the coefficient of efficiency:

($C.E. = \frac{frequency\ of\ surviving\ (\%\ M\ 1)}{100} X \frac{frequency\ of\ mutations\ (\%)}{100}$), (Krausse, 1968 by Mehandjiev et al., 1981), while mutagenic effectiveness was calculated on the basis of ratio ***Msd/L. Msd*** was the amount of mutations based on 100 M_2 plants and ***L*** was the lethality (Konzak et al., 1965 by Mehandjiev et al., 1981).

RESULTS AND DISCUSSION

Mutagenic efficiency (C.E. – Table 1) was different for all studied varieties. It was the highest in treatments with the lowest in lethality concentrations ($1{,}55\cdot10^{-3}$M NEU and $6{,}2\cdot10^{-3}$M EMS – $LD_{25\text{-}30}$) for variety Plovdiv 11M, while middle of lethality concentrations applied on variety Plovdiv 10 ($3{,}1\cdot10^{-3}$M NEU and $1{,}25\cdot10^{-2}$M EMS – $LD_{45\text{-}50}$) were more efficient. There were not found very clear dependences for varieties Dobroudjanski 7 and Dobroudjanski 2.

Mutagenic effectiveness (Table 1) was in dependence of studied varieties, applied mutagens and concentrations. There were found specific varieties' peculiarities. For example, the most effective for variety Dobroudjanski 7 were mutagenic treatments with middle of lethality concentrations, while for Plovdiv 10 the most effective were treatments with the highest of lethality concentrations of NEU and EMS. Despite higher values of **C.E.** and **Msd/L,** showed after application of middle ($3{,}1\cdot10^{-3}$M NEU and $1{,}25\cdot10^{-2}$M EMS – $LD_{45\text{-}50}$) or low ($1{,}55\cdot10^{-3}$M NEU and $6{,}2\cdot10^{-3}$M EMS – $LD_{25\text{-}30}$) by lethality concentrations of two applied mutagens, they are not the most efficient and effective for induction of wide mutation spectra and the highest mutation frequencies. As is known, mutation spectrum and frequency are very important indexes for discovery and selection of more interesting mutants with valuable breeding indications. It is interesting to point that not every time on higher mutagenic efficiency corresponded higher effectiveness. It is due to wideness of mutation spectra, different lethality in plants after application of mutagenic concentrations and different surviving of plants in M_1 generation. Therefore, when we have to choose the mutagenic treatment for creation of bigger mutation

diversity and selection of valuable common bean mutants, it is important to evaluate mutation frequency, spectra, coefficient of efficiency and mutagenic effectiveness (Mehandjiev et al., 1981; 1985).

Table 1. EMS and NEU mutagenic effectiveness and efficiency in induction of morphological mutations

Treatments	% lethality, in M_1 generation	Number of mutations	Mutation frequency, in %	***C.E.***	***Msd/L***	% lethality, in M_1 generation	Number of mutations	Mutation frequency, in %	***C.E.***	***Msd/L***
Variety Dobroudjanski 7						**Variety Plovdiv 11 M**				
Control pH 6,0	9,8	1	0,10 ± 0,10	0,0009	0,010	7,95	2	0,16 ± 0,11	0,0015	0,020
$6{,}2 \cdot 10^{-3}$ M HEK	81,73	106	10,09 ± 0,93	0,0184	0,123	81,13	181	14,76 ± 1,01	0,0278	0,182
$3{,}1 \cdot 10^{-3}$ M HEK	67,73	91	9,99 ± 0,99	0,0322	0,147	65,44	158	13,06 ± 0,97	0,0451	0,199
$1{,}55 \cdot 10^{-3}$ M HEK	51,34	91	7,23 ± 0,73	0,0352	0,141	49,51	149	11,78 ± 0,91	0,0595	0,238
Control pH 7,0	12,61	4	0,18 ± 0,09	0,0016	0,014	12,9	2	0,18 ± 0,13	0,0016	0,014
$2{,}5 \cdot 10^{-2}$ M EMC	78,8	95	7,32 ± 0,72	0,0155	0,093	75,9	150	12,43 ± 0,95	0,0300	0,164
$1{,}25 \cdot 10^{-2}$ M EMC	64,1	86	7,20 ± 0,75	0,0259	0,112	62,1	135	11,14 ± 0,90	0,0422	0,179
$6{,}2 \cdot 10^{-3}$ M EMC	50,7	77	3,45 ± 0,39	0,0170	0,068	49,2	102	8,33 ± 0,79	0,0423	0,169
Variety Dobroudjanski 2						**Variety Plovdiv 10**				
Control pH 6,0	8,7	2	0,19 ± 0,13	0,0017	0,022	8,56	1	0,09 ± 0,09	0,0008	0,010
$6{,}2 \cdot 10^{-3}$ M HEK	80,6	140	12,37 ± 0,98	0,0240	0,153	82,2	73	6,73 ± 0,76	0,0120	0,082
$3{,}1 \cdot 10^{-3}$ M HEK	65,8	144	11,36 ± 0,89	0,0388	0,173	65,4	49	4,21 ± 0,59	0,0146	0,064
$1{,}55 \cdot 10^{-3}$ M HEK	49,8	126	10,50 ± 0,88	0,0527	0,211	50,8	26	2,11 ± 0,41	0,0104	0,041
Control pH 7,0	13,3	2	0,15 ± 0,11	0,0013	0,011	13,3	1	0,09 ± 0,08	0,0008	0,007
$2{,}5 \cdot 10^{-2}$ M EMC	76,9	116	10,20 ± 0,90	0,0236	0,133	76,73	53	4,31 ± 0,58	0,0100	0,056
$1{,}25 \cdot 10^{-2}$ M EMC	62,1	122	9,50 ± 0,82	0,0360	0,153	62,9	37	3,21 ± 0,52	0,0119	0,051
$6{,}2 \cdot 10^{-3}$ M EMC	49,8	57	4,60 ± 0,60	0,0231	0,092	50,1	20	1,96 ± 0,43	0,0098	0,039

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New Observations on the Expression of the Gene *cl* for Circumlineated Patterns of Partly Colored Seed Coats - a Gene Carried by Recurrent Parent Line 5-593

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R. Prakken (1972) discovered the gene *cl* (for *circumlineatus*) in a cross between 'White J'(*T z cl Rk*) x 'Soldaat K' (*t z Cl* rk^d), which expressed circumlineated patterns of partly colored seed coats with either virgarcus or more restricted patterns. In the genetic background *P t z C J g B v*, he described the effects of *cl* as "the coloured part (or the separate units of it, even the finest colour dot) is sharply demarcated from the white part of the seedcoat by a narrow line (of a clear orange in *Rk*/- seeds and of a much darker colour in rk^d/rk^d ones), giving the impression of some sort of *precipitation* on the border between the coloured and uncoloured parts." There is no mention of a groove in the seed surface at the border between the colored and not colored (white) zones, as I have discovered in other materials carrying *cl*. For example, when I converted the genetic stock, *t z* virgarcus BC_3 5-593, to seed coat colors other than black, all progenies segregating for *t* and partly colored patterns were true breeding for *cl*. The three color stocks used were mineral brown (*v* BC_3 5-593), yellow brown (*b v* BC_3 5-593), and shamois (*g b v* BC_3 5-593). In the F_2 progenies from each backcross of the three color stocks to the black seeded virgarcus stock, the black virgarcus segregants never showed either the groove or the precipitation line, but all other non-black virgarcus segregants were true breeding for both the groove and the precipitation line. Other more expansive partly colored patterns were also true breeding for *cl* expression. From those results, I infer that 5-593 carries genotype *cl*. My next challenge was to convert 5-593 to the dominant allele, *Cl*, to create a new genetic stock.

I used 'Steuben Yellow Eye'(SYE) to study the inheritance of sellatus pattern of partly colored seed coats (Bassett, 1997). The sellatus zone in SYE is not circumlineated and has yellow brown color (with genotype *t Cl* z^{sel} *C J G b v Rk*). From the cross *t z* virgarcus (*G B V*, black) BC_3 5-593 x SYE, I derived two sister lines with sellatus pattern in yellow brown: F_4 *t cl* z^{sel} *b v* (circumlineated) and F_4 *t Cl* z^{sel} *b v* (not circumlineated). Both sister lines were crossed to the yellow brown tester stock *b v* (*T cl Z*) BC_3 5-593, and data were recorded on segregation for seed coat color and pattern. The results are presented in Table 1. The data are consistent with the hypothesis that the yellow brown tester stock is homozygous for *cl*. Subsequently, I converted the black sellatus pattern to three new stocks with either mineral brown, yellow brown, or shamois seeds. The segregation data from those backcross programs consistently showed that when a circumlineated sellatus parent was crossed with any of the three new color stocks, the F_2 progeny with sellatus pattern were always true breeding for *cl* expression (data not shown).

In my investigation of the inheritance of Anasazi type partly colored seed coats (Bassett et al., 2000), I used the Plant Introduction (PI) line 451802 (dark red kidney color) as the source of the bip^{ana} gene. I also made crosses with another Anasazi accession, PI 451801, which is black. In December of 2003, I examined the seeds of both PI lines again with a 15 X magnifier and observed a groove in the seed surface at the boundary of the colored and white zones, although the relief was greater in PI 451802 than in PI 451801. A reddish orange precipitation line was observed in the groove of PI 451802, but not in PI 451801. Stocks with Anasazi pattern in black were converted either to mineral brown, yellow brown, or shamois, and the F_2 progeny with Anasazi expression also were always true breeding for *cl* expression (data not shown).

During many years of breeding work, the patterns Anasazi, virgarcus, and sellatus were converted to four colors: black, mineral brown, yellow brown and shamois. Observations on the expression of *cl* in those stocks were recorded and summarized in Table 2. Clearly, there is a tendency for circumlineated pattern (both the groove and the precipitation line) to be suppressed with *V*, Anasazi being the exception. In PI 451801, the groove is expressed but not the precipitation line. In 5-593, neither the groove nor the precipitation line in expressed. With *B V*, only sellatus pattern shows evidence of restricted expression, viz., variable expressivity. For all other combinations of pattern and color, the *cl* expression is obvious and complete (for both components).

Table 1. Segregation for circumlineated (*cl/cl*) partly colored seed coat pattern (only *t/t* progeny are presented) from two crosses: Cross 1, F_4 *t Cl* z^{sel} *b v* sellatus x *b v* (*T cl Z*) self-colored BC_3 5-593 and Cross 2, F_4 *t cl* z^{sel} *b v* circumlineated sellatus x *b v* (*T cl Z*) self-colored BC_3 5-593.[z]

Cross no.	Expansa or ambigua		Sellatus		Ratio tested	χ^2	*P*
	Z/-		z^{sel}/z^{sel}				
	Cl-	*cl/cl*	*Cl*/-	*cl/cl*			
1	86		30	6	12:3:1	2.896	0.24
2	21			5	3:1	0.462	0.50

[z]Circumlineated seeds also have a groove in the seed surface at the boundary of the colored and white zones.

Table 2. Levels of expression of the circumlineatus gene *cl* in three partly colored seed coat patterns in four seed coat colors, i.e., the interaction of *cl* with genes for pattern and color.

Seed coat		Seed coat colors (with genotype)			
Patterns	Genotypes	Black *G B V*	Mineral brown *G B v*	Yellow brown *G b v*	Shamois *g b v*
Anasazi	*t Z* bip^{ana}	Obvious[z]	Obvious	Obvious	Obvious
Virgarcus	*t z Bip*	None	Obvious	Obvious	Obvious
Sellatus	*t* z^{sel} *Bip*	None	Variable[y]	Obvious	Obvious

[z]Variable for groove expression, depending on background genotype (see text).
[y]A frequency significantly below the expected 0.25 was observed.

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A Spontaneous Mutation (rk^{drv}) for Expanded Red Flower Veins t the rk^{d} Gene for Dark Red Seed Coat Color

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Recessive red seed coat colors in common bean are controlled at the red kidney locus *Rk* (Prakken, 1972; Smith, 1939 and 1961). Dry bean varieties with dark red kidney (garnet brown) seed coats carry the gene rk^{d} (Smith and Madsen, 1948) and usually carry other supporting recessive genes for seed coat color, viz., c^{u} and *v* or v^{lae}. Numerous genetic stocks have been developed at Gainesville by backcrossing recessive genes into a Florida dry bean 5-593 as the recurrent parent. A backcross program to transfer the rk^{d} gene from the dark red kidney variety 'Montcalm' into the recurrent parent *b v* BC_3 5-593 (yellow brown seed coat genetic stock), resulted in the development of a genetic stock with seed coat genotype c^{u} *g b v* rk^{d} BC_1 5-593. A single plant of c^{u} *g b v* rk^{d} BC_1 5-593 was observed to have a mutation for altered red flower veins, and this plant was crossed to the white banner stock *wb* BC_3 5-593 in Fall 1998. Four F_2 progenies from that cross were planted in the field in Spring 1999. A single F_2 plant selection for white flowers with red veins and dark red kidney seed coats was designated *C g b v* rk^{drv} BC_2 5-593 and was crossed with *b v* BC_3 5-593. Six F_2 progenies from that cross were planted in the field in Spring 2001. The proposed new gene symbol rk^{drv} has a superscript for "dark [red kidney] red vein [mutant]".

Under greenhouse conditions, the mutation expresses red veins in the wing petals of the flowers that are not the typical fine red veins that are a pleiotropic effect of the rk^{d} gene (Prakken, 1972). Instead, the vein color is "expanded" out in pink veins that have a much larger diameter. In plants grown in winter greenhouse conditions at Gainesville, Florida, the flowers appear to be a pale pink when viewed at a distance of one meter or more. At closer range one can see the distinct veins, but the red vein color is obviously more diffuse than in flowers with rk^{d} expression. Under May field conditions at Gainesville, Florida, the mutant expresses the fine red veins in wing petals that are typical of rk^{d}. In my experience, rk^{d} has poor to variable expressivity for red veins under field conditions at Gainesville, whereas the rk^{drv} gene has a much more reliable red vein expression under warm field conditions.

The absence of plants with cartridge buff seed coats among progeny with purple flowers (Table 1) is consistent with the hypothesis that *wb* may be an allele at (or very closely linked to) the *C* locus, but that hypothesis needs more direct testing to be substantiated. In any case, *wb* has no expression with *v*. Similarly, rk^{drv} has no expression with (is covered up by) *V*. There is no independent expression of the mutation for expanded red flower veins in plants with genotype *Rk*/- (Table 1). This is consistent with the hypothesis that the mutation has not occurred in a locus other than *Rk* and controls the trait by epistatic gene action.

The cross *C g b v* rk^{drv} BC_2 5-593 x *b v* BC_3 5-593 provides a more direct test of the hypothesis that the mutation for expanded red flower vein expression occurred at *Rk* (Table 2). All the F_2 progeny of that cross provide an opportunity for observable, independent segregation of dark red seed coat color and the red flower vein trait, but no independent segregation was observed (Table 2). The eleven plants having dark red kidney seed coats and white flowers without red flower veins were progeny tested in the greenhouse. All eleven F_2 plants had four F_3 progeny showing the red flower vein trait (Table 2). Thus, about 19% (11/57) of the F_2 plants

with rk^{drv}/rk^{drv} failed to express the expanded red flower vein trait due to variable expressivity of the gene.

I believe the mutant form of rk^{d} is a valuable mutant worth saving in the genetic stocks collection because rk^{drv} has more robust expression then rk^{d} under both greenhouse and field conditions at Gainesville. My previous experience with dark red kidney materials is that they tend to lose completely the ability to express red flower veins under field conditions. Early generations of breeding work with such materials showed some expression, but as further crosses and segregation occurred, the trait lost all ability to express under field conditions, and in some cases, even under warm greenhouse conditions (Bassett, unpublished data).

Table 1. Segregation for color in flowers and seed coats in the F_2 from the cross c^{u} *g b v* rk^{drv} BC_1 5-593 x *wb* BC_3 5-593 (*B V Rk*).[a]

V/- *Wb*/- -/-	*V*/- *wb*/*wb* -/-	*v*/*v* -/- *Rk*/-	*v*/*v* -/- rk^{drv}/rk^{drv}
Purple flowers and black (*B V*) or dark brown violet (*b V*) seed coats	White banner and black (*B V*) or dark brown violet (*b V*) seed coats	White flowers and various seed coats colors[b]	White flowers with red veins and dark red kidney seed coats
105	28	38	13

[a]The gene symbol rk^{drv} is used for the mutant with dark red kidney seeds and expanded expression of red flower veins.
[b]The seed coat colors included cartridge buff with c^{u}, mineral brown with *G B v* and yellow brown with *G b v*, and shamois with *g b v*.

Table 2. Segregation for color in the seed coats and flowers in the F_2 from the cross *C g b v* rk^{drv} BC_2 5-593 x *b v* BC_3 5-593.

Rk/- *G*/-	*Rk*/- *g*/*g*	rk^{drv}/rk^{drv} -/-	rk^{drv}/rk^{drv} -/-
White flowers and yellow brown seeds	White flowers and shamois seeds	White flowers with red veins and dark red kidney seeds	White flowers without red veins and dark red kidney seeds
171	52	46	11[a]

[a]F_3 progeny tests of the eleven plants demonstrated that they were true breeding for red flower veins, i.e., the failure to express under field conditions was due to variable expressivity of rk^{drv}.

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GENETIC CONTROL OF SEEDCOAT AND BANNER GREEN COLOR IN COMMON BEANS (*PHASEOLUS VULGARIS* L.)

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The green color of the different plant organs is due to the chloroplasts containing chlorophyll a (blue-green) and chlorophyll b (yellow-green) in various combinations in the different species and varieties. According to the plant organ, chlorophyll disintegration occurs and the green color transforms in white or some other at a certain stage of the plant orthogenesis.

In some varieties, earlier reduction of the seedcoat green color is observed and the seedcoat takes the color typical for maturity. The color can be white, dark yellow, brown, red, black, brick, etc. This character is controlled by a single recessive gene (*iw*). It is inherited independently from the *P* locus responsible for seedcoat color at maturity and from the *Y* locus controlling the yellow or green color of green pods (Baggett & Kean 1984; Dean 1970).

MATERIALS AND METHODS

The genetic control of seedcoat and banner green color was determined through the six progenies (P1, P2, F1, F2, BC1, and BC2) of the cross DG 91-10 (***BpcBpcIWIW***)/ Dobroudjanski Ran (***bpcbpciwiw***) during 2003.

The investigated characters were registered according to the scales given in Figure 1.

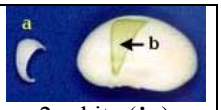
Green seedcoat color of green beans: a - *seedcoat without* cotyledons and b - *cotyledons without seedcoat*.	 1-green (***IW***)		 2-white (***iw***)
Banner green color.	 1-green (***Bpc***)		 2-white (***bpc***)

Fig. 1. Scales for green color of seedcoat and banner.

All F_1 plants and the backcross with the dominant parent had green color of the seedcoat and the banner.

In F_2 generation, both in seedcoat and banner the segregation was closest to the expected ratio 3 (green seedcoat, green banner) : 1 (white seedcoat, white banner) at $\chi^2 \leq 0.348$ and 0.314, for seedcoat and banner, respectively.

In the backcrosses with the recessive parent segregation ratio 1:1 was observed at $\chi^2 \leq$ 0.275 and 0.034, for seedcoat and banner, respectively.The hypothesis formulated in F_2 was thus

confirmed that green color is controlled by a single different dominant gene for both seedcoat and banner. We suggest the symbol ***Bpc*** for dominant allele controlling green color and ***bpc*** for recessive allele controlling the white color of the outer part of the banner.

Bagget & Kean (1984) have established that seedcoat white color in green beans (about two weeks before physiological maturity) is controlled by a single recessive gene *iw* (immature white).

The observed segregation (Table 1) of banner and seedcoat green color of 39 (***Bpc– IW–***) plants with green banner and green seedcoat : 3 (***Bpc– iwiw***) plants with green banner and white seedcoat : 13 (***bpcbpcIW–***) plants with white banner and green seedcoat : 12 (***bpcbpciwiw***) plants with white banner and white seedcoat rejected the zero hypothesis of an expected segregation ratio 9:3:3:1 at $\chi^2 = 37.37$, which was considerably higher than the critical value of 3.84.

Based on the frequency of the recombinant genotypes ***Bpc– iwiw*** and ***bpcbpc IW–*** corrected with the Kosambi function (1944), the distance between the two genes was calculated to 26 cM (Table 1) at LOD value 2.68, i.e. the statement that the two genes are linked is 474 times more correct than that they are not.

Table 1. Test for genetic control of banner (**Bpc**) and seedcoat (**IW**) green color in the cross BG 91-10 x Dobroudjanski Ran

	Banner—Seed Coat				*Banner*		*Seed Coat*	
	Bpc— IW—	***Bpc— iwiw***	***bpcbpc IW—***	***bpcbpc iwiw***	***Bpc —***	***bpcbpc***	***IW —***	***iwiw***
Observed segregation ratio	39	3	13	12	53	15	65	18
Expected segregation ratio	9	3	3	1	3	1	3	1
χ^2 test	37.37				*0.313*		*0.486*	
Recombination fraction	0.239							
Two-point distance (cM) bazed on Kosambi (1944) mapping function	26							
LOD score	2.68							

Banner and seedcoat green color was not affected by the environmental conditions and the used agronomy and we therefore think that they can serve as characters for Distinctness, Uniformity and Stability (DUC). We propose variety Abritus with white banner and seedcoat and breeding line DG 91-10 with green banner and seedcoat as suitable example varieties.

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ANTHOCYANINS IN TWO GENETIC RACES AND RECOMBINANT CULTIVARS OF BLACK BEAN (*Phaseolus vulgaris* L)

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Anthocyanins are a group of phenolic compounds, particularly flavonoids, commonly present in black, pink and red beans, in which these compounds contribute to the color of the shell. Anthocyanins have been recognized by their antiinflamatory activity, and they are associated with preventive effects against capilar fragility of blood vessels. Recently, the antioxidant properties of this kind of compounds have been considered as useful in the prevention of aterosclerosis and cancer of colon. On the other hand, its potential use as natural colorants in food, pharmaceutical and cosmetic industries, as an alternative to the use of synthetic colorants has been described[, 1, 2]. Anthocyanins content and profile were determined in 10 cultivars of black bean from two genetic races (five cultivars of the mesoamerican race and five cultivars of the Jalisco race) as well as in five recombinant cultivars. The aglicons of each anthocyanine were too evaluated.

Three groups of black bean cultivars, including five cultivars of the Mesoamerican Race (MR): Negro Jamapa, Negro Nayarit 80, Negro Medellin, Negro Veracruz and Negro Sinaloa; five cultivars of the Jalisco Race (JR): Negro Queretaro, Negro 151, Negro San Luis, Negro Puebla and Negro 152, and five cultivars considered as recombinats of the two prior races (RC); Negro Altiplano, Negro Puebla 152, Negro Perla, Negro OtomI and Negro Mecentral, were grown in 2002 under rainfall at the INIFAP´s Experimental Station at Texcoco, Mex. and used as study material. Total content of anthocyanins in shell and whole grain was determined by a conventional spectrophotometric method, and the anthocyanins profile and aglicons of the anthocyanins were analyzed by RP-HPLC[3].

Total content of anthocyanins in the whole grain of the studied black bean cultivars ranged from 37.7 to 71.6 mg/100 g and 10.1 to 18.1 mg/g in grain shell (Table 1), being bean shell the main reservory of this kind of compounds. Statistical differences (p=0.05) in the total content of anthocyanins in shell and whole grain were found among the different genetic races (Table 2). The dully shelled MR showed the highest total content of anthocyanins in shell (14.3 mg/g) and whole grain (14.3 mg/100 g), followed by the RC (13.0 mg/g and 49.6 mg/100 g) and JR (11.8 mg/g and 49.2 mg/100 g). Cultivars Negro Jamapa, Negro Perla, Negro Veracruz and Negro Nayarit 80, three of them of the MR and only one of the RC (Negro Perla), showed the highest total content of anthocyanins in shell of the studied cultivars, with values >14 mg/g. Three non-acylated anthocyanins were identified in the black bean cultivars: delphynidin-3-glucoside (D3G), petunidin-3-glucoside (P3G) and malvidin-3-glucoside (M3G) (Fig. 1). The proportions of each one of them varied inside and among races (Table 2). The respective aglicons for each anthocyanin were: delphynidin, petunidin and malvidin, respectivelly. Cultivars of the MR, JR and RC showed the highest relative percentages of D3G, P3G and M3G, respectivelly. Only cultivars Negro Veracruz and Negro Jamapa showed an additional non identified anthocyanin, with a retention time of 8.5 min under the analysis conditions.

The total content of anthocyanins in whole grain and shell of black bean cultivars, as well as the relative proportion of each anthocyanin was dependent of cultivar and race of origin, but anthocyanin profiles were the same among all the cultivars. MR cultivars were outstanding

because of their high total content of anthocyanins in shell and whole grain. The type of anthocyanins identified in black bean cultivars suggest the use of these compounds as antioxidants, more than as natural colorants.

Table 1. Total content of anthocyanins in whole grain and shell of 15 cultivars of black bean (averages by racial origen are presented).

CULTIVAR	ACN WG[1] (mg/100g)	ACN S[2] (mg/g)	CULTIVAR	ACN WG[1] (mg/100g)	ACN S[2] (mg/g)	CULTIVAR	ACN WG[1] (mg/100g)	ACN S[2] (mg/g)
Jalisco Race			**Mesoamerican Race**			**Recombinant Race**		
Negro Querétaro	64.14	12.58	Negro Jamapa	70.59	18.14	Negro Altiplano	71.86	17.28
Negro 151	51.72	12.48	Negro Nayarit 80	59.13	14.30	Negro Puebla 152	48.35	11.85
Negro San Luis	47.99	10.44	Negro Medellín	58.39	12.92	Negro Perla	47.06	15.48
Negro Puebla	44.23	13.10	Negro Veracruz	57.82	14.65	Negro Otomí	41.88	10.52
Negro152	37.66	10.21	Negro Sinaloa	50.36	11.56	Negro Mecentral	38.84	10.05
MEAN	**49.2 b**	**11.8 c**	**MEAN**	**59.3 a**	**14.3 a**	**MEAN**	**49.6 b**	**13.0 b**

Means with different letters in the same row and for the same trait are statistically different (Tukey, α=0.05).
[1]DMS=0.97 [2] DMS= 0.55
ACN= Total content of anthocyanins,WG= Whole grain, S= Shell

Table 2. Relative percentage of identified anthocyanins by race.

	D3G		P3G		M3G	
Race	**Retention time (min)**	**Relative %**	**Retention time (min)**	**Relative %**	**Retention time (min)**	**Relative %**
Jalisco	7.4	65.7	9.3	25.9	10.8	7.7
Mesoamerican	7.3	66.0	9.2	21.7	10.7	9.1
Recombinant	7.6	65.5	9.5	25.4	11.0	9.3

D3G= Delphynidin 3-glucoside, P3G= Petunidin 3-glucoside, M3G = Malvidin 3-glucoside

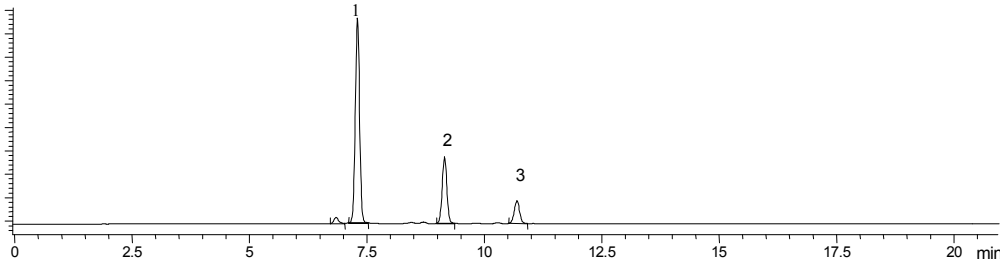

Figure 1. Thypical anthocyanin profile of black bean cultivars: 1) delphynidin-3-glucoside; 2) petunidin-3-glucoside, and 3) malvidin-3-glucoside.

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Evaluation of Microsatellite Diversity in Common Bean Parental Surveys

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Introduction

Microsatellite markers are highly polymorphic genetic markers that have proven useful for genetic mapping studies in plants. The utility of microsatellites derives from the fact that they detect length polymorphisms at genetic loci that have simple sequence repeats and as a result are highly variable. Microsatellites have been developed for common beans from both non-coding (genomic) and coding (gene-derived) sequences that contain simple sequence repeats (Yu et al., 1999, 2000; Gaitan-Solis et al., 2002 and Blair et al., 2003). The objective of this work was to characterize microsatellite diversity in three sets of common bean genotypes that are parents of mapping populations at CIAT. The parents included wild and cultivated germplasm from both the Mesoamerican and Andean gene-pools, while the microsatellites evaluated included gene-based and genomic derived markers as categorized by Blair et al. (2003).

Methodology

Plant Material: The 43 genotypes used in this study included 17 landraces (G685, G855, G2333, G3513, G4090, G4825, G5273, G11350, G11360, G14519, G19833, G19839, G21078, G21212, G21242, G21657, J117); 23 modern varieties and advanced lines from CIAT and other Latin American breeding programs (ICA Pijao, ICA Cerinza, Jalo EEP558, Jamapa, Tio Canela, BAT93, BAT477, BRB191, BAT881, DOR364, DOR390, DOR476, DOR714, MAM49, MAM38, MAR1, MD23-24, SEA5, SEA15, SEA 21, SEL1309, SEQ1027, VAX 6); and 3 wild accessions (G19892, G24404, G24390) of *Phaseolus vulgaris*. These genotypes were selected because they are the parents of over a dozen mapping populations being studied at CIAT for various traits (biotic or abiotic stress tolerance, nutritional quality and yield potential). The genotypes were grouped in 3 parental surveys that were carried out separately with common controls (DOR364, G19833). The proportion of Andean and Mesoamerican parents varied in each survey.

Genotyping: The genotypes were evaluated for allelic diversity with up to 150 microsatellite markers (of which 65 were gene-based and 85 were genomic) depending on the survey to which they belonged (150 for survey I, 148 for survey II and 97 for survey III) as shown in Table 1. The markers were amplified at different annealing temperatures according to the estimated melting temperatures of the primers. The reaction conditions were standardized as in Gaitan et al. (2002) and Blair et al. (2003) and markers that did not amplify (6 in survey I, 14 in survey II and none in survey III) were not considered further. The PCR products were resolved by electrophoresis for approximately one hour at 120 constant volts on silver-stained 4% polyacrylamide gels. Microsatellite alleles were sized by comparison to 10 and 25 bp molecular weight standards (Promega). The discriminating power (D) of each microsatellite was calculated by standard techniques (Tessier et al., 1999).

Results and Discussion

In all three parental surveys, the average number of alleles and discriminating power was higher for genomic microsatellites (ranging from 3.7 to 5.4 alleles and 0.467 to 0.578 discriminating power when including monomorphic markers, and 4.4 to 6.3 alleles and 0.613 to 0.692 discriminating power when excluding monomorphic markers) than for gene-based microsatellites (ranging from 2.8 to 3.3 alleles and 0.370 to 0.481 discriminating power when including monomorphic markers, and 3.2 to 4.1 alleles and 0.484 to 0.642 discriminating power when excluding monomorphic markers) (Table 1). The highest diversity was registered in parental survey I, which contained a good mix of Andean and Mesoamerican cultivated genotypes as well as wild accessions, compared to the other two surveys. Parental survey II had the lowest diversity values, mainly because it was predominantly made up of Mesoamerican genotypes only. Parental survey III contained a mix of Andean and Mesoamerican genotypes but no wild accessions.

When allele number was plotted against discrimination power (d), the higher diversity of genomic versus gene-based microsatellites was evident (Figure 1). Similarly, discrimination power of each microsatellite was positively correlated with the number of alleles produced at the locus (r=0.686 to 0.803). Null alleles were uncommon for both genomic and gene-based microsatellites but were scored as missing bands and therefore did not influence the estimation of discrimination power. Information from these parental surveys will be useful for genetic mapping of the various traits segregating in the genetic mapping populations under study.

Table 1. Average number of alleles and discriminating power (d) for genomic and genic microsatellites considering or not considering monomorphic markers as evaluated in each of three parental surveys.

Parental Panel	Marker Class	No. of Markers				Average No. Alleles		Average Disc. power (d)	
		Total	Poly	Mono	NA	w/o Mono	w/ Mono	w/o Mono	w/ Mono
Survey I	genomic	85	66	13	6	6.29	5.42	0.692	0.578
	gene-der.	65	48	16	0	4.06	3.30	0.642	0.481
	overall	150	114	29	6	5.35	4.47	0.671	0.535
Survey II	genomic	83	64	14	5	4.42	3.81	0.623	0.511
	gene-der.	65	46	10	9	3.20	2.80	0.564	0.464
	overall	148	110	24	14	3.91	3.38	0.598	0.491
Survey III	genomic	59	45	14	-	4.53	3.69	0.613	0.467
	gene-der.	38	29	9	-	3.83	3.16	0.484	0.370
	overall	97	74	23	-	4.26	3.48	0.563	0.429

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Isolation and Characterisation of the Seed Myo-Inolsitol-1-Phosphate Synthase (MIPS) Gene from Common Bean (*Phaseolus vulgaris* L.)

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The major form in which phosphorus occurs in plants is myo-inositol-1,2,3,4,5,6-hexa*kis*phosphate, commonly referred to as phytic acid or InsP6. This compound forms mixed salts with various mineral cations, e.g. potassium, magnesium, iron and zinc (phytates). It is generally assumed that the major role of InsP6 in plants is to act as a storage form for Pi and probably also for cations. Since InsP6 is the major form of phosphorus in seeds, total phosphorus and InsP6 level in the seed are usually positively correlated (Raboy, 2001, *TIPs*, 6: 458-462).

Due to its mineral-binding capacity, InsP6 affects the nutritional quality of food. Minerals, when bound to InsP6, are hardly or not absorbed in the intestine and are largely excreted, resulting in iron and zinc deficiencies, especially in developing countries, where food is mainly seed-based.

Recent studies have shown that dietary InsP6 might also have beneficial health effects, for example as an anticancer agent and anti-oxidant (Harland and Morris, 1995, *Nutr. Res.*, 15: 733-754). Clearly, the impact of dietary InsP6 must be considered on a case-by-case basis: infact while the negative effects of dietary InsP6 acid have their greatest impact on youth and growth in the developing world, the positive effects are of interest in the developed world where there is greater concern over pathologies of aging such as oxidative damage and cancer. Therefore, the modulation of the content of phytic acid in the seed, and particularly its reduction, is one of the major goals in seed crop genetic improvement (Raboy, 2001, *TIPs*, 6: 458-462).

Recently, it has been reported in soybean seeds that the mutation of the gene encoding for myo-inositol-6-phosphate synthase (MIPS) is correlated with a significant reduction in phytates and raffinosaccharide content (Hitz et al., 2002, *Plant Physiol.*,128: 650-660). In common bean the content of phytic acid is a seroius problem for human nutrition, particularly in countries of Central and South America, however no improved varieties are available for this character. In this context, we decided to test the possibility to produce, by chemical mutagenensis, mutants with an altered content of phytic acid.

The treatment of seeds with ethyl-methanesulphonate solutions can induce high frequencies of mutations in different monocots (D'Amato, 1965, *Radiat. Bot.*, 5: 303-316) and dicots, like common bean (Moh, 1969, *Mutat. Res.*, 7: 469-471; Moh, 1971, *Euphyt*., 20: 119-125; Motto et al., 1975, *Radiat. Bot*., 15: 291-299). In our experiment we used 7,000 F5 seeds of a breeding line under development at Istituto Sperimentale per l'Orticoltura. Following the technique described by Motto *et al.* (1975), seeds were dipped into a 48mM ethyl-methanesulphonate solution for 12 h at around 22 °C. The ratio seed/solution volume used was 2/1. After treatment, seeds were rinsed in demineralized water and sown in an open field. The first visible results were: a great reduction of seed germination (~50%), the presence of plants reduced in size, plants

with blind apex, plants with chlorophyll deficient sectors (chimeras). Only 2,028 plants were able to produce seeds, of which, 1,501 more than 100 seeds. The analysis for identification of mutants is in progress and is based on a measure of free Pi, taking into account that a reduced phytate content is positively correlated with an increase in free Pi (Pilu et al., 2003, *Theor. Appl. Genet.*, 107: 980-987).

At the mean time we started the isolation and characterisation of the seed specific form of MIPS in common bean.

MessengerRNA from early maturing cotyledons of Taylor's Horticultural (Asgrow) cultivar was used to isolate by RT-PCR a cDNA coding for the seed MIPS using as a reference gene for primer design the one isolated from soybean seeds (AF293970). The MIPS gene we isolated is about 74 % identical to a *P. vulgaris* MIPS gene expressed in vegetative tissues (PVU38920) and about 94% identical to the soybean seed gene we used as reference. A Southern blot analysis of MIPS gene organisation in *P. vulgaris* showed that no more than three gene are present. We analysed the expression of our MIPS gene in several tissues and developmental stages: developing cotyledons, flowers, 8 day seedling (roots, stems, cotyledons, leaves) and 18 day seedling (lower, medium and upper part of the stem, cotyledonary leaves, young leaves). The MIPS gene is highly expressed at very early stages of seed development (4 mm cotyledons) then its expression rapidly decreases (7-8 mm cotyledons). Probing of the same blot with a gene coding for the storage protein phytohemagglutinin showed that the expression of MIPS gene temporally precedes that of the storage protein. Positive hybridization occurred in flowers, while in 8 day developing seedlings MIPS expression was detected mainly in the stems and at low level in roots and leaves. In 18 day plantlets, MIPS gene is expressed in the upper part of the stem and to a lesser extent in the medium part of the stem and in young leaves.

To allow a more detailed characterization of the common bean seed MIPS, the production of specific antibodies raised against a recombinat form of the protein is in progress.

APA Locus Identification and Characterization from Common Bean (*Phaseolus vulgaris* L.) BAC Library

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Introduction

Lectin and lectin-related seed proteins (Arcelin/Phytohemagglutinin/α-Amylase Inhibitor) are encoded by a single locus, the APA locus, on linkage group 4 (Gepts 1999). In common bean (*Phaseolus vulgaris* L.) this locus shows a high variability. In fact the three major lectin-related components are present all together only in some wild accessions, while most of the wild and cultivated *P. vulgaris* contain only phytohemagglutinin (PHA) and α-amylase inhibitor (α-AI) and some wild Mesoamerican accessions contain only PHA and Arcelin (ARC). This variability indicates a very complex organization of the APA locus, which might be better understood by the isolation and comparison of the entire locus from genotypes with different sets of APA components.

In the recent years, Bacterial Artificial Chromosome (BAC) vectors have emerged as the system of choice for the cloning of whole genomes in large-insert libraries and have made valuable contributions to genome analysis and molecular genetics.
In order to characterize the complete APA locus we constructed a BAC library using the wild accession G12949, which contains the entire multigene lectin family (Arc/PHA/α-AI) (Lioi *et al*. 2003) and shows high resistance against seed eating insects.

Material and methods

The BAC library used for this study was custom made by Bio S&T, Montreal, Canada. High molecular weight DNA isolated from *P. vulgaris* (accession G12949 from CIAT) was cloned into *Hind*III-cut pIndigoBAC5 vector. The library consists of 30,720 clones maintained and grown on eighty 384-well microtiter plates. To identify BAC clones containing the multigene lectin family the entire library was gridded onto two 22.5 x 22.5 cm high-density filters double-spotted in a 4 x 4 array and probed with PHA, Arc4-II and α-AI clones isolated from the same genotype (Lioi *et al*. 2003).

Results and Discussion

Considering that the average insert size from 36 random clones is 135 Kbp and the genome size of common bean is about 637 Mbp (Arumuganathan and Earle, 1991), we estimate that our library covers at least 6 times the bean genome After hybridization on the two high-density filters with PHA, Arc4-II and α-AI probes, about 50 BAC clones were identified as positives. Among them, only 39 were confirmed as positives after a second screening using the two

specific PCR primers P1 and P2 (Mirkov *et al*. 1994). Our preliminary results confirm that the APA locus is very long, probably more than 250Kbp, in fact to cover the entire locus at least 4 overlapping clones will be needed. This result is in agreement with the finding of Kami *et al*. (2001), which analyzed the same locus in three different *P. vulgaris* genotypes. In addition, all our analyses suggest that PHA-E, ARC and PHA-L, besides being on the same locus, are more strictly associated to each other than α-AI. To obtain a more detailed figure on the APA organization the sequencing of a first BAC clone containing a large part of the APA locus is in progress.

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A Nitrate Reductase Gene Specific SSR Marker is Tightly Linked to a Major Gene Conferring Resistance to Common Bacterial Blight.

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Introduction

In developing SSR markers for mapping common beans, we found that one SSR marker from the nitrate reductase (NR) gene is tightly linked to a major gene conditioning resistance to CBB in the common bean line, HR67. This SSR marker explains about 70 % of the total phenotypic variation (Table 1).

Materials and methods

One RIL population of 112 F_5 lines, was developed by single seed descent from a cross between HR67 and OAC95-4 (Yu et al. 2000b). OAC 95-4 appears to be resistant only under field inoculation conditions (Tar'an et al. 2001), under greenhouse inoculation conditions OAC95-4 is slightly susceptible. Bacterial strains, their preparation, plant inoculation, culturing conditions, and disease severity rating were conducted in the same way as described by Yu et al. (2000b). SSR development was described by Yu et al. (2000a). Genomic DNA isolation, PCR amplification, and PCR product separation were conducted according to the procedures described by Yu et al. (2000b). Map integration and map distances among the SSR markers and the gene for disease resistance were analyzed by JoinMap 3.0.

Results and discussion

The genomic sequence of the NR gene is 4547 bp long. The coding region of the gene is 3980 bp, comprised of 3 introns and 4 exons (Fig. 1). The 5' SSR primer starts at 2671 bp of the genomic sequence and the 3' SSR primer ends at 2831 bp, which should amplify a 161bp DNA fragment (Fig. 1). Fig. 2 (H7) shows an integrated linkage group (LG) generated with JoinMap 3.0 from molecular marker data of our group and the University of Guelph (Tar'an et al. 2001). Table1 shows the association of the SSR marker to the major QTL.

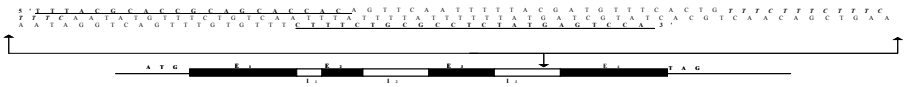

Figure 1. A diagram of the bean nitrate reductase (NR) gene illustrating the location and genomic sequence of the SSR marker

Table 1. One-way Analysis of Variances (ANOVA) and Kruskal-Wallis analysis (*K**) to determine molecular markers associated with CBB resistance of common beans

Locus	R^2	*F test* (P<0.0001)	*K** (P< 0.00001)
BC-420_{1000}	63.07	187.87***	47.7***
PV-tttc001	70.34	260.84***	55.7***

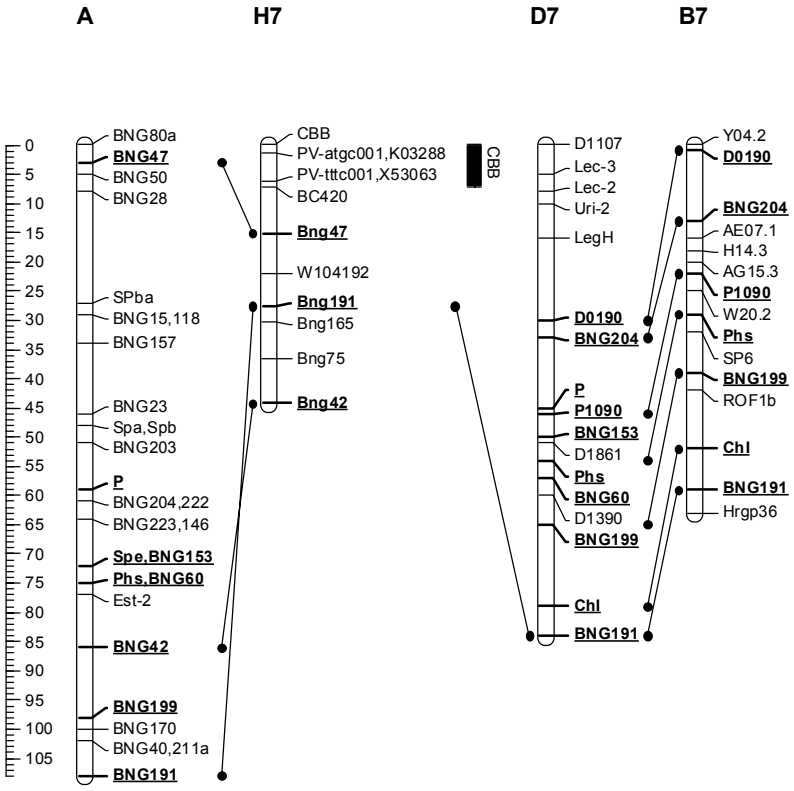

Figure 2. An integrated genetic linkage map (H7), drawn with MapChart, showing the map location of the common bacterial blight (CBB) QTL in HR67 and aligning it to the previous linkage groups developed at the University of Florida (linkage group A; Vallejos et al. 1992) and the University of California, Davis (linkage groups D7 and B7; Freyre et al. 1998).

Summary

We identified an SSR marker, PV-tttc001, that is located in the third intron region of the NR gene. This SSR marker is tightly linked to a major gene controlling resistance of common beans to CBB. This marker, which was positioned near the end of LG B7, explained about 70% of the phenotypic variation within the population (Table 1). Because of its co-dominant nature, the use of this marker for MAS would be more efficient than any of the markers developed so far for breeding common beans with high levels of resistance to CBB if XAN159 or HR67 were used as the resistance gene source.

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BEAN ROOT GROWTH UNDER DIFFERENT SOIL DRYING RATES
I. Growth of the Primary Root

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Introduction: The plant organ that first perceives the impact of soil water deficit is the root. Among other factors this deficit depends upon the drying rate of the soil. The growth of the primary root is orthotropic and its rapid elongation is important to the establishment and survival of the seedling. A soil water deficit inhibits the growth of roots, or if moderate, stimulates it (Creelman *et al*., 1990). The objective of the present work was to determine the primary root growth dynamics of bean seedlings subjected to different soil drying rates.

Materials and Methods: Two type-III bean (*Phaseolus vulgaris* L.) varieties were employed: Bayo Madero (BM) susceptible and Pinto Villa (PV) resistant to drought. The experimental design was a completely randomized factorial with eight replications. The treatments consisted of the combination of the two varieties and three soil drying rates: watered control (C), slow drying (SD) and fast drying (FD). The plants were grown in rhizotrons filled with 1.15 kg of dry sandy soil and placed in an environment-controlled chamber. The rhizotrons wood frames were made impermeable with paraffin and their front and back side walls were covered with glass panels. The front of the rhizotrons leaned at 60º angle with respect to the horizontal, so that the root system will grow resting against the glass panel. Beneath the front panel of the rhizotrons assigned to the FD treatment, a "mosquito screen" was affixed. The screen avoided the loss of soil when the glass panel was temporarily taken out. One seed was buried leaning against the front glass panel. The water loss was monitored by daily weighing of the system. This data and that of the soil dry weight were used to calculate the soil water content as follows: (g of water loss content/soil dry weight)x100. The control was maintained approximately at field capacity. Two days after the radicle emerged watering was withheld in SD and FD and the soil drying rate treatments began as follows: in SD water was lost only from the upper end of the rhizotron; in FD additional evaporation took place through the screen-covered wall when the back glass panel was taken off five hours daily during the light period. This allowed a fast and more uniform drying of the soil volume (2.5 cm thickness). In all treatments the daily elongation of various root categories was traced on the front glass panel using different colors for each day. Thus, a diagram of the root system was obtained as well as the information to calculate the root population growth rate. The tracing of the root ended when the plants under FD reached the permanent wilting condition. A separate determination was made of the growth rate of several individual secondary + adventitious roots which emerged the day after the treatment began. At the end of the experiment, the total length of each root population category and the dry weight of the shoot were determined.

Results and Discussion

Dynamics of soil drying. C showed small daily changes of soil water content, since the equivalent of the water lost by evaporation was replenished. After watering was withheld, the water loss was gradual in FD and SD, but faster in the former one (Figure 1).

Effect of soil drying rate on the primary root. When the front glass panel was taken off at the end of the experiment, it was observed that both the primary root and all the secondary + adventitious and tertiary roots were adhered on the glass panel (Figure 2).

Rate of elongation. No statistical differences on the daily rate of root elongation were found between varieties for any drying rate including C (data not shown). Within varieties the rate of elongation of FD presented a significant decrease with respect to C for both varieties (Figure 3). A higher rate of elongation of the primary root is important to reach quickly a deeper soil stratum

which usually has a higher water content. Such strategy favors the establishment and/or survival of the young plant.

Total root length. The length of the primary root was lower statistically with respect to C only in FD for BM, and in FD and SD for PV (Table 1).

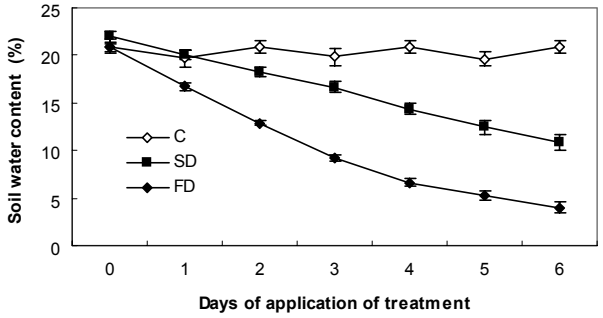

Figure 1. Soil water content in percentage of soil dry weight during the experiment, under three soil drying rates. C=watered control, SD=slow drying, FD=fast drying. The values are the average of eight replications. The vertical lines indicate ± standard error.

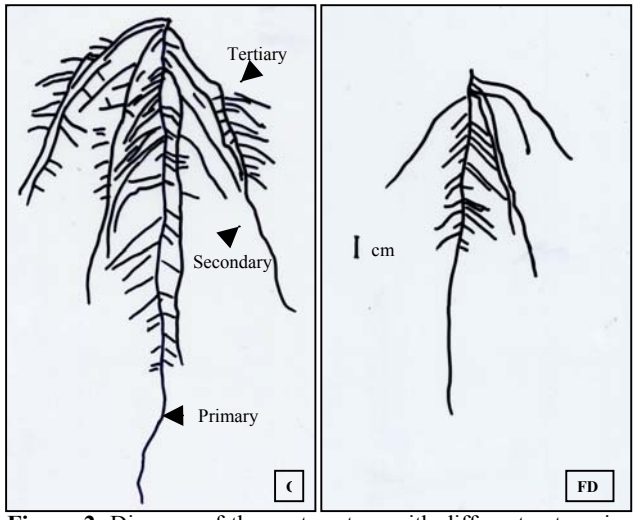

Figure 2. Diagram of the root system with different categories of root of the variety BM in the control (C) and fast drying (FD).

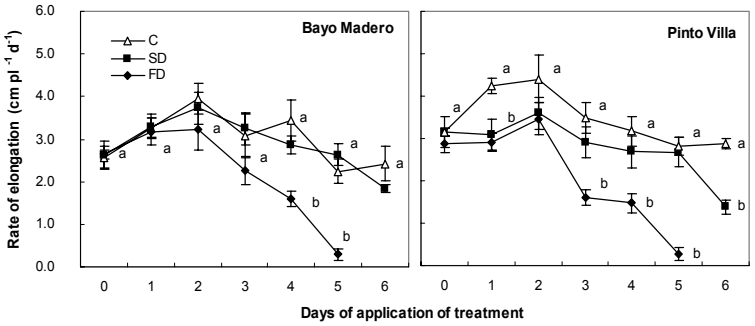

Figure 3. Rate of elongation of the primary root of two bean varieties, under three velocities of soil drying. pl=plant, d=day, C=watered control, SD=slow drying, FD=fast drying. The values are the average of eight replications. The vertical lines indicate ± standard error. For each day, different letters represent significant differences at P≤0.05 according to Tukey.

Table 1. Total length of the primary root at the end of the experiment in each treatment of the rate of soil drying for two bean varieties.

Variety	Rate of drying	Root length (cm) Primary
BM	C	24.07 a
	SD	23.50 a
	FD	17.75 c
PV	C	28.07 a
	SD	22.90 b
	FD	17.01 c

Bayo Madero=BM, Pnto Villa=PV, C=watered control, SD=slow drying, FD= fast drying. Different letters after the length indicate statistical difference at P≤ 0.05 according to Tukey.

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BEAN ROOT GROWTH UNDER DIFFERENT SOIL DRYING RATES
II. Growth of the Secondary+Adventitious and Tertiary Roots

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Introduction: The plant root system consists of roots of several categories: primary, secondary, adventitious and tertiary. The last three play an important role, because they are numerous and extensive. Rates of root emergence and elongation, number of roots and availability of photosyntates, supplied mainly by the cotyledons in the early growth of the seedling are important parameters. Smucker (1993), indicates that "Net root-system geometry results from the combined expression of dominant apical meristems that successfully compete for plant photosyntates". Number of roots and root length contribute to soil volume exploration and absorption activity. Rate of emergence might be important for the rate of root replacement. The establishment and/or survival of the young plant under different soil drying rates might be related to the root number and length. The objective of the present work was to determine several characteristics pertaining to the dynamics of growth of secondary+adventitious and tertiary roots of the bean seedling subjected to the different soil drying rates.

Materials and Methods. These are indicated in part I Ontiveros-Cortés, *et al*. 2004 in this BIC volume. Since it was not possible to discriminate visually, secondary from adventitious roots (emerging from the hypocotyl) both were considered under "secondary+adventitious."

Results and Discussion

Effect of Soil Drying upon Secondary+Adventitious Roots

Rate of emergence: No statistical differences were found on the rate of emergence of secondary+adventitious roots either between varieties or soil drying rates. Within varieties differences with respect to C were found only in PV, both under SD and FD (Figure 1).

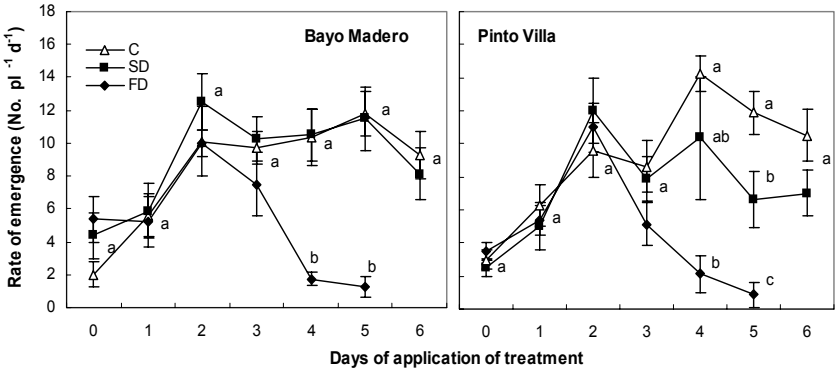

Figure 1. Number of secondary+adventitious roots emerged per day in two bean varieties, under three soil drying rates of the soil. C=watered control, SD=slow drying, FD=fast drying. The values are the average for eight replications. The vertical lines indicate ± standard error. For each day different letters represent significant differences at P≤0.05 according to Tukey.

Number of secondary+adventitious roots. The number of secondary+adventitious roots are in part related to their rate of emergence. Control of PV had more roots than control of BM. In both varieties FD presented the lowest number of secondary+adventitiuous roots. This number was statistically different from those of SD and C (Table 1). The number of roots of PV under SD was 18% less than that of its control. This might contribute to slow down the water depletion of the soil.

Rate of elongation of the secondary+adventitious root population. For this variable, no statistical differences were detected between varieties of any of the soil drying rates. Within varieties the pattern

was similar to that of the rate of root emergence. FD exhibited the lowest rate of elongation in both varieties. Under SD the PV variety diminished significantly its rate with respect to C (Figure 2).

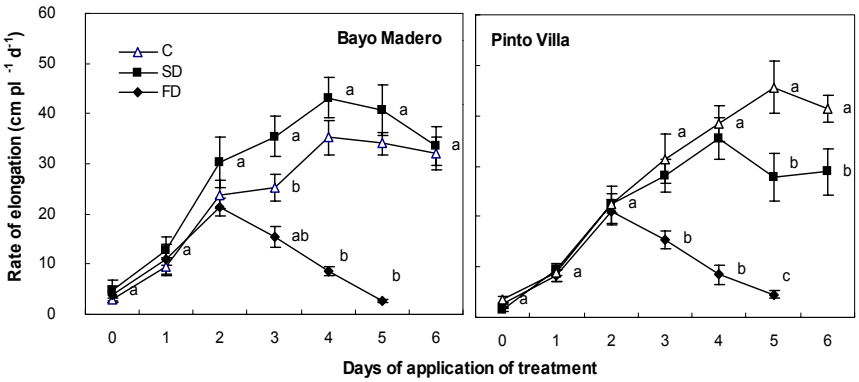

Figure 2. Rate of elongation of the secondary+adventitious root population of two bean varieties, under three rates of soil drying. C=watered control, SD=slow drying, FD=fast drying. The values are the average for eight replications. The vertical lines indicate ± standard error. For each day different letters represent significant differences at P≤0.05 according to Tukey.

Total length of the secondary+adventitious root population. This variable was lower with respect to C only in FD for BM, and in SD for PV (Table 1). This response shows that PV tends to inhibit the root elongation, even under a moderate soil drying rate. Passioura (1983) indicated that drought resistance might increase when the size of the root system diminishes.

Rate of elongation of an individual root. The rate of elongation of an *individual* secondary or adventitious root was similar to that of the primary root.

Effect of soil drying rate upon the tertiary root

Tertiary roots appeared at the fourth day of soil drying. They were observed only under SD and C, with no statistical differences between varieties for those variables which were evaluated also in the case of the secondary+adventitious roots. Tertiary roots were absent in FD (Table 1) which might be ascribed to the short duration of the experiment.

Table 1. Length and total number of roots at the end of the experiment for several root categories in each treatment of soil drying rates.

Variety	Rate of drying	Root length (cm)		Number of roots	
		Secondary+adventitious	Tertiary	Secondary+adventitious	Tertiary
BM	C	162.61 a	23.93 a	57.88 a	23.88 a
	SD	199.70 a	18.85 a	58.13 a	25.75 a
	FD	61.59 c	-	25.75 b	-
PV	C	190.64 a	14.26 a	60.88 a	19.75 a
	SD	141.54 b	15.95 a	50.25 a	16.63 a
	FD	54.81 c	-	26.13 b	-

Bayo Madero=BM, Pnto Villa=PV, C=watered control, SD=slow drying, FD=fast drying. The values are the average for eight replications. For each column different letters represent significant differences at P≤ 0.05 according to Tukey.

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GENOTYPIC VARIATION IN ADVENTITIOUS ROOTING UNDER LOW PHOSPHORUS AVAILABILITY IN COMMON BEAN (*Phaseolus vulgaris* L.)

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Introduction

Since soil phosphorus content and availability are highly heterogeneous in most soils, generally being highest in surface horizons and decreasing with depth, root architectural traits have been proposed as an important adaptive mechanism for effectively and efficiently acquiring phosphorus under low-P input systems (Lynch and Brown 2001). Architectural traits like basal root angle have been correlated with low phosphorus adaptation (Bonser, Lynch *et al.* 1996). However, because adventitious roots are often the shallowest portions of a bean root system with an initial horizontal growth habit, they might explore topsoil horizons more efficiently than other root types (Miller, Ochoa *et al.* 2003). This study attempted to characterize the phenotypic variation and genetic regulation of adventitious rooting when phosphorus is limiting.

Materials and Methods

A $F_{5:8}$ recombinant inbred (RIL) population of 84 lines derived from the F_2 generation of the cross between G2333 and G19839, two parents contrasting for adventitious rooting and other root traits at the seedling stage, was used in this study. G2333 (Colorado de Teopisca) is a small seeded climbing type IV Mexican landrace of the Mesoamerican gene pool and G19839 is a large seeded bush type III Peruvian landrace of the Andean gene pool.

Both parents and the 84 RILs were planted in the field in an Andisol in Darien, Colombia and in nutrient solution under greenhouse conditions at University Park, PA. In both experiments genotypes were evaluated under high and low phosphorus conditions. The field experiment was amended with 45 kg P_2O_5 per hectare as triple super phosphate (TSP) in high P plots and 7.5 kg P_2O_5 per hectare as TSP for low P plots. For high and low P treatments in hydroponics a 1.5 % (w:v) of solid-phase-buffered alumina-P (Lynch, Epstein *et al.* 1990) providing a constant availability of 100 μM or 1 μM P in solution were added. The experimental design was a RCBD with split plot arrangement of treatments with phosphorus level as a main plot and genotypes as subplots with 3 replicates and 2 sub samples in the field and 5 replicates in the greenhouse.

Six weeks after sowing the upper 30 cm of the root system was carefully extracted from the soil in the field. All adventitious roots were counted and sub samples of adventitious and basal roots were collected for image analysis. For the hydroponics experiment the entire root system was harvested 14 days after germination. Adventitious roots were counted and both adventitious and a sub sample of basal roots were preserved in 25% ethanol for image analysis. In both experiments shoot and root biomass by root type were recorded. Total root length was obtained by scanning samples and analyzing them with WinRhizo Pro software (Regent Instrument Inc., Quebec City, Quebec, Canada) and used for calculating specific root length (SRL).

Results and Discussion

Except for the number of adventitious roots in the field, genotypic differences were observed between parents and among RILs for the ability to produce adventitious roots as well as the responsiveness of adventitious rooting under phosphorus stress (Table 1). G19839 had as many

adventitious roots in the field as G2333, but not in the greenhouse, where G2333 produced twice as many adventitious roots as G19839. We interpret this as a phenological effect since the field data were collected at 6 weeks after planting whereas the seedling data were collected at two weeks after transplanting to the nutrient solution. Under controlled conditions all root parameters evaluated were significantly greater in G2333 than in G19839. Broad sense heritability values were moderate to high for all root traits evaluated (Table 1)

We observed significant phenotypic differences among RILs and also transgressive segregation for all adventitious root traits when comparing the ranges of the RIL population with the means of the parents, indicating useful genetic variation for the ability to produce adventitious roots as well as the responsiveness of adventitious rooting to phosphorus availability. Identifying contrasting genotypes for a particular root trait within a RIL population with a common genetic background will provide us with a valuable tool for investigating the mechanisms by which plants sense and respond to phosphorus stress.

A genetic linkage map using SSR, SCAR, and RAPD markers in this RIL population is in the final developmental stage at CIAT and will facilitate the identification of quantitative trait loci (QTL) or genetic factors conditioning adventitious and other root traits that might be important for low phosphorus adaptation in common bean.

Table 1. Phenotypic differences between parents and among RILs, and broad sense heritability (h^2) for some adventitious root traits in a RIL population of G2333 x G19839.

Adventitious root parameters	Phosphorus level	Parents			RILs		
		G19839	G2333	Prob.$_P$	Range	Prob.$_{RIL}$	h^2
Number / Field (n = 6)	High	34.4	32.5	ns	15.4 - 54.0	***	0.76
	Low	29.1	32.1	ns	18.3 - 50.5	***	0.68
Number / Greenhouse (n = 5)	High	7	14.1	***	2.6 - 20.8	***	0.57
	Low	8.4	14.9	***	0.4 - 21.4	***	0.66
Biomass (g) / Greenhouse	High	2.1	5.4	***	0.4 - 10.9	***	0.62
	Low	1.8	5.7	***	0.1 - 9.4	***	0.63
Root Length (m) / Greenhouse	High	39.8	131.7	***	10.6 - 274.5	***	0.70
	Low	30.4	139.3	***	1.3 - 289.7	***	0.66
SRL (m g^{-1}) / Greenhouse	High	105.2	275.7	***	42.1 - 287.0	***	0.52
	Low	123.4	243.0	***	152.4 - 277.0	ns	-

*** Significant differences between the two parents (Prob.$_P$) or among RILs (Prob.$_{RIL}$) at P=0.001.

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Segregation of Heat Tolerance in Five Snap Bean F_2 Generations

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Diallel analysis of 10 snap bean parents indicated 'Brio', 'Carson', 'Cornell 502' and 'CT 70' possess significant positive general combining ability (GCA) for yield components under high temperature stress (**Table 1**) (Rainey and Griffiths, 2004). Five hybrids were advanced based on GCA and parental performance ('Cornell 502' × 'Brio', 'Cornell 502' × 'Carson', 'Cornell 502' × 'CT 70', 'Cornell 502' × 'HB 1880', 'Cornell 502' × 'Labrador'). However, incidence of significant negative GCA among parents with high yield potential under temperature stress, and lack of correlation between GCA and parental performance indicates the need to evaluate advanced generations for improvement of yield under heat stress in common bean.

200 F_2 plants from each cross were screened at 32°C day/28°C night along with parents and a heat-sensitive control ('Majestic') in 90 m^2 greenhouses of the Department of Horticultural Sciences, NYSAES, Geneva, NY in 2003. Temperature treatment was imposed 2 weeks after seedling emergence and continued until most plants ceased flowering and pods were beginning to dry.

Plants were given a heat tolerance rating based on visual assessment of pod number, seeds per pod and harvest index, described in **figure 1**. Heat-sensitive plants retaining parthenocarpic pods (or pins) were counted separately from those without any pods or pins.

'Cornell 502' × 'HB 1880' had the highest number of individuals in the '3 or '4' categories, and had best overall pod quality under heat stress based on visual qualitative assessment. No significant distortion towards high temperature tolerance or sensitivity categories was observed in four of the five F_2 generations, indicating the involvement of additive gene action, and plant numbers in the '1' and '4' categories suggested multiple gene control of heat tolerance. Despite the observation that both parents are good combiners, 'Cornell 502' × 'CT 70' had 65% of individuals in the heat sensitive '1' and '2' categories. This observation supports results from the diallel analysis that indicate high yield potential and GCA of parents under heat stress may not be the best predictor of progeny yield under heat stress.

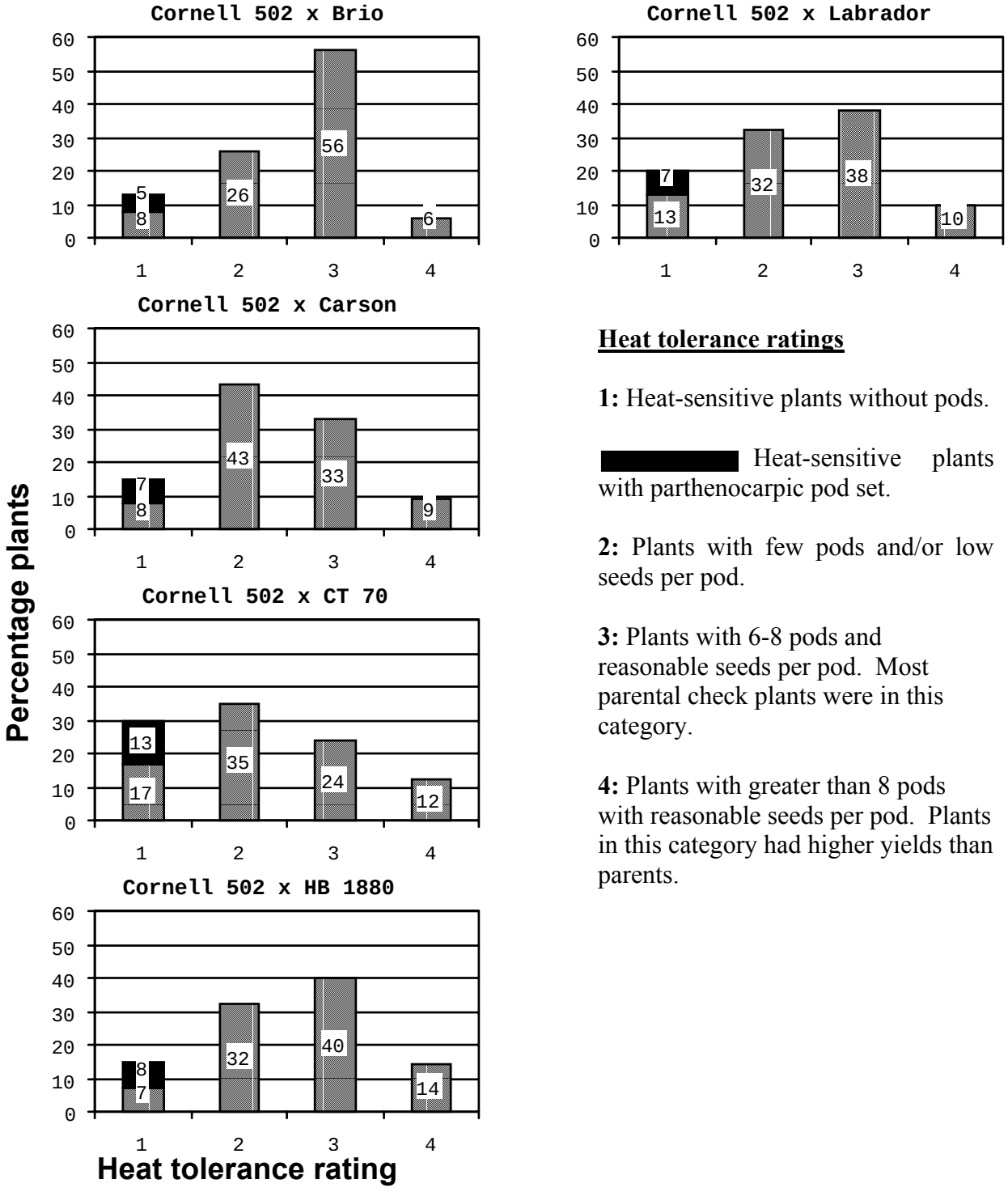
Table 1. GCA for select yield components calculated for 10 snap bean parents. Values obtained from screening F_1 progeny and parents under high temperatures (32°C day/28°C night).

Genotype	Source	Pod No.	Seed No.	Seed/Pod
Barrier	Alpha Seeds	-1.03*	-3.90*	*-2.09**
Brio	Seminis	0.57	2.38*	0.07
Carson	Syngenta	0.38	3.51*	0.33***
Cornell 502	Cornell	1.27*	5.90***	0.15*
CT 70	Cornell	0.01	4.00*	0.33***
HB 1880	Syngenta	-0.05	-2.01	0.04
Hystyle	Harris Moran	-0.76*	-3.24*	-0.29*
Labrador	Seminis	-0.26	-4.37*	-0.35***
Opus	Asgrow	-0.31	-2.07*	-0.27*

Venture	Syngenta	0.18	-0.20	0.20*

***Indicates significance at P ≤ 0.05. *** Indicates significance at P ≤ 0.0001.**

Figure 1. Percentage of plants in each category rating heat tolerance for five F_2 generations screened in high temperature (32°C day/28°C night) greenhouse environments. Numbers of missing, dead or dwarf plants are not included.



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Gas exchange of *Phaseolus* species under saline field conditions

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Introduction

High salt content in soils reduces plant growth (aboveground dry matter and root dry matter). Salinity also decreases leaf area, stomatal density, and gas exchange on leaves. Stomata plays a crucial role in the control of CO_2 uptake in plants grown under saline conditions because crop yield can be reduced due to low photosynthesis rate and stomatal conductance in bean plants (Pascale *et al.*, 1999). In a previous report, we found that some cultivars of *Phaseolus* species were tolerant to saline field conditions (Escalante *et al*., 2003). The objective of the present work was to determine stomatal conductance and transpiration rate in bean plants (*Phaseolus vulgaris* L. and *Phaseolus coccineus* L.) grown under different saline field conditions.

Material and Methods

The study was carried out in Montecillo, Mexico (19º19' N, 98º54' W, 2250 m of altitude) during rainy season (June-September, 2000) and with a temperate climate. Seeds of *Phaseolus vulgaris* L. cv. Bayomex and *Phaseolus coccineus* L. cv. Ayocote were sown with a plant density of 6.25 plants m^{-2}. The design was a random block with four replications. The soil with high salinity had a pH of 8-8.7 and an electro-conductivity of 7-14 dS m^{-1}. The soil with low salinity had a pH of 6.8-7.5 and an electro-conductivity of 2-5 dS m^{-1}. All the plots were fertilized with 100-100-00 NPK. Measurements of stomatal conductance, transpiration rate and leaf temperature were taken using a portable steady-state porometer Model LI-1600 (Licor Instruments, Nebraska) at pod filling stage.

Results and discussion

In *P. vulgaris*, the stomatal conductance and transpiration rate were lowest under high salinity conditions (Table 1). However, leaf temperature was highest because leaves closed the stomata. In fact, it is well known that the transpiration on leaves functions as a cooling system. Increasing salinity progressively decreased leaf water vapor conductance, and the rate of CO_2 assimilation decreased gradually, too. Two-thirds of this reduction in CO_2 assimilation rate at high salt level (80 mM NaCl) was attributable to stomatal conductance in *Phaseolus* species (Pascale *et al.*, 1999).

Table 1. Stomatal conductance (gs), transpiration rate and leaf temperature in plants of *P. vulgaris* L. and *P. coccineus* L. grown under low and high saline conditions. Montecillo, México.

Species	Salinity conditions	g_s (mmol m^{-2} s^{-1})	Transpiration (mmol H_2O m^{-2} s^{-1})	Leaf temperature (^oC)	Biomass (g m^{-2})	Seed yield (g m^{-2})
Phaseolus vulgaris	Low	420.5 a	10.9 a	21.8 a	251.0 a	145.8 a
	High	155.3 b	5.0 b	25.0 b	210.7 b	114.5 b
Phaseolus coccineus	Low	299.4 a	8.1 a	22.9 a	305.6 a	152.5 a
	High	246.9 b	7.4 a	24.1 a	116.4 b	50.0 b

Average radiation=1880 (μmol m^{-2} s^{-1})

Also, *P. coccineus* reduced stomatal conductance under high saline conditions; however, transpiration rate did not show significant differences with the plants grown under low and high salinity. In both species, salinity caused a reduction in biomass and seed yield due to a low gas exchange (low stomatal conductance), but *P. coccineus was* more tolerant than the *P vulgaris* plants under saline conditions during pod-filling stage.

Conclusions

In conclusion, *P. vulgaris* is more sensitive to a decrease in gas exchange, biomass, and seed yield under high saline field conditions than *P. coccineus*.

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Canopy reflectance of *Phaseolus* species under saline field conditions

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Introduction

In Mexico, soils of some agricultural regions contain high levels of salts (sodium salts). High salinity in soils reduces plant growth, leaf area (low canopy), and seed yield (Subbarao and Johansen, 1994). *Phaseolus vulgaris* L. is considered as salt sensitive species; however, *Phaseolus coccineus* (ayocote) L. has been classified a tolerant to salt conditions (Subbarao and Johansen, 1994). Remote sensing (spectral reflectance) can be used to assess biomass, leaf area, absorbed radiation, and total chlorophyll with different reflectance indices such as normalized vegetative indices (NDVI and GNDVI) and a ratio analysis of reflectance spectra index for chlorophyll a (RARSa) (Araus *et al.*, 2001). For that reason, the objective of the present work was to determine differences in NDVI, GNDVI and RARSa indices between *P. vulgaris* and *P. coccineus* under saline field conditions.

Material and Methods

The study was carried out in Montecillo, Mexico (19º19' N, 98º54' W, 2250 m of altitude) during the rainy season (June-September, 2000) and with a temperate climate. Seeds of *Phaseolus vulgaris* L. cv. Bayomex and *Phaseolus coccineus* L. cv. Ayocote were sown with a plant density of 6.25 plants m^{-2}. The design was a random block with four replications. The soil with high salinity has a pH 8-8.7, and an electro-conductivity of 7-14 dS m^{-1}. The soil with low salinity had a pH of 6.8-7.5 and an electro-conductivity of 2-5 dS m^{-1}. All the plots were fertilized with 100-100-00 NPK. Canopy reflectance was measured with a portable spectroradiometer (FieldSpec, USA) at pod filling stage.

Results and Discussion

Canopy reflectance of bean plants was reduced under saline conditions in *P. vulgaris* and *P. coccineus* (Figure 1). *P. coccineus* had an apparently lower canopy reflectance than did *P. vulgaris*. Indeed, *P. coccineus* had the lowest biomass and seed yield under high salinity (Table 1).

In both species, the two vegetative indices (NDVI and GNDVI) were higher in plants grown under low salinity than under high salinity. A high NDVI or GNVI value implies high biomass and seed yield as was reported in bean plants grown under different nitrogen and phosphorous levels (Gutierrez *et al.*, 2003a, b).

The RARSa index also was reduced under saline growth conditions in plants of *P. vulgaris* and *P. coccineus*. The RARSa index indicates an apparently low chlorophyll content on leaves due to high salt content in the soil.

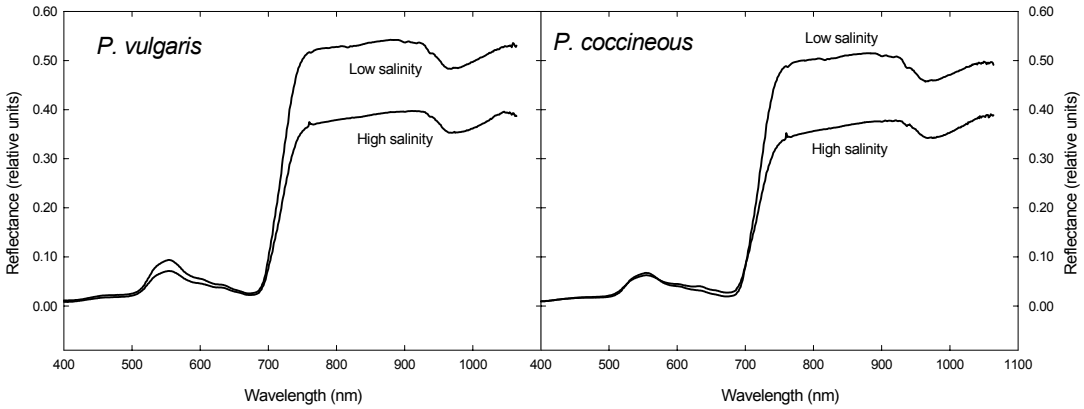

Figure 1. Canopy spectral reflectance in plants of *P. vulgaris* L. and *P. coccineus* L. under low and high salinity levels. Montecillo, Mexico.

Table 1. Spectral reflectance indices of *P. vulgaris* L. and *P. coccineus* L. plants grown under saline field conditions. Montecillo, Mexico.

Species	Salinity conditions	NDVI	GNDVI	RARSa	Biomass ($g\ m^{-2}$)	Seed yield ($g\ m^{-2}$)
Phaseolus vulgaris	Low	0.916 a	0.738 a	0.069 a	251.0 a	145.8 a
	High	0.874 b	0.666 b	0.045 b	210.7 b	114.5 b
Phaseolus coccineus	Low	0.931 a	0.774 a	0.095 a	305.6 a	152.5 a
	High	0.833 b	0.702 b	0.037 b	116.4 b	50.0 b

Conclusions

In conclusion, spectral reflectance indices, biomass and seed yield are reduced under saline field conditions. NDVI, GNDVI and RARSa indices estimated a plant growth and chlorophyll reduction under saline conditions.

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BIOMASS AND SEED YIELD OF COMMON BEAN IN SOLE CROP AND INTERCROP

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INTRODUCTION

In Mexico, about 30% of the common bean and maize are produced in intercropping systems, where the growth and seed yield of the common bean are generally reduced by competition in resources used by intercrop component species (Francis, 1986). The objective of this study was to determine whether the seed yield and its components of common bean are reduced when they are sown with sunflower.

MATERIALS AND METHODS

The study was conducted in Montecillo, Méx., (19º 29' N, 98º 53' W and 2250 m of altitude) in summer, 2002. There were six treatments: three intercrops of common bean *Phaseolus vulgaris* L. cvs. Canario, Bayomex (determinate type) and Michoacan (indeterminate type) and one of sunflower (cv. Victoria), and three sole crops of common bean. Pure stands and intercrops were sown at population density of 4.2 (bean) and 8.3 (sunflower) plants m^{-2} on 25 may, 2002 in a dry clay soil with pH 7.8 and content of organic matter and total nitrogen of 3.8% and 0.2% respectively. All experiments were fertilized with 100-100-00 NPK. The experimental design was randomized blocks with 4 replicates. At physiological maturity (final harvest) we evaluate total biomass, seed yield (8% humidity) its components and harvest index (seed yield/total biomass).

RESULTS AND DISCUSSION

Table 1 shows a summary of biomass, seed yield and its components. The bean cultivars showed a decrease in biomass and yield seed when they were sown with sunflower. However, the sowing combined of sunflower and common bean cv. Canario increased grain yield in bean, possibly due to the improvements that the cultivation of this oil crop in alkaline soils provides. Michoacan (sole or intercropped) gave the highest biomass (1336 and 1600 g m^{-2}), and seed yield (289 and 306 g m^{-2}). The harvest index showed a decrease in the intercropped Michoacan (19.3%) over the sole Michoacan (22%). Canario (sole or intercropped) gave the lowest biomass (277 and 424 g m^{-2}) and seed yield (42 and 30 g m^{-2}). The number of pods (r^2= 0.86**) and number of seeds (r^2=0.83**) showed a high relationship with seed yield. These results suggest that it is possible to increase the production of beans in alkaline soils by the combination of bean and sunflower.

Table 1. Biomass, harvest index, seed yield and its components of 3 cultivars of common bean (sole and intercropping with sunflower cv. Victoria), Montecillo, México.

TRAT	B	HI	YIELD	WS	S	P	S/P
	gm^{-2}	%	gm^{-2}	g	m^{-2}	m^{-2}	m^{-2}
Sole Mich	1336 a	22.0 a	289 a	21.8 e	1060 b	252 b	35 ab
Sole Bayo	573 bc	9.7 bc	60 bc	22.7 de	262 c	80 c	29 b
Sole Canario	277 c	15.2 abc	42 c	34.0 ab	84 c	50 c	15 c
Intercropped Mich	1600 a	19.2 a	306 a	28.7 bc	2708 a	494 a	45 a
Intercropped Bayo	783 b	16.5 ab	123 b	28.0 cd	326 c	95 c	28 b
Intercropped Canario	424 bc	7.2 c	30 c	35.7 a	61 c	27 c	17 c
Prob F	**	**	**	**	**	**	**
CV (%)	20	25	24	9	21	26	14
$DSH_{0.05}$	387.9	8.7	81.4	5.9	373.6	100.9	9.8

** = $P \leq 0.01$.

B = Biomass, HI = Harvest index, Yield = Seed yield, WS = Weight 100 seeds, S = Number seeds m^{-2}, P = Number pods m^{-2}, S/P = Number seed per pod m^{-2}.

CV = Coefficient of variation, HSD = Honestly significant difference test 0.05.

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EFFECT OF IRRIGATION ON 'BURKE' AND 'OTHELLO' PINTO BEAN GROWN IN CENTRAL WASHINGTON

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Introduction

Bean grown in Washington in rotation with small grain and potato under irrigation. Bean was produced for seed to export to domestic or international markets. Most bean acreage in Western Washington was not irrigated and yield was lower than bean grown in Eastern Washington where irrigation and heat unit are very important for high yield and high quality bean. In any drought years, irrigation is very limited and mostly reserved for perennial crops and other cash crops. Breeders continue to work on high yielding and high quality new bean lines with resistance to viral, bacterial and fungal diseases. There was very limited information on environmental factors like soil fertility, micro nutrients and water requirement to produce an optimum yield. In this experiment, 'Burke' and 'Othello' pinto bean were seeded in a solid rows 56-cm apart within rows and about 10 cm apart in the row.

Materials and Methods

This irrigation study was conducted on Shano silt loam soil at the WSU Research Unit in Othello. Bean was planted on 96 rows of 200 ft long for each cultivars. Research plots were pre-irrigated uniformly before land preparation. Soil nutrient levels were adjusted 100 lb/a (112 $kg.ha^{-1}$) of N, 50 lb/a (56 kg ha^{-1}) of P_2O_5. Eptam and Sonalan were applied pre-plant during land preparation with the rates of 4 pt material per acre (4.7 l ha^{-1}) and 2 pt per acre (2.3 l ha^{-1}) respectively. Seeds were treated with a mixture of 5% Apron XL and 10% Maxim 4FS and planted using a 4-row cone seeder on May 29, 2003. All treatments were furrow irrigated uniformly until July 15. There were 6 irrigation treatments with 7 replications.

Irrigation treatments were:

Trt 1: irrigated on July 15 only
Trt 2: irrigated on July 15 and 22.
Trt 3: irrigated on July 15, 22 and 29
Trt 4: irrigated on July 15, 22, 29 and August 4
Trt 5: irrigated on July 15, 22, 29, August 4 and 11
Trt 6: irrigated on July 15, 22, 29, August 4, 11 and 18 or control.

Each irrigation treatment is 24 hours set and PAM (polyacrylamide) was used to reduce the sediments in runoff water. Each treatment consisted of 16 rows and only 4 middle rows were harvested. Plots of 4 rows by 20 ft long were cut, windrowed and threshed using Hege small plot combine. Seed moisture at harvest was less than 8%. Bean was cleaned and weighed for yield. Hundred seed weight was also recorded for comparison.

Discussion

Under normal irrigation for bean production in Washington, Othello matures at about 80 to 85 days after planting and 3 to 4 days earlier than Burke. In 2003 growing season, weather condition was excellent for bean production. Mean temperature in Othello, Washington was 2^{0} F higher in 2003 than in 2002 (73, 70, and 64 F in July, August and Sept 2003) and having only 2 days with 100 and 102.8 F in July. The drought stress during the treatment period was not very

profound as last year. Soil moisture in the silt loam soil may be still high when the treatment started Therefore, no severe symptom of drought stress had been observed. The delayed maturity of the plots receiving maximum irrigation were observed. The canopy of the drought stress treatment 1 (only one irrigation since full canopy development) plots were not severely reduced as during last year. There were significant difference in seed yields and 100-seed weight of both cultivars at PF>F=0.0001 and 0.0004. Average yield of Burke was 200 lbs/a higher than Othello. Burke responded to irrigation much better than Othello in this study. Othello gradually increased yield with irrigation and produced optimum yield at 4 irrigations. Burke responded positively to irrigation and also maximized at 4 irrigations (Table 1). Seed of Othello is normally smaller (36 to 39 g per 100 seeds) than Burke (41 to 45 g per 100 seeds). Irrigation water increased seed size of both cultivars significantly as it did to seed yields.

Conclusion

Results of this study show that both cultivars responded to irrigation very well by increasing yield and seed size. Both cultivars Burke and Othello can produce high yield and good quality bean if grown in the soil with adequate soil moisture. However, the optimum production of both cultivars is enough soil moisture up to 21 days before harvest or after full pod development. Additional irrigation water after that stage may delay harvest but it did not increase yield or seed size significantly.

Table 1. Effects of Irrigation Treatment on Yield (lb/a) and 100-seed Weight (g/100 seeds) of Burke and Othello Grown in 2003.

	Burke 2003		Othello 2002		Othello 2003	
Trt	Yield	100-wt	Yield	100-wt	Yield	100-wt
1	3043 c	41.3 d	2875 c	34.2 bc	3044 c	39.5 c
2	3266 bc	43.4 c	3437 bc	32.2 d	3415 ab	40.8 b
3	3409 b	41.7 d	3950 b	32.8 cd	3359 bc	40.9 b
4	4025 a	45.1 ab	3714 bc	34.8 b	3518 ab	40.2 bc
5	4141 a	44.4 bc	3663 bc	35.3 b	3704 ab	42.7 a
6 (control)	4139 a	46.1 a	5716a	38.0a	3748 a	43.2 a
Mean	**3670**	**43.7**	**3893**	**34.5**	**3465**	**41.2**
CV (%)	**7.35**	**2.08**	**15.6**	**2.9**	**9.46**	**1.98**
Pr>F	**0.0001**	**0.0001**	**0.0004**	**0.0001**	**0.0045**	**0.0001**
LSD (.05)	**294.5**	**1.37**	**916.0**	**1.52**	**357.7**	**1.23**

Each value is the average of 4 replications in 2002 and 7 replications in 2003.
Means in a column with the same letter are not significantly different

TOTAL PHENOLIC CONTENT IN CANNED BEANS

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Introduction

Phenolic compounds in beans have been studied primarily because they reduce mineral and protein absorption and because of their potential role in the "hard-to-cook" phenomenon. However, current research suggests that plant phenolics may reduce the incidence of disease (1). Bean extracts have been shown to have antimutagenic (2,3) and antioxidant activities (4,5). Two animal studies show that feeding beans inhibits cancer (6,7). Epidemiological studies show an inverse relationship between the consumption of legumes and the incidence of chronic diseases such as cardiovascular disease, cancer, diabetes, and other degenerative disorders (reviewed in 8). Therefore, in societies that suffer from chronic diseases and seldom have mineral or protein deficiencies, bean phenolics likely promote good health. However, identification of protective components in beans and their mode of action are just beginning to be investigated. Takeoka et al (9) identified three anthocyanin glucosides in black beans and Beninger and Hosfield (5,10) identified anthocyanins, flavonol glycosides, and proanthocyanidins (condensed tannin) in seed coats of several bean varieties. Work to date has focused on raw beans or isolated seed coats rather than cooked or canned beans. Herein we report the total phenolic content of beans as they would be consumed. Since some consumers discard the brine from canned beans, we determined total phenolics in the brine, beans, and beans plus brine.

Materials and Methods

Commercially canned beans were kindly provided by Bush Brothers and Company. Three fractions were prepared for each type of bean: a) the entire contents of a can, b) the brine, and c) the beans. The water content for each fraction was estimated from published data and sufficient methanol and concentrated HCl was added to yield a mixture of 70% methanol: 29% water: 1% HCl. The beans and/or brine were homogenized in the methanol:HCl mixture and then sonicated for 1 hr. The sonicated homogenate was placed in a refrigerator overnight and then centrifuged. The supernatants were condensed under vacuum and low heat (45 °C). The condensed extracts were assayed for total phenolic content by the Folin-Ciocalteu assay and the results are expressed as (+)-catechin equivalents.

Results and Discussion

Table 1 shows that a wide range in total phenolics exists in canned beans (56 to 223 mg of (+)-catechin equivalents). The quantity of phenolics that leached from the bean into the brine ranged from 17 to 47 mg of (+)-catechin equivalents. The quantity of phenolics migrating from the beans into the brine was fairly consistent with the exception that only a small quantity of phenolics was in the brine from pinto beans. More than half of the phenolics were in the brine of baby butter, large butter, and garbanzo beans. If bean phenolics are shown to promote health, then a significant portion of the potential health benefits are lost when the brine from these varieties is discarded.

Conclusions

There is a wide range in phenolic content of canned beans and more than 50% of the phenolics originally in beans are leached into the brine of bean varieties that posses smaller quantities of phenolics.

Table 1. Total phenolics in canned beans (mg of (+)-catechin equivalents per can).

	Brine	Beans	Beans + Brine
Baby Butter	37	25	56
Large Butter	34	30	64
Navy	28	43	66
Purple Hull	34	43	77
Garbanzos	45	37	76
Black eyed peas	33	60	86
Pinto	17	83	100
Crowder	34	68	98
Red	23	92	124
Light Red Kidney	43	79	149
Dark Red Kidney	38	88	159
Black	47	175	223
Average ± SD	34 ± 9	64 ± 41	107 ± 49

Acknowledgements

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GENOTYPIC AND SEASONAL EFFECTS ON SEED PARAMETERS AND COOKING TIME IN DRY, EDIBLE BEAN

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INTRODUCTION

Dry bean seeds are good source of protein, starch and nutrients such as fibre, vitamins and minerals and are well suited to meet demands of health-conscious consumers. Soaking and cooking are traditional forms of processing. Cooking time is one of the major criteria used in evaluating cooking quality. The cooking quality of dry beans is affected by varietal differences, composition of seeds and physical properties such as size or weight, seed volume, seed coat (Boros 2003; Boros & Wawer 2003; Castellanos *et al*., 1995; Shellie & Hosfield, 1991; Elia *et al*. 1997). Weather conditions prevailing during crop cultivation, as a major element of environment, significantly influence seed physical and functional characteristics and cooking time (Illiadis 2001, Coskuner & Karababa 2003). The purpose of this work was to study the effect of climatic conditions during growing season on quality seeds properties and cooking time in a group of dry bean cultivars adapted to local conditions.

MATERIALS AND METHODS

Seeds of twenty-one dry bean cultivars of Polish origin from field experiment grown in 1999, 2000 and 2001 in randomized, complete block design at IHAR Radzików were used in this study. The Food and Feed 1250 apparatus was used for seed protein content determination. The percentage of testa in seed is an average of 10 seeds in 3 replications. Water absorption of the entries and conductivity of soaking water were determined on samples of known mass of 50 seeds soaked in distilled water for 18 hours at 25 ^{o}C temperature. Bean cooking time was estimated with a 25-seed Mattson pin-drop cooker (Jackson & Varriano-Marston 1981). Cooking time was calculated as a time from initial cooking until the time when 20 of pins penetrate seeds in cooker (CT 80%). Data were subjected to analysis of variance (ANOVA).

RESULTS

Large differences in air temperature and in the amount as well as the distribution of rainfall during 3 growing seasons (May - September) were noticed, mainly at seed filling and maturation of dry bean. Results of the variance analysis have shown significance of genotype, year and genotype x year components of variance (table1).

Table1. Mean squares from ANOVA analysis of variance for dry bean seed parameters and cooking time of 21 dry bean genotypes grown for 3 seasons.

Sources	Mean squares		
traits	Genotype(G)	Harvest years (HY)	GxHY
df	20	2	40
100 seed mass (g)	1781.55**	2808.81**	89.36**
Testa content (%)	4.42**	6.43**	0.22**
Protein content (%)	5.69**	339.46**	4.54**
Water absorption (%)	190.79**	60.94**	53.85**
Coductivity (µS $cm^{-1}g^{-1}$)	430.47**	23.78**	85.93**
Cooking time (min)	78.22**	85.24**	11.79**

The year effect was significant for all seed parameters measured (table 2.). The ranges averaged over 3 seasons indicated large differences among genotypes, particularly for 100-seed weight, testa content and cooking time. The means and the ranges of tested traits among the cultivars used in this study were

similar or slightly higher than reported earlier (Boros & Wawer 2003, Shellie& Hosfield 1991). Significance of genotype x year interaction indicates that genotypes responded differently in each season. The most likely temperature and rainfall distribution had the major effect on genotype x year interaction.

Table 2. Seasonal means, grand means and ranges for seed quality parameters of 21 dry bean genotypes

Traits	1999	2000	2001	Grand Mean	Range
100 seed mass (g)	34.52 b	46.89 a	36.34 c	39.5	17.1- 66.8
Testa content (%)	8.16 a	7.63 b	8.21 a	8.0	7.1 – 9.0
Protein content (%)	23.19 b	22.49 a	21.61 c	22.4	21.2 – 24.0
Water absorption (%)	103.6 a	102.1 b	101.7 b	102.4	96.8 – 111.0
Conductivity ($\mu S\ cm^{-1}g^{-1}$)	24.2 b	24.2 b	25.3 a	24.6	16.2 – 30.4
Cooking time (min)	19.97 a	17.89 b	19.84 a	19.2	15.24 – 26.2

To examine nature of experimental variability, variance component analysis for all traits was performed. Examining variance components estimates indicated that genotypic component was substantially larger than season and genotype x season components, indicating that genotypic effect predominated over environmental effects for tested parameters. Obtained results differed to some extent with that reported by Hosfield et al. (1984) and Balasubramanian et al. (1999) who found that both, year and genotype x year effects were larger than genotype effect for most quality traits.

Table 3. Spearman's rank correlation between seasonal pairs for 21 dry bean cultivars

Traits	Seasonal comparison		
	1 vs.2	1 vs. 3	2 vs.3
100 seed weight (g)	0.8125**	0.8818**	0.9093**
Testa content (%)	0.7343**	0.8347**	0.7737**
Protein content (%)	0.1633	0.0557	0.4367**
Absorption (%)	0.3741**	0.6522**	0.4765**
Conductivity ($\mu S\ cm^{-1}g^{-1}$)	0.2627*	0.6861**	0.3118**
Cooking time (min)	0.5310**	0.5660**	0.4918**

Spearman's coefficient of rank correlation between pairs of seasons showed significant values of tested traits indicating that cultivars ranked similarly from season-to-season, mainly for hundred seed weight and testa content, to lower extent for absorption and cooking time and differently ranked for protein (table3.). The data, likewise other (Shellie & Hosfield 1991, Hosfield et al.1984, Balasubramanian et al.1999) suggests that several year of testing are needed for proper assessment of cultivar performance for processing seed quality.

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Kinetics of Cooking for Sugar Beans

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INTRODUCTION

Farm sales of U.S. dry beans were $1.8 billion during 1998-2000 (USDA 2001). On average, 14% of the U.S. population consumes dry beans daily. Because of the large supply and relatively low profit margin of canned and dry-bagged beans, growers and processors would be interested in value-added bean products. Sugar beans (cooked beans with a sugar coating) are sold in small packages and consumed in Japan and Asian countries as a snack, similar to honey-coated peanuts in the U.S. A potential U.S. market for such bean products exists, due to increased consumer interest in eating foods for health ("nutraceuticals" or "functional foods"), and awareness of the health benefits of beans and a plant-based diet in general.

Because of the lower labor costs, foreign processors in some countries can afford to use labor-intensive changing-temperature batch processes. U.S. processors cannot compete unless a continuous constant-temperature process is used. The objective of this study was to determine the textural kinetic parameters for bush cranberry beans. These parameters can be used to scale up a commercial process and increase market share for US dry bean growers and processors.

MATERIALS AND METHODS

Beans. Bush cranberry beans were obtained dry from AgriAnalysts LLC (Frankenmuth, MI). They were soaked in 23ºC distilled water, four times their mass, with 0.08 % of the water mass of sodium bicarbonate and 0.06% of the water mass of sodium phosphate for 12 hours.

Isothermal experiments. Beans were separated into 50-g samples and placed in mesh bags after soaking. Beans were cooked for up to two hours in distilled water at temperatures of approximately 90°C, 95°C, or 99°C. Samples were taken at 5, 15, 30, 60, 105, and 120 minutes. At each sampling time, samples were immersed in ice water to rapidly cool the beans to 23ºC.

Dynamic heating experiments. These tests represented typical batch commercial processes used overseas. For each bean class, beans were separated into 60-g samples and placed in mesh bags after soaking. Cooking was done in steam-jacketed kettles. The beans were cooked for up to 40 minutes in distilled water beginning at 25-30 °C, and increasing to 98°C in approximately 15 minutes. Samples were removed at 90°C, and every 5 minutes after reaching 98°C. After removal, beans were cooled rapidly in an ice bath to 25-30 °C

Texture Analysis. Texture was measured using a TMS-90 Texture Analyzing system with a CS-1 Standard Shear Compression Cell at 23º ± 1ºC. Maximum force (Newtons) required to crush a target sample size of 15 g ± 0.3 g drained beans was measured at a shear press speed of 4. For each cooking condition, triplicate samples were run.

Data Analysis for Texture

First-order model using isothermal data.

The first order reaction equation was: $\ln(F - F_\infty(T)) = -kt + \ln(F_0 - F_\infty(T))$

where F (Newtons) was the recorded force required to crush the beans; $F_\infty(T)$ (Newtons) was the lowest achievable firmness at a constant bean temperature T; k (min^{-1}) was the rate constant; t was cooking time (in minutes); F_o was the initial firmness (Newtons).

Equivalent cooking time and temperature

A U.S. processor would like to convert an arbitrary dynamic heating process to a constant-temperature process. The equivalent isothermal cooking time at a given constant temperature T_E is (Dolan 2003) $\ln(t_E) = \ln\beta + (\Delta E / R)(1/T_E - 1/T_r)$

RESULTS AND DISCUSSION

Figure 1 shows an example of firmness versus cooking time for bush cranberry beans. Rate constant for softening at 99ºC was k = 0.060 ± 0.004 min^{-1} and ΔE= 96 kJ/g mol.

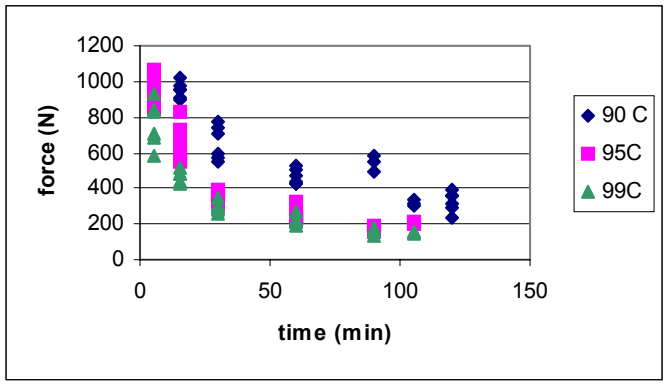


Figure 1. Firmness vs. cooking time for bush cranberry beans.

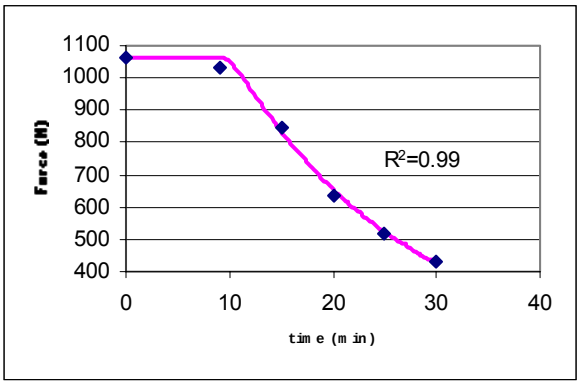
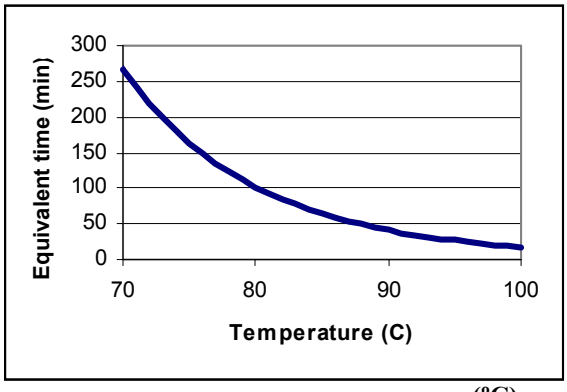


Figure 2. First-order predicted firmness (line) and experimental firmness (points) vs. cooking time

Figure 2 shows predicted values of bush cranberry bean firmness over time.

Equivalent cooking time and temperature

Figure3 shows an example of an equivalent cooking time-and-temperature curve for bush cranberry beans. Fig. 3 shows that the arbitrary dynamic heating process was equivalent to cooking 19 min @ 99ºC, 41 min @ 90ºC, and 102 min. @ 80ºC. The rapid lengthening of cooking time at lower temperatures was the result of the exponential decrease of softening rate with temperature. Fig. 3 is an example of a convenient nomograph that processors could use to design cooking times for any given dynamic cooking process.



(ºC)

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USE OF HYDRATION, GERMINATION AND α-GALACTOSIDASE TREATMENTS TO REDUCE OLIGOSACCHARIDES IN DRY BEANS

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Introduction

Celiac disease is caused by an allergic reaction to gluten, resulting in impaired nutrient absorption, pain, and distress. Since a gluten-free diet is the only known successful treatment for celiac disease, gluten-free flour is especially important for these people. One option for gluten-free products is the use of flour made from dry beans. In addition to the benefits of protein and fiber to the diet, dry beans may also have cancer-preventive benefits, making bean flour potentially attractive to the population at large (Hangen and Bennink 2002).

There has been a surplus of beans in the U.S. due largely to flatulence problems in people who consume dry beans. These problems are from a combination of oligosaccharides (stachyose and raffinose), resistant starch and fiber within the dry beans. Soaking, germination, heating, fermentation or enzyme treatments have been suggested to reduce oligosaccharide content of the beans to mitigate the flatulence problem. However, many of these treatments require either extensive amounts of time, energy or both.

In the following experiment, Michigan black, red and navy beans (all *Phaseolus vulgaris*) were treated by hydration, germination and α-galactosidase treatment in an effort to reduce raffinose and stachyose. The objective of this investigation is to determine a cost-effective process of oligosaccharide reduction to be used for large-scale bean flour production.

Materials and Methods

Preparation

Control Group (n=3): Beans (10 g) from each class were finely ground and measured for moisture content (MC) (n=3).

Hydration Group (n=3): Beans were soaked for 5 hours at 23ºC, since 90-95% of maximum weight gain occurs during this time. Beans (10 g) were removed from the water, ground and measured for MC.

Germination Group (n=3): Beans were placed in a germinating chamber at 26.7°C, 100% humidity, for 48 hours. Roots were broken off, and beans (10 g) were ground and measured for MC.

Enzyme Group (n=3): Beans were prepared identically to the control with 700 μL of commercial α-galactosidase (KCN00100, Novozymes, Inc., Franklinton, NC) added during extraction. Reaction time was approximately 1 hour.

Oligosaccharide extraction and measurement

70% (v/v) ethanol solution was added to each sample at a 10 mL/g dry mass ratio. The sample was thoroughly mixed, sonicated for 1 hour and centrifuged (2000 x g, 5min). Stachyose and raffinose levels were measured using an HPLC with a refractive index detector. A Rezex RNM Carbohydrate column (7.8 x 300 mm) and a Phenomenex guard column (7.8 x 50 mm) were used to separate oligosaccharides. HPLC grade water was used as mobile phase at 0.4

mL/min. Column and detector temperatures were maintained at 85 and 45°C, respectively. Standard curves were determined using raffinose and stachyose standards.

Results and Discussion

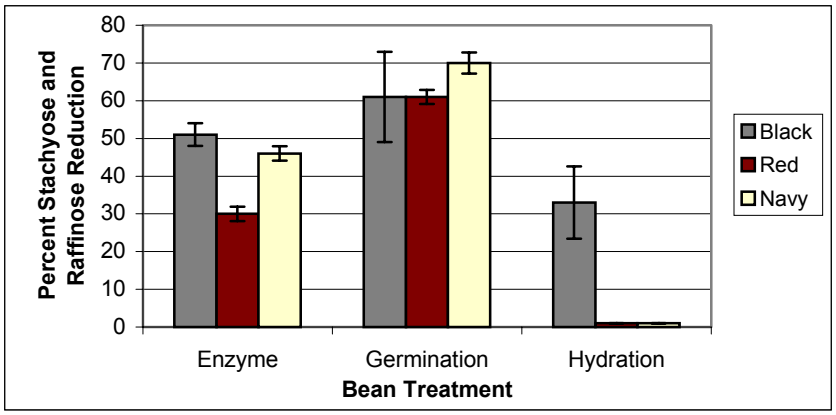

Figure 1. Percent reduction of oligosaccharides with enzyme, germination and hydration treatments for black, red and navy beans.

Germination showed the greatest reduction of oligosaccharides (Fig. 1). This was expected for all bean types as germination induces a variety of other enzymes that may have a role in oligosaccharide reduction. Also, the longer incubation times (48 hours) and optimum conditions (26.7˚C, 100% humidity) effectively increase enzyme activity. However, these conditions require extensive time and energy for conventional bean flour processing. Furthermore, nutritive quality of the bean is significantly diminished during germination, as much of the nutrient reservoirs are utilized (Elías et al. 1975).

Hydration showed the least oligosaccharide reduction (Fig. 1). Though black beans showed a 33% loss of oligosaccharides, the high relative standard deviation and the lack of navy and red bean oligosaccharide reductions indicate that there is little benefit with using hydration techniques for 4 hours at room temperature. Other investigators have shown benefits with certain hydration regimes; however, these processes require larger time and heat (Siddhuraju and Becker 2001), which may affect industrial feasibility.

Enzyme treatment results indicate that oligosaccharide levels decreased from 30 to 51% (Fig. 1). This occurred at room temperature for only one hour; suggesting that at even less-than-optimum enzyme conditions, significant oligosaccharide reduction may occur. These lower energy and shorter time requirements make enzyme treatment promising for potential industrial implementation.

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Evaluation of Water Absorption Rate in Niche Market Dry Beans

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Introduction

In our discussions with small-scale growers and their customers, we have found that dry bean color, size, cooking time and flavor are of predominant interest. Dry beans require more time to cook than most legume grain crops, and can vary from 1½ to 8 hours depending on variety. Varietal differences in cooking time are therefore an important consideration for consumers in the United States as well as in Africa and Latin America.

Previous studies have indicated an indirect relationship between cooking time and water absorption in dry beans. In these studies, genotypes that absorbed the most water cooked comparatively faster than genotypes that absorbed less water. In November 2003 we conducted a preliminary experiment at WSU Vancouver Research and Extension Unit to measure water absorption of 1-year old dry beans (harvested in 2002) and freshly harvested beans (harvested in 2003). The Experiment included 49 varieties harvested in 2002 and 111 varieties and breeding lines harvested in 2003. In this report we will only present data for 46 entries that were harvested in both 2002 and 2003.

Materials and Methods

First we conducted a preliminary study to determine if there was a difference between water absorption in tap water as compared to distilled water at our location. Based on these results (there were no significant differences in the 15 varieties that we randomly tested in 4 replications) we used only tap water for our water absorption experiment. We randomly selected and weighed 25 beans of each variety harvested in 2002 and 2003. The beans were soaked for 16 hours at room temperature in beakers each containing 1-liter of tap water (also at room temperature). After soaking, beans were drained, dried with blotting paper, and weighed. Water absorption was calculated as the percent increase in weight. The study was replicated 4 times. The percent increase in weight after soaking was statistically analyzed separately for dry bean varieties harvested in 2002 and 2003, and we compared percent increase in weight between the two years.

Results and Discussion

Dry bean varieties varied significantly in their ability to absorb water. In addition, the age of the bean significantly affected its ability to absorb water. Freshly harvested and 1-year-old beans of niche-market varieties tended to absorb more water than varieties in major market classes in this study, such as Navy and Light Red Kidney (Table 1). For example, Red/Old Fashioned Soldier, Maine Yellow Eye, French Shell Flambeau, Vermont Cranberry and Trout/Jacobs Cattle were among the beans with the greatest water absorption regardless of bean age. One-year-old beans of 5 varieties (UI-537, NW-63, G-18689, Serene, and ICB-10-5-14) absorbed significantly more water than freshly harvested beans of those varieties. In general, 1-year-old beans absorbed less water than freshly harvested beans (95% versus 101%, respectively). This study indicates that:

there are significant differences among dry bean varieties with regard to water absorption; the age of beans significantly impacts water absorption; and many niche market varieties may be faster cooking than many varieties in major market classes.

Table 1. Percent increase in fresh weight of dry beans after soaking for 16 hours.

Variety	**2003**	**2002**	**Variety**	**2003**	**2002**
Old Fashioned Soldier	116.88	109.04	Black Coco	101.30	90.58
Montcalm	113.89	104.23	Montezuma Red	100.01	97.77
Main Yellow Eye	113.61	103.83	Thort	99.92	91.45
French Shell Flambeau	112.19	106.27	Chider's Golden	99.69	102.22
Vermont Cranberry	111.53	112.64	UI-537	99.56	105.53
Trout/Jacob's Cattle	110.37	111.04	Norstar	98.42	93.36
Kintoki	109.84	81.99	Hutterite	97.42	84.88
French Flageolet	109.41	94.63	Speckled Bays	97.25	73.50
Dwayne Baptiste's	108.63	103.25	Maefax	97.17	96.75
CELRK	108.54	105.43	Andrew Kent	97.09	87.68
UI-911	107.38	105.91	Candy	96.99	80.44
Molasses Face	106.78	102.43	Major	96.98	78.77
Cardinal	106.44	100.48	NW-63	96.93	109.92
Mansell Magic	104.16	101.93	Mrocumiere	96.74	79.73
Tounge of Fire	104.12	97.40	Ireland Creek	95.61	67.31
Cannellini	103.77	89.27	G-18689	94.84	106.15
Zert	102.96	96.10	Arthur	94.74	98.91
Beka	102.20	95.87	Pinks	94.00	99.91
Etna	102.10	86.48	Kardinal	93.74	60.62
Lake Kivu	101.81	100.94	Orca	91.69	97.72
Midnight Black Turtle	101.68	105.86	Serene	88.34	100.77
Magpie	101.66	96.04	LeBaron	77.95	54.87
Norwegian	101.52	105.87	ICB-10-5-14	55.38	91.00
Overall Average				**100.50**	**94.93**
P Value				**0.0000**	**0.0000**
Comparative P value				**0.0036**	

MONOSACCHARIDES AND OLIGOSACCHARIDES CONTENT IN DRY BEANS MEASURED BY HPLC

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Introduction

Legumes are very complex foods that contain practically all nutrients. They are high in protein and carbohydrates. Of the latter, starch is most predominant, though monosaccharides, disaccharides and oligosaccharides are also present (Sánchez-Mata *et al.*, 1998). Within the oligosaccharides category, of particular importance are the raffinose family oligosaccharides (RFO), such as raffinose, stachyose and verbascose where galactose is present due to α-1.6 links, and so are also known as α-galactosides. The most abundant oligosaccharide in most legumes is stachyose, followed by raffinose and verbascose (Sánchez-Mata *et al*., 1998; Burbano *et al*., 1999). The RFOs are the cause of flatulence in humans and animals because they lack the α-galactoside enzyme. On the contrary, glucose, fructose and sucrose determine nutritive quality. Some research works have studied the content of these compounds in common beans under very different situations and methods of analysis, and in some case genetic parameters have been estimated (McPhee *et al*., 2002). The objective of this paper is to study the content of mono and oligosaccharides in different varieties of the common bean (*Phaseolus vulgaris*, L.), used for human consumption, and grown in conventional and organic conditions, so as to determine possible differences among varieties as a basis for future quality breeding.

Materials and Methods

Three different varieties of common beans (*Phaseolus vulgaris*, L.) were studied: Fru-4 (long white seed) which is purportedly flatulent, Jacob's cattle (red spotted seed), considered non-flatulent (Murphy, 1973) and Anasazi X A58 (black spotted seed), where the Anasazi family is possibly non-flatulent.

Each variety was submitted to two different cultivation systems: conventional and organic.

The content in the monosaccharides glucose (Gl) and fructose (F), the oligosaccharides sucrose Sa), raffinose (Ra) and stachyose (St), and oligosaccharides possibly stemming form the hydrolysis of raffinose and stachyose (GPH) was measured in the bean flour using HPLC. The GPH content may have been produced during the boiling process when making the flour.

The extracts obtained were diluted with 2 mL of double-distilled water, injected using Sep-Pack C18 cartridges and were successively passed through filters of 0.45 µm and 0.22 µm (Millex 6V Millipore). The Sep-Pack cartridges were activated before use with 5 mL of methanol followed by 5 mL of double-distilled water. A sample of 20 µl of this extract was analysed in a Waters Mode 45 Solvent chromatograph which was equipped with a Waters 2410 refraction index detector and HP 3396 Series II integrator. A Kromasil 100 NH_2 5µm (20 x 0.46 mm) analytic column (Teknokroma, Bellefonte, PA, USA) was used with a acetonitrile-water mobile phase at a 80:20 ratio. The sugars were identified and measured by comparing the fructose, glucose, sucrose, lactose, raffinose and stachyose patterns at 0.5% (Muzquiz, 1992). The carbohydrate content per 100 g of sample was analysed by means of an analysis of variance, using a factorial design with repetitions.

Results and Discussion

The analysis of variance shows significant differences among varieties for the majority of the characters, and only for Gl+F between cultivation systems. However the interactions are significant in many cases. The average values of the characteristics studied in the three genotypes within the two cultivation systems are shown in Table 1. In comparing the results it can be concluded that the most desirable variety for low flatulence is Jacob's cattle because of its undetectable levels of raffinose and stachyose in both cultivation systems. The next most desirable variety would be Anasazi X A58 in

organic cultivation as it is the sample that had the lowest content of non-digestible oligosaccharides (raffinose + stachyose + GPH). Anasazi X A58 under conventional cultivation and Fru-4 under either system are the least desirable. In addition, the Jacob's cattle variety shows high amounts of oligosaccharide sweeteners (glucose + fructose + sucrose), mainly in conventional cultivation. Although the Anasazi X A58 variety shows the highest sweetener amounts in conventional cultivation, it can produce flatulence problems due to being the richest in non-digestible saccharides. Finally, the Fru-4 variety can be considered to be in an intermediate position for the characteristics under consideration.

The use of HPLC has been effective in differentiating genotypes and cultivation systems, mainly for the carbohydrates glucose, fructose, sucrose and GPH. For raffinose and stachyose the experimental conditions must be changed in order to obtain greater accuracy.

Table 1. Average content of mono, di and oligosaccharides in the different types of common beans (wet basis).

		Sugars (g/100 g, wet basis)				
Bean		**Gl+F**	**Sa**	**GPH**	**Ra**	**St**
Fru-4 (long white seed)	Conventional	7.58146 (0.583)	0.87891 (0.036)	0.62235 (0.363)	Non-detectable	0.50360 (0.367)
	Organic	5.57631 (1.040)	1.02030 (0.179)	0.79398 (0.239)	Non-detectable	1.02800 (.0.974)
Jacob's cattle (spotted red seed)	Conventional	20.86312 (0.631)	1.03394 (0.032)	0.55985 (0.202)	Non-detectable	Non-detectable
	Organic	10.47667 (0.317)	0.80065 (0.452)	0.99040 (0.181)	Non-detectable	Non-detectable
Anasazi x A58 (spotted black seed)	Conventional	23.72705 (1.150)	3.15322 (0.150)	1.47266 (0.212)	0.38213 (0.129)	0.19663 (0.226)
	Organic	7.70617 (0.116)	3.85067 (0.058)	0.35273 (0.394)	0.04076 (0.382)	Non-detectable
Cultivation System						
Organic		7.91972 [a]	1.89054 [a]	0.71237 [a]	0.01358 [a]	0.34266 [a]
Conventional		17.39054 [b]	1.68869 [a]	0.88495 [a]	0.12737 [a]	0.23341 [a]
Varieties						
Fru-4		6.57888 [a]	0.94961 [a]	0.70818 [a]	[a]	0.76580 [a]
Jacob's cattle		15.66989 [b]	0.91729 [a]	0.77513 [a]	[a]	
Anasazi X A58		15.71661 [b]	3.50195 [b]	0.91269 [a]	[b]	0.09832 [b]

The values are the averages of the three numbers. The values in parentheses are the standard deviations. For each character, different letters indicate significant differences between averages for $\alpha = 0.05$.

Acknowledgments

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Effects of Reciprocal Crossing on the Cooking Time of Dry Bean

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Introduction

The cooking time of dry beans has been of great concern by the plant breeders in Brazil. Many researchers have observed variability for this character (Costa et al., 2001) and obtained some information on its genetic control (Hosfield, 2001; Belicus et al., 2001 and Elia et al, 1996). Much has been discussed in respect to the influence of the tegument on the water absorption by the grain and consequently its cooking ability (Elia et al, 1996). Nevertheless, it has not been cited if the cooking time is dependent only on the tegument or if it also depends on the embryo, and specially on the cotyledon's constitution. Such findings would be of great relevance to breeders due to the fact that the characteristics related to the tegument belong to a different generation of the embryo and cotyledons which show xenia effect. This experiment was carried out aiming to check the contribution of the integument characteristics´ and or cotyledons on the cooking ability of beans.

Material and Method

Crossing were made between C1-107 as the female parent and Carioca-80, Amarelinho and G2333 as the male parents as well as the reciprocals. While the F_2 generation has been obtained, more crossings were repeated aiming to obtain the seeds of the parents, F_1 and F_2 with the same age. Three months after the harvesting of those generations, the cooking test was set up using the JAB-77 minor type experimental cooker. Average and variance of each population and generation were estimated.

Result and Discussion

C1-107 line had the fastest cooking time (Table 1), as previously shown by Costa et al. (2001). It can be inferred that the cooking time of F_1 generation was similar to the line used as female, both in cross and its reciprocal. However, the F_2 average was similar to the parent of longer cooking time. Considering the fact that in the F_1 generation, the tegument comes from the female parent and the F_2 generation corresponds to the F_1 generation, it can be concluded that the characteristics associated to the tegument are responsible for the beans cooking time. Later evaluations will be made in order to confirm these results.

Table 1: Averages, Variances and the number of grains evaluated for the cooking time in minutes, for the following crossings: C1-107 x G2333, C1-107 x Amarelinho, C1-107 x Carioca-80 and their reciprocals.

		Averages	Variances	Grain Number
Parents	CI-107	39,3077	44,7308	13
	G2333	51,0769	78,9103	13
F_1* Generation	♀CI-107 x G2333 ♂	39,4211	142,5906	19
	♀G2333 x CI-107 ♂	52,6471	170,8414	34
F_2 Generation	♀CI-107 x G2333 ♂	48,9357	136,9239	140
	♀G2333 x CI-107 ♂	50,5360	204,8152	125
Parents	CI-107	36,5000	64,4545	12
	Amarelinho	50,2000	89,2000	05
F_1* Generation	♀CI-107 x Amarelinho ♂	34,2353	100,8520	34
	♀Amarelinho x CI-107 ♂	46,4000	282,8333	25
F_2 Generation	♀CI-107 x Amarelinho ♂	52,4643	359,8180	84
	♀Amarelinho x CI-107 ♂	60,7067	389,1020	75
Parents	CI-107	35,6250	63,6359	24
	Carioca-80	52,4000	96,2571	34
F_1* Generation	♀CI-107 x Carioca-80 ♂	34,3333	39,2941	18
	♀ Carioca-80 x CI-107 ♂	52,4000	96,2571	15
F_2 Generation	♀CI-107 x Carioca-80 ♂	46,4238	291,6058	151
	♀ Carioca-80 x CI-107 ♂	50,7329	261,2040	146

* Generation related to the embryo.

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CANNING QUALITY OF BEAN CULTIVARS FROM DIFFERENT CLASSES CONSUMED IN MEXICO

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Common bean is a traditional food staple in Mexico. Since ancestral times, the diet of the population has been based on the consumption of a legume-cereal complement: bean-corn. Beans are consumed after submitting them to a time consuming process, which includes grain soaking and cooking, and the seasoning of cooked bean. Recently, the consumption of canned beans has increased since this product is considered as a ready to use food. Since the development of the bean canning industry in this country, it has gradually grown, particularly in the decade of the 90´s, i.e., from 1994 to 1999 the increase in the production of cans was 1,237%[1], and it is still growing. At the present, two types of beans are being handled as raw material for the canning industry, black and light colored beans and only one factory is canning beans from different commercial classes separately. The knowledge of the canning quality traits of the cultivars grown in Mexico is important for the industry and for the breeders, therefore, in this note we report on the traits of four commercial classes that are grown in the main bean producing areas of Mexico.

Samples (100 g) from the bean classes: pinto, bayo (cream), flor de mayo (pink) (lighted colored beans) and black beans, were blanched at 80°C for 3 min, canned in 303 X 406 cans with 350 ml of brine (1.25% saline solution) and cooked in a retort at 115.6ºC for 45 min.[2] Canned beans were stored one week and afterwards tested for: drained weight, bean volume, broken grains in cooked beans, and grain and broth color (hue, chroma and luminosity), viscosity of broth and cooked grain texture.

The grain of the studied commercial classes showed distinctive physical traits in: grain color, size and appearance, although small differences among cultivars within each commercial class were also present. The pinto class showed the highest values for drained weight and volume of canned beans (247.0 g and 246.3 ml, respectively), followed by flor de mayo and black classes; whereas the bayo class showed the lowest values for those traits (232.8 g and 219.4 ml, respectively) (Table 1). Pintos can be considered as a high yielding class for the production of canned beans in both types of presentations: whole grain and refried beans. The bayo class showed the lowest proportion of broken grains in canned beans (11.5 %), and consequently broths of low viscosity (42.8 cps), thus, it can be considered as an outstanding class for bean canning as whole grain in spite of its low yield. This class is used in central and northern Mexico for soupy dishes. Broken grain percent was the trait with the highest variability among the studied traits.

The three commercial classes of light colored beans: flor de mayo, pinto and bayo showed similar color traits (hue, chroma and luminosity) in broth and in canned grains, therefore, the mixture of clear colored beans can render canned products with similar color properties, and the possible use of mixtures should be studied.

On the other hand, the intermediate yields (in weight and volume) of black bean and their particular color characteristics for broth and cooked grain, make them suitable as raw material for canned black bean, as whole grain and refried beans.

Unexpectedly, the canned grain of the pinto and bayo classes, known by their characteristic long cooking times, showed lower grain firmness values than those considered as representative of the classes of fast cooking beans (flor de mayo and black beans) (Fig. 1). Those results might be due to the sticky consistence of flor de mayo and black cooked beans and the existence of smaller spaces among cooked grain (caused by the relatively small size of cooked grain), enhanced by the method used for texture measurement (cell extrusion in a Texture Analyzer).

Table 1. Basic statistics for canning quality traits in four commercial classes of common bean.

	Flor			Pinto			Bayo			Black		
Trait	***Mean***	***D S***	***C V***	***Mean***	***D S***	***C V***	***Mean***	***D S***	***C V***	***Mean***	***D S***	***C V***
DW (g)	239.55	17.88	7.46	247.02	12.89	5.22	232.83	18.76	8.06	239.32	15.26	6.38
CGV(ml)	239.30	22.87	9.56	246.31	26.74	10.9	219.38	12.17	5.55	238.63	24.27	10.2
BG (%)	38.93	20.62	53.0	32.53	18.53	57.0	11.75	11.53	98.1	28.31	14.49	51.2
B Hue (°)	50.80	2.47	4.86	57.84	6.12	10.6	52.66	1.22	2.32	44.48	2.87	6.45
B Chroma	19.43	3.73	19.2	16.61	3.36	20.2	18.18	2.35	12.9	12.58	2.79	22.2
B Lum	28.84	5.78	20.0	30.20	5.30	17.5	28.37	3.38	11.9	15.65	3.96	25.3
CGHue(°)	48.21	3.45	7.16	51.04	4.66	9.13	49.81	1.90	3.81	34.23	3.00	8.76
CGChroma	24.89	2.82	11.3	25.41	5.44	21.1	24.85	2.28	9.18	10.47	1.35	12.9
CGLum	31.37	3.14	10.0	32.30	4.35	13.5	30.74	3.22	10.5	17.27	2.14	12.4
Visc (cps)	118.95	113.1	95.1	123.36	89.1	72.2	42.75	21.09	49.3	125.54	69.61	55.4

DW= Drained weight, CBV= 100 g dry bean volume after canning, *BG*= Broken grain, B= Broth, CG=Canned grain, Visc= Viscosity.

The canning quality traits studied showed variability among commercial classes (specially broken grains). The specific uses of the bean grain by canning industry can be determined for the different commercial classes, on the basis of their particular attributes.

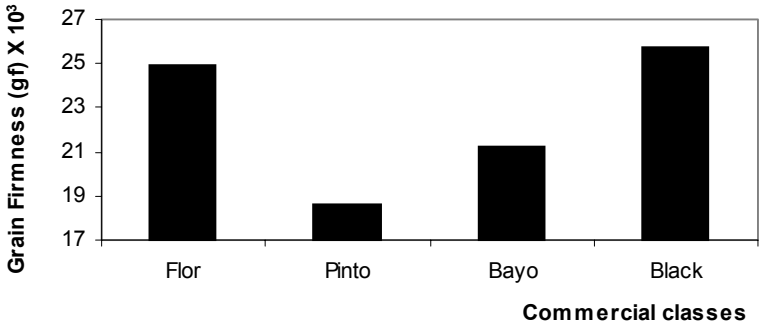

Fig. 1. Texture of canned bean in four commercial classes of common bean.

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Combining Ability and Heterosis for Agronomic Traits in Snap Bean

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Introduction

Studies considering combining ability in snap bean has showed heterosis occurrence for many important agronomic traits (Leal et al. 1979; Rodrigues et al. 1998; Carvalho et al. 1999). However, obtaining economic viable hybrids in snap bean has received less attention.

In a breeding program that uses hybridization, it is important to determine the genotypes combining ability with potential use as parents. Therefore, diallel analysis is an appropriate technique being a genetic-statistical method which helps to indicate superior parents and identified promisers crosses (Cruz and Regazzi, 2001).

According Griffing (1956), a diallel design means all possible crosses among **p** lines. Further parents and hybrids evaluation allow to select superiores genotypes (Barelli et al. 2000, Cruz and Regazzi, 2001).

Abreu (2001) evaluated the genetic diversity in climbing snap beans accessions of the germplasm bank of Darcy Ribeiro North Fluminense State University (UENF). This study was carried out using multivariate analysis and it was concluded that there was significant variability among accessions. Therefore, the present research aimed to study the combining ability of the most five divergents accessions of the UENF´s germplasm bank in order to identify superiors parents and hybrids and to start a breeding program with snap bean specifically for North of Rio de Janeiro State.

Material and Methods

The snap bean accessions UENF 1429, UENF 1432, UENF 1442, UENF 1445 and UENF 1448, from vegetables germplasm bank at UENF, Rio de Janeiro, Brazil, were chosen because of their divergent morphological and agronomic traits identified by Abreu (2001) and used as parents in a complete diallel without reciprocals. The hybrids were confirmed by flower color and RAPD markers. The populations consisting of 15 treatments were assessed in the greenhouse at the UENF, in a randomized complete block with fifteen replications. The Griffing´s diallel analysis (1956) was carried out using GENES program (Cruz, 2001).

Results and Discussion

The diallel analysis indicated the predominance of additive gene effects for mean weight of pods, mean number of seeds per pod, mean insertion of the first pod and mean height of pods; while the non-additive effects were predominant for mean number of pods.

Based on estimated general combining ability estimates, the accessions UENF 1429, UENF 1432 and UENF 1445 were indicated for using ´per se` in plant breeding programs.

In Brazil, despite the fact that there is no snap-bean hybrid available, probably due to the high cost for the seed production, the heterosis for most of the traits related to the yield and their primary components is a favorable argument towards the use of heterosis in this crop. In the present investigation, for example, the best hybrid for total number of pods per plant was 17% superior to the high parent; for total weight of pods per plant, the best hybrid was 76% superior to the high parent; and for mean number of seeds per pod, the best hybrid was 7% superior to the high parent.

The hybrids UENF 1429 x UENF 1432, UENF 1429 x UENF 1445 e UENF 1445 x UENF 1448 have high potential to obtain superior lines in advanced generations.

Genes with additive action were responsible for the expression of the major traits evaluated. The accessions UENF 1429, UENF 1432 and UENF 1445 were indicated for using ´per se` in plant breeding programs. There is a high potential to obtain superior genotypes in the following crosses: UENF 1429 x UENF 1432, UENF 1429 x UENF 1445 and UENF 1445 x UENF 1448.

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SNAP BEAN -APHID -VIRUS COMPLEX: EFFICACY OF NEONICOTINOID SEED TREATMENT TO REDUCE THE INCIDENCE OF VIRUSES

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Viruses commonly transmitted by aphids caused major problems for New York snap bean growers in 2001. Below average bean yields were associated with fields that had high numbers of aphids as well as infection by *Alfalfa mosaic virus* (AMV), *Cucumber mosaic virus* (CMV) and several aphid-transmitted *Potyviruses*. The problem was widespread and included production areas from the mid-west to the western regions of New York and Ontario.

The strategies to control this problem are limited; however, new chemistry seed treatments (Taylor, 2003) have the potential to arrest secondary spread of the viruses. Gaucho (imidacloprid) and Cruiser (thiamethoxam) are registered as a seed treatment on snap beans and other large-seeded legumes in the US. Both compounds have systemic activity to control aphids.

The objective of this study was to determine if seed treatments reduce the incidence of aphid-transmitted viruses, such CMV and AMV. To achieve this objective, field research was conducted in multiple plantings through the growing season, and at two locations to test efficacy of Gaucho and Cruiser.

The experiment was conducted at two locations, NYSAES's Fruit and Vegetable Research Farm in Geneva (central New York state) and in a commercial field north of Batavia, NY (western New York State). Seeds (var. 'Hystyle') were planted in Geneva on June 4, July 2 and July 17 and north of Batavia on June 8, July 1 and July 15. The experiment had 2 treatments (Gaucho 480 at 63 g [a.i.]/ 100 kg of seed and Cruiser at 30 g [a.i.]/ 100 kg of seed) plus a nontreated control arranged in a RCBD replicated 5 times. We were only interested in whether or not there were significant main treatment effects (planting date and seed treatment) and not if an interaction existed between these main effects. "Non-treated" seed were treated with Lorsban to control seed maggots.

During bloom to early pin stage, ten plants were randomly sampled from one row in each plot to determine the percentage of plants infected with AMV and CMV. Viruses were detected using ELISA. Data were analyzed using an analysis of variance procedure of SAS and means were compared using a Fisher's protected LSD at $P<0.05$.

Snap beans were infected with high levels of AMV and CMV at both locations and in all plantings (Table 1). At both locations, percentages of plants infected with AMV were greatest in plots planted in early July, whereas percentages were significantly lower for those planted in early June and mid July. In Batavia, percentages of plants infected with CMV also were greatest in plots planted in early July, followed by plots planted in mid July and then early June. In Geneva, percentages of CMV-infected plants did not differ among plantings. Aphids were more active (dispersing) throughout western NY during late July and August than in June (data not shown). Thus, greater dispersal activity by aphids later in the season could have resulted in higher virus incidence in later bean plantings. However, we can not explain why virus incidences were lower in the late plantings (mid-July) than in the middle ones (early July).

Gaucho and Cruiser seed treatments did not reduce the incidence of AMV and CMV at either location for any of the three plantings; data have been combined for all plantings and are

shown in Table 1. These results indicate that the seed treatments did not affect the aphid's ability to transmit AMV and CMV to snap bean. Thus, the use of Gaucho or Cruiser to prevent aphids from transmitting AMV, CMV and possibly other non-persistently transmitted, stylet-borne viruses should not be expected. In contrast to these results, Cruiser provided good control of potato leafhopper, *Empoasca fabae* (Nault et al., 2004).

Table 1. Mean percentage of 'Hystyle' snap beans infected with *Alfalfa mosaic virus* (AMV) and *Cucumber mosaic virus* (CMV) in early-, middle- and late-planted plots sown with either insecticide-treated or untreated seed in New York in 2002.

		Mean percentage of plants infected[3]			
		Geneva		Batavia	
Planting[1]	n	AMV	CMV	AMV	CMV
Early	15	43 b	53 a	16 b	20 c
Middle	15	76 a	52 a	67 a	74 a
Late	15	29 b	50 a	29 b	44 b
Pr > F		<0.0001	0.9541	<0.0001	<0.0001
Treatment[2]					
Untreated	15	45	51	33	41
Gaucho 480	15	54	55	37	45
Cruiser	15	49	48	42	52
Pr > F		0.4927	0.7219	0.5143	0.2143

[1] Data averaged across all treatments. Early plantings (Geneva: 6/4; Batavia: 6/8), middle plantings (Geneva: 7/2; Batavia: 7/1) and late plantings (Geneva: 7/17; Batavia: 7/15).

[2] Data averaged across all plantings.

[3] Percentage infection based on number of infected plants from a random sample of 10. Viruses were detected using ELISA.

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EVALUTION OF COMMON BEAN ELITE LINES DEVELOPED IN BRAZIL FOR ANGULAR LEAF SPOT RESISTANCE

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Angular leaf spot (ALS) caused by the fungus *Phaeoisariopsis griseola* (Sacc.) Ferraris, attacks the common bean (*Phaseolus vulgaris* L.) in Brazil and in other bean growing regions of the world. We started a recurrent selection bean breeding program to create superior varieties with "carioca-type" grains adapted to Brazil. One of the goals of this program is to pyramid disease resistance genes into these lines in order to obtain durable resistance to several pathogens, including *P. griseola*. As a natural step of the program we evaluated the resistance/susceptibility of 32 "carioca-type" elite lines to seven different pathotypes of *P. griseola* collected in Brazil (Nietsche et al. 2001).

Twelve seeds of each line were sown in the greenhouse and tested with each of the seven pathotypes. The plants were inoculated with suspensions of *P. griseola* (2×10^4 spores/mL) and incubated for two days in a mist chamber (20°C ± 1 and relative humidity > 95%). Twenty-one days after inoculation the plants were scored visually for the disease symptoms using a 1-9 scale (van Schoonhoven & Pastor-Corrales, 1987). Plants with grades 1 to 3 were considered resistant and with grades 4 to 9, susceptible.

The Andean cultivar AND 277, which was used as control, was resistant to all pathotypes tested, confirming the results obtained by Carvalho *et al*. (1998). The "carioca-type" grains cultivar Pérola and eight other lines were susceptible to all pathotypes of *P. griseola* tested (Table 1). However, nine lines were resistant to five of the seven pathotypes tested. Lines UTF-0013, LP 98-20 and VC 3 were the most resistant ones, showing complete resistance (grade 1) to five of the pathotypes tested, while lines UTFB-0018, VC 5 and VC 2 displayed complete resistance to three of the pathotypes (Table 1).

These results confirm the observation that most elite lines used in Brazil are susceptible or show resistance to only few *P. griseola* pathotypes, due to the wide pathogenic variability of the fungus (Sartorato, 2002). The most resistant cultivars with complementary resistance to different *P. griseola* pathotypes will be included in the recurrent selection program and crossed to obtain genotypes with "carioca-type" grains and with a wide resistance spectrum to angular leaf spot.

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Table 1. Reaction of common bean lines to *Phaeoisariopsis griseola* pathotypes 31-39 (a), 31-17 (b), 63-23 (c), 31-23 (d), 63-19 (e), 63-31 (f) e 63-39 (g).

Cultivar	Gene Pool	Pathotype with incompatible reaction							Pathotype with compatible reaction						
AND 277	Andean	a	b	c	d	e	f	g							
UTF-0037	Mesoamerican	a	b	c	d	e								f	g
UTF-0029	Mesoamerican	a	b	c	d	e								f	g
UTF-0013	Mesoamerican		b	c	d	e								f	g
UTFB-0022	Mesoamerican	a	b	d	c									f	g
GEN 12	Mesoamerican	a	b	c	d	e								f	g
LP 98-20	Mesoamerican		b	c	d	e	f		a						g
VC 2	Mesoamerican		b	c	d	e	f		a						g
VC 3	Mesoamerican		b	c	d	e	f		a						g
VC 5	Mesoamerican		b	c	d	e	f		a						g
Talismã	Mesoamerican	a	b	c	d								e	f	g
GEN 12-2	Mesoamerican	a		c	d	e				b				f	g
UTFB-0018	Mesoamerican	a		c	d	e				b				f	g
OPS-82	Mesoamerican	a		c	d		f			b			e		g
UTF-0030	Mesoamerican	a	b					g			c	d	e	f	
UTF-0031	Mesoamerican	a	b					g			c	d	e	f	
UTF-0019	Mesoamerican	a	b					g			c	d	e	f	
CNFC 9437	Mesoamerican	a						g		b	c	d	e	f	
LP 98-31	Mesoamerican	a						g		b	c	d	e	f	
FT Bonito	Mesoamerican	a	b								c	d	e	f	g
Vi-4599C	Mesoamerican			c		e			a	b		d		f	g
IAPAR-81	Mesoamerican	a								b	c	d	e	f	g
CNFC 9500	Mesoamerican	a								b	c	d	e	f	g
IPR-Juriti	Mesoamerican								a	b	c	d	e	f	g
Vi-4899C	Mesoamerican								a	b	c	d	e	f	g
Pérola	Mesoamerican								a	b	c	d	e	D	g
CNFC 8017	Mesoamerican								a	b	c	d	e	f	g
VC 4	Mesoamerican								a	b	c	d	e	f	g
Vi 669C	Mesoamerican								a	b	c	d	e	f	g
LP 98-76	Mesoamerican								a	b	c	d	e	f	g
Carioca 1070	Mesoamerican								a	b	c	d	e	f	g
LH-11	Mesoamerican								a	b	c	e	d	f	g

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Pathogenic diversity of *Phaeoisariopsis griseola* in Costa Rica.

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In Costa Rica, the common bean (*Phaseolus vulgaris* L.) is one of the most important components of diet, and the principal source of protein for human population. This crop is attacked by a large number of diseases. Angular leaf spot (ALS), caused by the fungus *Phaeoisariopsis griseola* (Sacc.) Ferraris, is widespread, and its incidence and severity, has increased in the last ten years in Costa Rica (Araya & Araya, 2000).

The reaction of bean varieties to natural population of the pathogen vary considerably from one location to another, because of the presence of multiple races, that infect determined varieties of bean, with a different range of severity.

A major part of bean production comes from medium, small and subsistence farmers, who have significant yield reduction and therefore economical looses due to the presence of *P. griseola* and other pathogens. The development and use of resistant cultivars offer the only economical means for disease control (Sartorato *et al*., 2000). With that intention since the middle of the ninetieth decade, different sources of resistance and potential gene donator have been evaluated in Costa Rica (Araya & Araya, 2000).

Breeding programs frequently need to develop cultivars with new sources of resistance because the lifetime of a resistant cultivar is very short (Sartorato *et al*., 2000). The effective use of resistance to angular leaf spot depends on the understanding of pathogenic variability and geographical distribution of the causal agent.

The objective of this study was to identify pathotypes of *P. griseola* from isolates collected in Costa Rican agricultural regions to guide genetic improvement programs in order to develop commercial varieties with resistance to angular leaf spot.

Pathogenic diversity of *P. griseola* have been investigated by inoculating 60 different isolates of this pathogen on a set of 12 differential cultivars established at the Centro Internacional de Agricultura Tropical (CIAT). Table 1 presents the *P. griseola* pathotypes identified in the different agricultural regions of Costa Rica. Isolates exhibited a different virulence pattern when inoculated on differential cultivars. From the 60 isolates evaluated, 20 different pathotypes were identified, from which, 19 induced compatible reactions with mesoamerican cultivars only, and 17 with Andean and Mesoamerican. The results have demonstrated the great diversity, virulence and non-specificity of Mesoamerican pathotypes. Among all the pathotypes, the most virulent was the pathotype 31-63 that infected 10 differentials. Pathotypes 0-0, 0-7, 0-53, 31-47 and 38-55 were the most frequent (Table 1). Isolates collected at the same agricultural region showed differences in their virulence patterns. The high frequency of pathotypes 0-0, 0-7 and 0-53 suggest the possibility to detect resistant gene in Andean cultivars to be incorporated in bean breading programs in the country.

Resistance sources to angular leaf spot should be selected using Mesoamerican germplasm and inoculating the most frequent and virulent isolates, to guarantee the covering of the major scope of diversity and to accumulate the greater number of genes or resistance factors.

Table 1. Distribution of pathotypes and isolates of *Phaeoisariopsis griseola* identified in the principal agricultural regions of Costa Rica.

Pathotype	Agricultural Region*					Total number of isolates
	CN	BR	CH	HN	HA	
0-0	5	1		1		7
0-7	3	1		1		5
0-53	2		2	3		7
1-1				3		3
1-3					2	2
2-46				2		2
8-30		3				3
9-7				3		3
13-13	1					1
13-23		1				1
20-21		1				1
31-19	3					3
31-47	4			1		5
31-57		2				2
31-63		1				1
34-63				3		3
38-55	4	1				5
42-57	1					1
49-55			3	1		4
52-57	1					1
TOTAL	24	11	5	18	2	60

* (CN) Central, (BR) Brunca, (CH) Chorotega, (HN) Huetar Norte, (HA) Huetar Atlántica.

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ANALYSIS OF THE PATHOGENIC VARIABILITY OF *Phaeoisariopsis griseola* IN BRAZIL

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Understand the pathogenic variability and the knowledge of broadly based sources of resistance to angular leaf spot are fundamental for any breeding program. Consequently, the main goals of this work was to identify among the *Phaeoisaripsis griseola* (Sacc.) Ferraris differential cultivars, those with widest resistance spectra for this pathogen in Brazil. All data were colleted from the previously published papers on the Brazilian isolates of this fungus that were classified by using the differential series developed by Pastor Corrales and Jara (Fitopatologia Colombiana 19:15-24. 1995)

Our analyses showed that between 1996 and 2002, it was identified a total of 51 *P. griseola* pathotypes in Brazil (Table 1). Pathotypes 31-39, 63-31, 63-23, 63-39, 63-47, 63-55 and 63-63 are the most frequent and wide distributed, and are commonly found in the States of Goiás and Minas Gerais. Some of these pathotypes were also identified in bean samples from the States of Roraima, Paraiba, Pernambuco, Alagoas, Santa Catarina and Espírito Santo. The pathotypes 5-07, 2-23, 7-23, 7-31, 7-39, 9-23, 11-19, 11-39, 13-23, 13-55, 15-07, 15-23, 15-31, 15-33, 15-47, 15-55, 29-55, 31-07, 31-15, 31-17, 31-21, 31-31, 31-33, 31-47, 31-53, 31-55, 39-23, 45-39, 47-31, 47-47, 55-23, 55-39, 57-23, 59-23, 59-47, 61-41 and 63-35 were identified in only one Brazilian State. Although the number of collected isolates was different from each State, our data showed that the Goiás State presented the highest *P. griseola* variability (25 pathotypes) followed by the State of Minas Gerais (20 pathotypes) (Table 1).

Differential Mesoamerican cultivars México 54, Cornell 49-242 and BAT 332, with seeds originated from CIAT´s Germoplasm Bank, are the most resistant ones to this disease in Brazil, and are incompatible with 36, 27 and 23 *P. grisola* pathotypes. The association of genes present in these three resistant sources will confers resistance to all the 50 identified pathotypes, except for the pathotype 63-63. The Andean genes of cultivars G 5686 and Amendoin are also important sources conferring resistance to 30 and 21 pathotypes, respectively.

Table 1. Pathotypes of *Phaeoisariopsis griseola* identified in Brazil.

	Virulence phenotype of differential cultivars													States[g]										
	1[a]	2	3	4	5	6	7	8	9	10	11	12	Pathotype	GO	MG	RR	SC	PE	ES	PB	BA	MS	AL	PR
1	+	-	-	-	-	-	+	+	+	-	-	-	05-07[b]			X								
2	+	-	+	-	-	-	+	+	+	-	+	-	02-23[b]	X										
3	+	+	+	-	-	-	+	+	+	-	+	-	07-23[b]					X						
4	+	+	+	-	-	-	+	+	+	+	+	-	07-31[b]					X						
5	+	+	+	-	-	-	+	+	+	-	-	+	07-39[b]	X										
6	+	-	-	+	-	-	+	+	+	-	+	-	09-23[e]	X										
7	+	+	-	+	-	-	+	+	-	-	+	-	11-19[c]								X			
8	+	+	-	+	-	-	+	+	+	-	-	+	11-39[b]	X										
9	+	-	+	+	-	-	+	+	+	-	+	-	13-23[b]	X										
10	+	-	+	+	-	-	+	+	+	-	+	+	13-55[b]			X								

Table 1. (Cont.)

Virulence phenotype of differential cultivars														States[g]										
	1[a]	2	3	4	5	6	7	8	9	10	11	12	pathotype	GO	MG	RR	SC	PE	ES	PB	BA	MS	AL	PR
11	+	+	+	+	-	-	+	+	+	-	-	-	15-07[b]			X								
12	+	+	+	+	-	-	+	+	+	-	+	-	15-23[b]	X										
13	+	+	+	+	-	-	+	+	+	+	+	-	15-31[b]		X									
14	+	+	+	+	-	-	+	-	-	-	-	+	15-33[c]							X				
15	+	+	+	+	-	-	+	+	+	-	-	+	15-39[b]	X	X									
16	+	+	+	+	-	-	+	+	+	+	-	+	15-47[b]		X									
17	+	+	+	+	-	-	+	+	+	-	+	+	15-55[b]			X								
18	+	-	+	+	+	-	+	+	+	-	+	+	29-55[c]				X							
19	+	+	+	+	+	-	+	+	+	-	-	-	31-07[d]		X									
20	+	+	+	+	+	-	+	+	+	+	-	-	31-15[e]	X										
21	+	+	+	+	+	-	+	-	-	-	+	-	31-17[d]		X									
22	+	+	+	+	+	-	+	-	+	-	+	-	31-21[d]		X									
23	+	+	+	+	+	-	+	+	+	-	+	-	31-23[b,d,f]	X	X									
24	+	+	+	+	+	-	+	+	+	+	+	-	31-31[b,c,d,e]	X										
25	+	+	+	+	+	-	+	-	-	-	-	+	31-33[d]		X									
26	+	+	+	+	+	-	+	+	+	-	-	+	31-39[b,c,d]	X	X	X							X	
27	+	+	+	+	+	-	+	+	+	+	-	+	31-47[e]	X										
28	+	+	+	+	+	-	+	-	+	-	+	+	31-53[d]		X									
29	+	+	+	+	+	-	+	+	+	-	+	+	31-55[c]						X					
30	+	-	+	+	-	+	+	+	+	-	+	-	39-23[c]										X	
31	+	+	+	+	+	-	+	+	+	-	-	+	45-39[c]					X						
32	+	+	+	+	-	+	+	+	+	+	+	-	47-31[e]	X										
33	+	+	+	+	-	+	+	+	+	-	-	+	47-39[e]	X				X						
34	+	+	+	+	-	+	+	+	+	+	-	+	47-47[b]			X								
35	+	+	+	-	+	+	+	+	+	-	+	-	55-23[b]			X								
36	+	+	+	-	+	+	+	+	+	+	+	-	55-31[e,f]	X	X									
37	+	+	+	-	+	+	+	+	+	-	-	+	55-39[c]									X		
38	+	-	-	+	+	+	+	+	+	-	+	-	57-23[e]	X										
39	+	+	-	+	+	+	+	+	+	-	+	-	59-23[e]	X										
40	+	+	-	+	+	+	+	+	+	+	-	+	59-47[e]	X										
41	+	-	+	+	+	+	+	-	-	+	-	+	61-41[d]		X									
42	+	+	+	+	+	+	+	+	+	-	-	-	63-07[d,e]	X	X									
43	+	+	+	+	+	+	+	+	+	+	-	-	63-15[e,f]	X			X							
44	+	+	+	+	+	+	+	+	-	-	+	-	63-19[c,d]		X				X					
45	+	+	+	+	+	+	+	+	+	-	+	-	63-23[b,c,d,e,f]	X	X		X							
46	+	+	+	+	+	+	+	+	+	+	+	-	63-31[b,c,d,e,f]	X	X		X		X			X		X
47	+	+	+	+	+	+	+	+	-	-	-	+	63-35[c]				X							
48	+	+	+	+	+	+	+	+	+	-	-	+	63-39[c,d,f]		X				X	X				
49	+	+	+	+	+	+	+	+	+	+	-	+	63-47[c,d,e]	X	X					X	X			
50	+	+	+	+	+	+	+	+	+	-	+	+	63-55[c,d]	X	X			X						
51	+	+	+	+	+	+	+	+	+	+	+	+	63-63[d,e,f]	X	X		X							
R	0	9	7	8	21	30	0	6	7	36	23	27	Total	25	20	7	6	5	4	3	2	2	2	1
S	51	42	44	43	30	21	51	45	44	15	28	24												

[a]1=Don Timóteo, 2=G 11796, 3=Bolón Bayo, 4=Montcalm, 5=Amendoin, 6=G5686, 7=PAN 72, 8=G 2858, 9=Flor de Mayo, 10=México 54, 11=Bat 332, 12=Cornell 49-242.

[b]Pastor Corrales & Paula Jr., 1996 (RENAFE, doc. 69, v.1, p.239-241); [c]Isolates from Embrapa Arroz e Feijão and identified by Nietsche, 2000, [d]Nietsche, 2000 (Thesis DSc., UFV-Brazil), [e]Sartorato, 2002a (VII Congresso. Nacional de Pesquisa de Feijão. Viçosa, p.120-124), [f]Sartorato, 2002b (Fitopatol. bras., v.27, p.78-81).

[g]GO=Goiás, MG=Minas Gerais, RR=Roraima, SC=Santa Catarina, PE=Pernambuco, ES=Espírito Santo, PB=Paraíba, BA=Bahía, MS=Mato Grosso do Sul, AL=Alagoas, PR=Paraná.

DEVELOPMENT OF SCAR MARKERS LINKED TO COMMON BEAN ANGULAR LEAF SPOT RESISTANCE GENES

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Angular leaf spot (ALS), caused by fungus *Phaeoisariopsis griseola* (Sacc) Ferraris, is considered the most important disease in many common bean (*Phaseolus vulgaris*) growing areas. ALS in Brazil is responsible for significant yield losses. The development and use of resistant cultivars is considered to be the most viable method for controlling this disease. The use of molecular markers linked to the resistance genes can be very useful to monitor these genes in breeding programs. Several RAPD markers linked to ALS resistance have been identified, however, this type of marker is sensitive to PCR amplification conditions, which reduce its reproducibility among different laboratories. SCAR markers, on the other hand, are highly specific RAPD-derived markers which do not show such limitations. The objective of this work was to develop SCAR markers from RAPD markers previously identified as linked to ALS resistance genes *Phg-ON* (markers OPBA16, OPAA19, and OPM02) and *Phg-1* (marker OPH13), present in different common bean cultivars (Carvalho et al., 1998; Faleiro et al., 2003). The RAPD markers were amplified, fractionated in 1.5% agarose gels, the bands of interest were excised and purified with the Gel Extraction Kit (QIAgen), and cloned into the pGEM-T Easy vector (Promega). After transformation of competent DH5α *Escherichia coli* cells, the positive clones (white colonies) were confirmed by PCR. The clones were sequenced in an ABI Prism 377 (Perkin Elmer), the SCAR primers were designed, synthesized and tested in populations segregating for genes *Phg-ON* or *Phg-1* (Table 1). The amplification conditions are described on Table 2. These SCAR markers can now be used in marker assisted common bean breeding programs. They are presently being in the BIOAGRO/UFV breeding program, which aims to pyramid disease resistance genes in different commercial bean varieties.

Table 1. Segregation analysis and the genetic distances (centiMorgan - cM) between the SCAR markers and the ALS resistance genes.

Locus tested	*Parents*			**Expected ratio**[a]	Observed ratio	χ^2	*Prob. (%)*	**cM**
	Susceptible		*Resistant*					
SCARAA19/*Phg-ON*	TO	x	ON	9:3:3:1	63:5:4:53	292.75	0.00	10.1
SCARBA16/*Phg-ON*	TO	x	ON	9:3:3:1	64:4:3:54	307.57	0.00	7.1
SCARM02/*Phg-ON*	TO	x	ON	9:3:3:1	65:2:3:55	322.84	0.00	5.3
SCARH13/*Phg-1*	Vermelho	x	AND 277	9:3:3:1	148:4:7:38	119.71	0.00	5.6

[a] **Considering independence between the loci.**

Table 2. Sequences and amplification conditions for SCAR markers linked to angular leaf spot resistance genes.

Marker	Primer sequence (5'- 3')[a]	PCR profile				Size (bp)
		Cycles	Denaturation	Annealing	Extension	
SCARAA19	F: TGAGGCGTGTCAATGGATATAA R: GAGGCGTGTTGATAATTCTGG	35	94 °C/30 s	56 °C/1 min	72 °C/90 s	650
SCARBA16	F: TTCCACGTCTATTTTGCATCA R: CACGCATCACGCAGAACT	35	94 °C/30 s	58 °C/1 min	72 °C/90 s	560
SCARH13	F: GACGCCACACCCATTATGTT R: GCCACACAGATGGAGCTTTA	35	94 °C/30 s	59 °C/1 min	72 °C/90 s	520
SCARM02	F: CAACGCCTCATTAAATTGGA R: CGCCTCTAAACGGGAGAAAC	35	94 °C/30 s	58 °C/1 min	72 °C/90 s	460

[a] F = forward; R = reverse

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Occurrence and Distribution of Andean, Afro-Andean and Mesoamerican Pathotypes of *Phaeoisariopsis griseola* in Kenya

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Introduction

Angular leaf spot (ALS) caused by *Phaeoisariopsis griseola* is the most widely distributed and damaging diseases of common bean (*Phaseolus vulgaris* L.), causing yield losses as high as 80% (Saettler, 1991). Understanding the population diversity structure and distribution of such an economically important pathogen is, therefore, an important prerequisite in designing strategies for developing and deploying durable resistance. Previous studies have revealed high levels of pathogenic and genetic variation in *P. griseola* (Liebenberg and Pretorius, 1997; Mahuku *et al*., 2002), but little is known about the pathogen's diversity and distribution in Kenya and the implication of this diversity when developing resistant bean varieties. The objective of this study was, therefore, to characterise the virulence diversity occurring in *P. griseola* and map-out its distribution in Kenya.

Materials and Methods

One hundred isolates of *P. griseola* were obtained from naturally infected bean leaves collected from farmer's fields in different agroecological zones in Kenya. Virulence characterisation of the isolates was performed by inoculating a set of 12 bean differential cultivars obtained from CIAT with the pathogen at a concentration of $2x10^4$ conidia ml^{-1}. Six of the bean cultivars were large-seeded varieties of Andean origin and the other six were small-seeded Mesoamerican genotypes. Scoring for ALS severity based on a scale of 1 to 9 (Van Schoonhoven and Pastor-Corrales, 1987) was done 17 days after inoculation. Plants showing grades 1 to 3 were considered resistant and those showing grades 5 to 9 were susceptible. Disease grades were obtained for all isolate-cultivar interactions and the data analyzed by ANOVA and General Linear Model (GLM) procedure using SAS system computer package (SAS Institute Inc. Cary, USA). Isolates with similar disease reactions were grouped together to form a physiological race.

Results and Discussion

P. griseola isolates varied significantly ($P \leq 0.05$) in their virulence and were grouped into 44 races. Based on the set of differential cultivars that they infected, isolates were divided into three virulence groups; Andean, Afro-Andean and Mesoamerican. Thirteen of the isolates were Andean, 17 were Afro-Andean and 70 were Mesoamerican. Andean isolates were found to exclusively infect Andean differential cultivars whereas Afro-Andean isolates mostly infected Andean cultivars plus one or two Mesoamerican genotypes. Mesoamerican isolates, on the other hand, mainly infected Mesoamerican genotypes but also infected some Andean cultivars. Out of the 44 *P. griseola* races identified, 50% were represented by only one isolate while 11% had more than five isolates. Compared to Andean and Afro-Andean, Mesoamerican races were more frequently isolated in all the districts covered in this study (Figure 1).

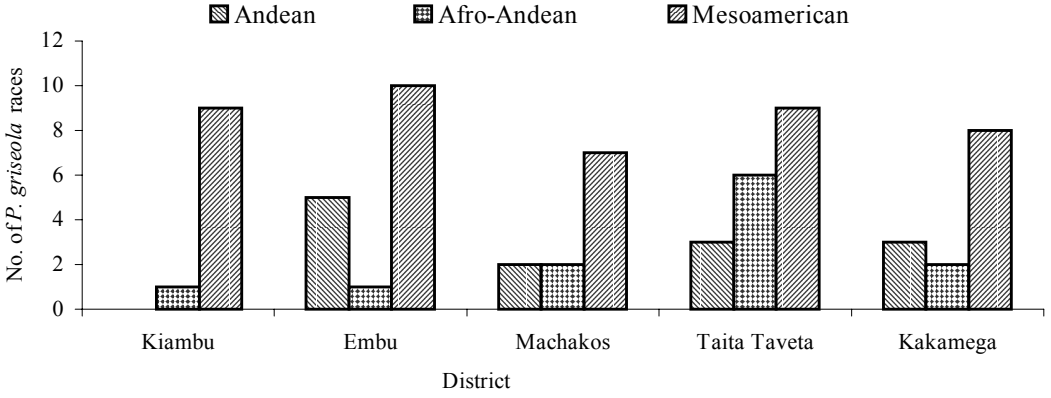

Fig. 1 Occurrence of Andean, Afro-Andean and Mesoamerican races of *P. griseola* in Embu, Kakamega, Kiambu, Machakos and Taita Taveta districts

This study revealed enormous virulence diversity in *P. griseola* population in Kenya, which must be taken into consideration when developing and deploying ALS resistance. Mesoamerican isolates are the most virulent and their wide distribution in the bean producing areas poses an immense danger to the crop. There is, therefore, an urgent need to develop bean cultivars with durable ALS resistance as a foundation for integrated disease management strategies, especially for small-scale farmers who are resource-constrained.

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ANALYSIS OF THE PATHOGENIC VARIABILITY OF *Colletotrichum lindemuthianum* IN BRAZIL

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Understand the pathogenic variability and the knowledge of broadly based sources of resistance are fundamental points in a breeding program. Consequently, the main goal of this work was to identify, among the *Colletotrichum lindemuthianum* differential cultivars, those with most ample resistance spectra for this pathogen in Brazil. All data were collected from the most important previously published papers of the Brazilian isolates of this fungus that were classified by using the differential series developed by Pastor Corrales (CIAT - Document 113, p. 249, 1992).

Our analyses showed that between 1994 and 2002, it was identified a total of 50 *C. lindemuthianum* pathotypes in Brazil (Table 1). Pathotypes 65, 73, 81 and 87 are the most frequent and wide distributed in the country, and are commonly found in the States of Paraná, Santa Catarina, Góias and Distrito Federal. Pathotypes 1, 5, 17, 67, 79, 85, 86, 93, 96, 102, 105, 111, 117, 121, 123, 125, 137, 193, 217, 320, 321, 339, 343 and 585 were identified in only one Brazilian State. Although the number of collected isolates was different from each State, our data showed that the Paraná State presented the highest *C. lindemuthianum* variability (29 pathotypes) followed by the States of Goiás (17 pathotypes), Santa Catarina (16 pathotypes) and Rio Grande do Sul (14 pathotypes) (Table 1).

Differential cultivars carrying the gene *Co-4* and its alleles and genes *Co-6* and *Co-5*, individuality or in association with others genes, are those that confer the highest resistance to this disease in Brazil. Consequently, the cultivars G 2333 (*Co-4*^{2}, *Co-5* and *Co-7*), PI 207262 (*Co-4*^{3} and *Co-9*) and TO (*Co-4*) are incompatibles with 50, 45 and 44 *C. lindemuthianum* pathotypes, respectively. The cultivars AB 136 (*Co-6_ co-8*) and TU (*Co-5*) confers resistance to 50 and 49 pathotypes, respectively. The allele *Co-1*^{2} of Andean cultivar Kaboon, is the principal Brazilian Andean resistance source to anthracnose and is incompatible with 36 pathotypes originally collected mainly from the States of Espirito Santo, Mato Grosso do Sul, Minas Gerais, Bahía, Distrito Federal and Rio Grande do Sul. The *Co-2* gene of Cornell 49-242 confers resistance to 29 pathotypes, in several Brazilian States, including pathotypes 65, 81 and 87, widely distributed in Brazil (Table 1).

References (Table 1):

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Table 1. Pathotypes of *C. lindemuthianum* identified in Brazil.

	Virulence phenotype of differential cultivars														Brazilian States[i]													
	1[a]	2	3	4	5	6	7	8	9	10	11	12	Pathotype	PR	GO	SC	RS	MG	ES	BA	DF	MS	PE	PB	SP	RJ	SE
1	+	-	-	-	-	-	-	-	-	-	-	-	1[b,f]	X														
2	+	-	+	-	-	-	-	-	-	-	-	-	5[c]				X											
3	+	+	+	-	-	-	-	-	-	-	-	-	7[d,e,f]	X		X							X					
4	-	-	-	+	-	-	-	-	-	-	-	-	8[d,h]		X			X										
5	+	-	-	-	+	-	-	-	-	-	-	-	17[c]				X											
6	+	+	+	-	+	-	-	-	-	-	-	-	23[c,d,f]		X		X			X			X					
7	+	+	+	+	+	-	-	-	-	-	-	-	31[c,e]	X			X											
8	+	+	+	-	+	+	-	-	-	-	-	-	55[c,d]	X		X	X											
9	-	-	-	-	-	-	+	-	-	-	-	-	64[d,f]	X			X		X									
10	+	-	-	-	-	-	+	-	-	-	-	-	65[c,d,e,f,g,h]	X	X	X	X	X	X	X	X	X		X				
11	+	+	-	-	-	-	+	-	-	-	-	-	67[d]						X									
12	+	-	+	-	-	-	+	-	-	-	-	-	69[e,f,g]	X	X		X	X			X								
13	+	+	+	-	-	-	+	-	-	-	-	-	71[f,g]		X					X								
14	-	-	-	+	-	-	+	-	-	-	-	-	72[d,f,g]	X			X		X									
15	+	-	-	+	-	-	+	-	-	-	-	-	73[c,d,e,f,g]	X	X	X	X		X		X	X			X	X		
16	+	+	-	+	-	-	+	-	-	-	-	-	75[d,g]			X			X									
17	+	-	+	+	-	-	+	-	-	-	-	-	77[g]	X	X	X	X											
18	+	+	+	+	-	-	+	-	-	-	-	-	79[d]						X									
19	+	-	-	-	+	-	+	-	-	-	-	-	81[d,e,f,g,h]	X	X	X		X		X	X	X	X		X			
20	+	+	-	-	+	-	+	-	-	-	-	-	83[d,g]		X			X										
21	+	-	+	-	+	-	+	-	-	-	-	-	85[g]					X										
22	-	+	+	-	+	-	+	-	-	-	-	-	86[f]			X												
23	+	+	+	-	+	-	+	-	-	-	-	-	87[d,e,f,g,h]	X	X	X	X	X	X	X	X		X	X				
24	+	-	-	+	+	-	+	-	-	-	-	-	89[d,e,f,g]	X	X	X		X				X						X
25	+	-	+	+	+	-	+	-	-	-	-	-	93[g]	X														
26	+	+	+	+	+	-	+	-	-	-	-	-	95[d,e,g]	X		X												
27	-	-	-	-	-	+	+	-	-	-	-	-	96[g]	X														
28	+	-	-	-	-	+	+	-	-	-	-	-	97[d,f,g]	X	X		X											
29	+	-	+	-	-	+	+	-	-	-	-	-	101[d,g]	X						X	X							
30	-	+	+	-	-	+	+	-	-	-	-	-	102[d]	X														
31	+	-	-	+	-	+	+	-	-	-	-	-	105[g]	X														
32	+	-	+	+	-	+	+	-	-	-	-	-	109[f,g]	X	X	X												
33	+	+	+	+	-	+	+	-	-	-	-	-	111[f,g]			X												
34	+	-	+	-	+	+	+	-	-	-	-	-	117[d]		X													
35	+	+	+	-	+	+	+	-	-	-	-	-	119[d,e]		X			X		X	X		X					
36	+	-	-	+	+	+	+	-	-	-	-	-	121[f]			X												
37	+	+	-	+	+	+	+	-	-	-	-	-	123[g]	X														
38	+	-	+	+	+	+	+	-	-	-	-	-	125[g]		X													
39	+	+	+	+	+	+	+	-	-	-	-	-	127[g]	X	X													
40	+	-	-	+	-	-	-	+	-	-	-	-	137[f]	X														
41	+	-	-	-	-	-	+	+	-	-	-	-	193[g]	X														
42	+	-	-	+	+	-	+	+	-	-	-	-	217[f]			X												
43	+	-	-	+	+	+	+	+	-	-	-	-	249[f]	X		X												
44	-	-	-	-	-	-	+	-	+	-	-	-	320[f]	X														
45	+	-	-	-	-	-	+	-	+	-	-	-	321[g]	X														
46	+	-	-	-	+	-	+	-	+	-	-	-	337[b]															
47	+	+	-	-	+	-	+	-	+	-	-	-	339[d]									X						
48	+	+	+	-	+	-	+	-	+	-	-	-	343[d]									X						
49	+	-	+	-	-	-	+	+	+	-	-	-	453[c,d]	X			X											
50	+	-	-	+	-	-	+	-	-	+	-	-	585[d]						X									
S	43	19	24	21	23	15	41	5	6	1	0	0																
R	7	31	26	29	27	35	9	45	44	49	50	50	Total	29	17	16	14	9	9	7	7	6	5	2	2	1	1

[a]1=Michelite, 2=Michigan Dark Red Kidney, 3=Perry Marrow, 4=Cornell 49-242, 5=Widusa, 6=Kaboon, 7=México 222, 8=PI 207262, 9=TO, 10=TU, 11=AB 136, 12=G 2333. [i]PR=Paraná, GO=Goiás, SC=Santa Catarina, RS=Rio Grande do Sul, MG=Minas Gerais, ES=Espiritu Santo, BA=Baía, DF=Distrito Federal, MS=Mato Grosso do Sul, PE=Pernambuco, PB=Paraíba, SP=São Paulo, RJ=Rio de Janeiro, SE=Sergipe.

Characterization of *Colletotrichum lindemuthianum* Isolates by using Differential Cultivars

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Introduction

Among diseases of the common bean, anthracnose is one of the most important diseases caused by *Colletotrichum lindemuthianum* (Sacc.et Magn.) Scrib., which has demonstrating wide pathogenic variability. In Brazil, the characterization was limited to the Southeast and South regions, while in Santa Catarina state the last information about identification of diversity of *C. lindemuthianum* races was reported by Balardin et al. (1990). In this study our objective was to determine the variability in *C. lindemuthianum* in Santa Catarina.

Material and Methods

Eighteen isolates of *C. lindemuthianum* were collected between 2002 and 2003 from common bean cultivars grown in Guatambú, Ponte Serrada, Campos Novos and Ituporanga in Santa Catarina state. The fungus was isolated from stem or pods showing anthracnose symptoms. Monosporic cultures of each isolate were grown and maintained for short periods on tubes containing either PDA (potato-dextrose agar) or pods culture medium, incubated at 22^{o}C for 14 days. The virulence phenotype of each isolate was characterized, using a set of 12 common bean differential cultivars. The protocol for inoculation was as follows: 14-day-old bean plants with fully developed first trifoliate leaves were spray-inoculated with a spore suspension (1.2 x 10^{6} spores ml^{-1}) of each isolate of *C. lindemuthianum*. After seven days of inoculation in a mist chamber, seedlings were evaluated for their disease reaction using a scale from 1 to 9 (Balardin et al., 1990; Pastor-Corrales, 1991). Plants with disease reaction scores from 1-3 were considered resistant, whereas plants that were rated from 4-9 were considered susceptible.

Results and Discussion

The reactions of set differential cultivars to the 18 isolates of *C. lindemuthianum* demonstrated the presence of races 17, 65, 67, 73, 75, 83, 89, and 101 (Table 1). In addition, five new races were characterized as 67, 73, 75, 83, and 101. In a previous report, races alpha (17), epsilon (65), and 89 had been identified in Santa Catarina (Oliveira et al., 1973; Menezes and Dianese, 1988; Balardin et al., 1990). Beside that, these authors identified the races beta, delta, mexican I, brazilian I, kappa, mu, and gamma, in Santa Catarina. Later, in the same state, Rava et al. (1994) identified the race 55 using the binary system.

Race 67 showed high frequency, comprising 22% of the total sample. This race was found in two different locations. In the other hand, six different isolates (three for each one) had given rise to the races 73, and 101. The race 73 was the only common and widespread. The results showed the existence of variability of the *C. lindemuthianum* pathogen in Santa Catarina. Therefore is evident that the bean-breeding program must consider the frequency of the races identified in this work, providing new studies with the proposal to keep the information about the variability of this pathogen in this region up to date. Based on the resistance reactions, the differential cultivars PI 207262, TO, TU, AB 136, and G 2333 are the main resistance sources to the eight identified races.

Table1. Reaction of differential cultivars to *Colletotrichum lindemuthianum* isolates collected in Santa Catarina state

Isolate	Local	Race	Differential cultivars*											
			A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**
1	Guatambú	83	S	S	R	R	S	R	S	R	R	R	R	R
2	Guatambú	67	S	S	R	R	R	R	S	R	R	R	R	R
3	Guatambú	101	S	R	S	R	R	S	S	R	R	R	R	R
4	Ponte Serrada	65	S	R	R	R	R	R	S	R	R	R	R	R
5	Guatambú	75	S	S	R	S	R	R	S	R	R	R	R	R
6	Guatambú	73	S	R	R	S	R	R	S	R	R	R	R	R
7	Ponte Serrada	73	S	R	R	S	R	R	S	R	R	R	R	R
8	Campos Novos	17	S	R	R	R	S	R	R	R	R	R	R	R
9	Campos Novos	67	S	S	R	R	R	R	S	R	R	R	R	R
10	Guatambú	83	S	S	R	R	S	R	S	R	R	R	R	R
11	Guatambú	101	S	R	S	R	R	S	S	R	R	R	R	R
12	Ponte Serrada	75	S	S	R	S	R	R	S	R	R	R	R	R
13	Ponte Serrada	65	S	R	R	R	R	R	S	R	R	R	R	R
14	Ponte Serrada	101	S	R	S	R	R	S	S	R	R	R	R	R
15	Campos Novos	67	S	S	R	R	R	R	S	R	R	R	R	R
16	Ituporanga	73	S	R	R	S	R	R	S	R	R	R	R	R
17	Campos Novos	89	S	R	R	S	S	R	S	R	R	R	R	R
18	Campos Novos	67	S	S	R	R	R	R	S	R	R	R	R	R

*A- Michelite (1); B- Dark Red Kidney (2); C- Perry Marrow (4); D- Cornell 49-242 (8); E- Widusa (16); F- Kaboon (32); G- Mexico 222 (64); H- PI 207262 (128); I- TO (256); J- TU (512); K- AB136 (1024); L- G2333 (2048).
*S- Susceptible, R- Resistant.

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The Mesoamerican Anthracnose Resistance Gene, *Co-4²*, Does Not Confer Resistance in Certain Andean Genetic Backgrounds

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Introduction

The highly anthracnose resistant Mexican landrace, G2333, carries three characterized anthracnose resistance genes: *Co-4²*, *Co-5*, and *Co-7* (Pastor-Corrales et al., 1994; Young et al., 1998). The most effective gene in this pyramid is *Co-4²*, which conferred resistance to 33 out of 34 different races of *Colletotrichum lindemuthianum* collected from 9 different countries in the Americas (Balardin et al., 1997). Three SCAR markers linked to *Co-4²* have been developed to facilitate the use of this gene in anthracnose resistance breeding: SAS13 at 0.39 cM from the *Co-4²* gene (Young et al., 1998; Melotto and Kelly, 2001), SH18 at 4.27±2.37 cM from the gene and SBB14 at 5.87±1.93 cM from the gene (Awale and Kelly, 2001). SAS13 has successfully been used to introduce *Co-4²* into highly susceptible pinto bean through marker-assisted backcrossing in the absence of pathogen screening (Miklas and Kelly, 2002).

Materials and Methods

The three SCAR markers, SAS13, SH18 and SBB14, were used to introduce *Co-4²* into four Ecuadorian bush bean lines through backcrossing (Table 1). The source of *Co-4²* was a single backcross of Red Hawk, a dark red kidney variety, to SEL 1308. SEL 1308 was derived from one backcross of Talamanca to G 2333. Of the three characterized resistance genes in G 2333, SEL 1308 carries only *Co-4²* (Young et al., 1998).

Table 1. Ecuadorian parent lines' seed color, hundred seed weight and pedigrees

Ecuadorian Line	Seed Color	g/100 seeds	Pedigree
Cocacho	Canario	~50 g	landrace
Paragachi	Red Mottle	~45 g	may be selection from PVA1441
Yunguilla - INIAP-414	Red Mottle	~50 g	G13922//G2172/G6474
ARME-2	Red Mottle	~45 g	AND 1005/Paragachi

In each generation of crossing the plants carrying *Co-4²* were selected using the SCAR markers. Three backcrosses were made to each Ecuadorian recurrent parent. After the crossing was completed, plants from the BC_3F_1, BC_2F_2, and, in some cases, BC_1F_2 generations were inoculated with *C. lindemuthianum* in the greenhouse and tested with the SCAR markers.

Results and Discussion

In backcross populations generated from the lines Yunguilla and ARME-2, plants carrying the markers for *Co-4²* were resistant to anthracnose in greenhouse inoculations (Table 2). However, the marker assisted backcrossing did not work as expected in the Cocacho and Paragachi derived populations. In these two populations, plants exhibited only partial resistance to anthracnose (Table 2). In the Paragachi backcross populations, *Co-4²* appears to be conferring the observed partial resistance, since the marker data and disease reaction were dependent as determined by the χ^2 Test for Independence (Table 3).

In the Cocacho populations, however, *Co-4²* does not confer partial resistance since the marker data and observed disease reaction were independent (Table 3).

Table 2. Results from greenhouse inoculations and marker analysis of backcross populations derived from each Ecuadorian recurrent parent

Disease Reaction	Recurrent Parent	Marker Result	
		Present	**Absent**
RESISTANT[1]	Cocacho[2]	11	5
	Paragachi[2]	30	1
	Yunguilla[3]	20	3
	ARME-2[3]	17	0
SUSCEPTIBLE	Cocacho	22	13
	Paragachi	12	17
	Yunguilla	0	4
	ARME-2	0	11

[1] partial resistance in Cocacho and Paragachi populations; complete resistance in Yunguilla and ARME-2 populations
[2] BC_3F_1, BC_2F_2, and BC_1F_2 plants inoculated with an Ecuadorian race 0 isolate of *C. lindemuthianum*
[3] BC_3F_1 and BC_2F_2 plants inoculated with a Mexican race 2 isolate of *C. lindemuthianum*

Table 3. χ^2 Test for Independence of *Co-4*^{2} marker results and disease reactions in backcross populations derived from Cocacho, Paragachi, Yunguilla, and ARME-2

Recurrent Parent	**χ^2-value**	**Significance**
Cocacho[1]	0.17	not significant
Paragachi[1]	21.89	<0.001
Yunguilla[2]	13.42	<0.001
ARME-2[2]	28.00	<0.001

[1] BC_3F_1, BC_2F_2, and BC_1F_2 plants inoculated with an Ecuadorian race 0 isolate of *C. lindemuthianum*
[2] BC_3F_1 and BC_2F_2 plants inoculated with a Mexican race 2 isolate of *C. lindemuthianum*

Conclusions

One possible explanation for the failure of marker-assisted selection for *Co-4*^{2} in the Cocacho and Paragachi populations is that a recombination between the gene and the markers occurred early in population development. However, this is unlikely since SAS13 is tightly linked to *Co-4*^{2} and multiple plants carrying the markers were used as parents in each generation. Another possible explanation is that *Co-4*^{2} requires the presence of another complimentary gene (or genes) which is present in Middle American germplasm but absent in certain Andean landrace genotypes like Cocacho and Paragachi. This situation limits the usefulness of marker-assisted selection for the introduction of *Co-4*^{2} into genotypes which lack the complimentary gene(s) and highlights the necessity of incorporating direct selection, through disease screening, into breeding projects utilizing molecular markers to select for disease resistance genes.

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BEAN REACTIONS TO 24 PATHOTYPES OF *Colletotrichum lindemuthianum*

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Common bean is one of the most important leguminous crops in Brazil. The Embrapa Rice and Beans, together with others bean breeding programs, has developed several genotypes that show one or more relevant characteristic to the farmers. However, common bean has shown to be very susceptible to innumerous diseases. Anthracnose, caused by the fungus *Colletotrichum lindemuthianum* is one of the most important bean disease in Brazil. This disease can be controlled by several methods including cultural practices, chemicals and cultivar resistance. The objective of this test was to evaluate the reaction of bean cultivars/lines to *C. lindemuthianum*.

To accomplish this objective it was used the bean cultivars/lines Magnífico, Carioca Rubi, Perola, Talisma, Arua, Piata, CNFC 7806, CNFC 7813, CNFC 9504, CNFC 10276, CNFC 10281 (carioca), Uirapuru, Soberano, Diamante Negro, Valente, CNFP 7776, CNFP 10120, CNFP 10138, CNFP 10150 (black), Radiante (cranberry), Timbó (purple) and Marfim (small beige) and 24 pathotypes of *C. lindemuthianum* including 1, 7, 23, 55, 64, 65, 69, 71, 73, 77, 79, 81, 87, 89, 95, 97, 102, 117, 321, 343, 453 and 2047. The pathotypes 97 and 453 were represented, in this test, with two isolates each. Entries were sown in plastic tray, ten seeds/entry, 6 entries/tray + the susceptible control (IPA 7419). Monosporic cultures of each pathotype were grown in test tubes containing bean pods partially immersed in agar-agar cultures medium and incubated at 20 ± 2°C for 10-12 days. After this period of time pods were transferred to a Becker containing sterilized water to obtain a spore suspension. Each entry was inoculated by spraying (DeVilbiss no. 15) the bean leaves and stem with a spore suspension adjusted to a concentration of 1.2×10^6 spore mL^{-1}. After inoculation trays were transferred to a humid chamber for 48 hours and, then, moved to greenhouse benches. Symptoms were evaluated 8-10 days after inoculation by using a 1 (no symptoms) to 9 (dead plant) scale where 1 to 3 were considered resistant and 4 to 9 susceptible.

Among the 23 tested cultivars, only Arua, Piata, CNFP 10120, CNFP 10138 and CNFP 10150 showed resistant reaction to all pathotypes (Table 1). Even though these cultivars were considered resistant to all pathotypes to some of them they showed at least one susceptible plant. Cultivar Arua and lines CNFP 10120 and CNFP 10138 showed only one susceptible plant to the pathotypes 64, 65 and 453, respectively. CNFP 10150 and Piata presented at least one susceptible plant to the pathotypes 77, 79 and 89 and 1, 7, 77, 97, 117 and 321, respectively. This results indicate that for these pathotypes these genotypes are still segregating and need to undergo another selection phase. Others cultivars presented different reactions according to the pathotype tested (Table 1). DNA from each cultivar has already been extracted to test them with all the SCAR markers found in the literature.

Table 1. Number of pathotypes to which the 22 bean genotypes were R (resistant), R/S (resistant/susceptible), S/R (susceptible/resistant) and S (susceptible) to 24 pathotypes of *Colletotrichum lindemuthianum*.

Genotype	Grain type	R		R/S		S/R		S	
		NP	% P	NP	% P	NP	% P	NP	% P
Magnífico	Carioca	1	4.2	2	8.3	7	29.2	12	50.0
Carioca Rubi	Carioca	1	4.2	3	12.5	4	16.7	16	66.7
Pérola	Carioca			4	16.7	1	4.2	19	79.2
Aruã	Carioca	23	95.8	1	4.2				
Piatã	Carioca	18	75.0	6	25.0				
Requinte	Carioca	9	37.5	5	20.8	2	8.3	8	33.3
Pontal	Carioca	11	45.8	4	16.7	2	8.3	7	29.2
CNFC 9504	Carioca	6	25.0	13	54.2	3	12.5	2	8.3
CNFC 10276	Carioca	12	50.0	4	16.7	1	4.2	7	29.2
CNFC 10281	Carioca	13	54.2	4	16.7			7	29.2
Talismã	Carioca	5	20.8	10	41.7	2	8.3	7	29.2
Uirapuru	Black	6	25.0	8	33.3	2	8.3	8	33.3
Soberano	Black	9	37.5	12	50.0			3	12.5
Dia. Negro	Black			2	8.3			22	91.2
Valente	Black	12	50.0	3	12.5	2	8.3	7	29.2
CNFP 7776	Black	9	37.5	5	20.8	1	4.2	9	37.5
CNFP 10120	Black	23	95.8	1	4.2				
CNFP 10138	Black	23	95.8	1	4.2				
CNFP 10150	Black	21	87.5	3	12.5				
Radiante	Cranberry			2	8.3			22	91.7
Timbó	Purple	9	37.5	3	12.5	4	16.7	8	33.3
Marfim	Small beige	19	79.2	4	16.7	1	4.2		
IPA 7419								24	100.0

R = number of pathotypes with 100% resistant plants.
R/S = number of pathotypes in which there was more resistant than susceptible plants (segregating).
S/R = number of pathotypes in which there was more susceptible than resistant plants (segregating).
S = number of pathotypes with 100% susceptible plants.
NP = number of pathotypes.
% P = percentage of pathotypes.

DEVELOPMENT OF SCAR MARKERS LINKED TO COMMON BEAN ANTHRACNOSE RESISTANCE GENES *Co-4* and *Co-6*

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Anthracnose, caused by fungus *Colletotrichum lindemuthianum* (Sacc. & Magn.) Scrib., is among the main diseases of the common bean (*Phaseolus vulgaris* L.) in Brazil an in other bean-growing areas around the world. This disease may cause total yield loss when susceptible cultivars are grown under environmental conditions appropriate for the pathogen proliferation. The development of resistant cultivars is an alternative strategy to control of the disease. New cultivars have to be developed along the years because of the high pathogenic variability of the fungus. Several RAPD markers have been identified and used to facilitate the development of resistant cultivars. Because of the reproducibility problems associated with the RAPD technique these markers can be used to derive SCAR markers which are more specific and reproducible. The objective of this work was to develop SCAR markers from RAPD markers linked to anthracnose resistance genes *Co-4* (RAPD markers OPY20 and OPC08) and *Co-6* (RAPD markers OPAZ20 and OPZ04) previously identified in our laboratory (Alzate-Marin et al., 1999; Alzate-Marin et al., 2000; Arruda et al., 2000). The RAPD bands of interest were excised from 1.5% agarose gels and purified with the Gel Extraction Kit (QIAgen). The DNA fragments were cloned into the pGEM-T Easy vector (Promega), which was used to transform competent DH5α *Escherichia coli* cells. The positive clones were sequenced in an automated sequencer (ABI Prism 377, Perkin Elmer), and the SCAR primers were designed, synthesized and tested in appropriate populations segregating for resistance genes *Co-4* or *Co-6* (Table 1). The sequences of the SCAR primers and the amplification conditions are depicted on Table 2.

These SCAR markers are now being used in the common bean breeding program developed at the BIOAGRO/UFV, which aims to pyramid resistance genes in different commercial bean cultivars grown in Brazil.

Table 1. Segregation analysis and genetic distances (centiMorgans - cM) between SCAR markers and anthracnose resistance genes *Co-4* or *Co-6*.

Locus tested	*Parents*			**Expected ratio**[a]	Observed ratio	χ^2	*Prob. (%)*	**cM**
	Resistant		*Susceptible*					
SCARY20/*Co-4*	Rudá	x	TO	9:3:3:1	115:3:0:44	177.56	0.00	1.2
SCARC08/*Co-4*	Ouro Negro	x	TO	9:3:3:1	69:5:4:47	227.21	0.00	7.8
SCARAZ20/*Co-6*	***Rudá***	***x***	***AB 136***	9:3:3:1	176:13:2:48	149.57	0.00	7.1
SCARZ04/*Co-6*	Michelite	x	AB 136	9:3:3:1	63:0:1:17	63.23	0.00	2.9

[a] **Considering independence between the loci.**

Table 2. Sequences and amplification conditions for SCAR markers linked to anthracnose resistance genes *Co-4* and *Co-6*.

Marker	Primer sequence (5' – 3')[a]	PCR profile				Size (bp)
		Cycles	Denaturation	Annealing	Extension	
SCARAZ20	F: ACCCCTCATGCAGGTTTTTA R: CATAATCCATTCATGCTCACC	35	94 °C/30 s	60 °C/1 min	72 °C/90 s	845 [b]
SCARZ04	F: GGCTGTGCTGATTAATTCTGG R: TGCTCATTTTATAATGGAGAAAAA	45	94 °C/30 s	45 °C/2 min	72 °C/90 s	567 [c]
SCARY20	F: AGCCGTGGAAGGTTGTCAT R: CCGTGGAAACAACACACAAT	35	94 °C/30 s	65 °C/1 min	72 °C/90 s	830 [b]
SCARC08	F: AGAATGCCTTTAGCTGTTGG R: CAGAGAGGCTAGGCTTATCG	35	94 °C/30 s	65 °C/1 min	72 °C/90 s	910 [b]

[a] F = forward; R = reverse
[b] Coupling phase; [c] Repulsion phase

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Analyses of the Pathogenic Variability of *Uromyces appendiculatus* in Some Regions of Brazil

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Common bean (*Phaseolus vulgaris* L.) rust, caused by *Uromyces appendiculatus*, is among the most important fungal diseases affecting this crop in Brazil and in other parts of the world. Several studies have demonstrated the extensive pathogenic variability of *U. appendiculatus* in the States of Santa Catarina, Rio Grande do Sul, Goiás and Minas Gerais. However, the use of distinct sets of differential cultivars made the comparison among the obtained data somewhat difficult. This fact also prevented the identification of sources with wide resistance spectra in these regions. Consequently, the main goal of this work was to identify, among the *U. appendiculatus* differential cultivars, those with the widest resistance spectra for this pathogen. Data were collected from previously published paper with *U. appendiculatus* isolates characterization from Brazilian States of Santa Catarina, Rio Grande do Sul and Goiás (Santos & Rios, 2000), and from our recent characterization of isolates from the State of Minas Gerais (Souza et al., 2002). On these two isolates characterization it was used the differential series proposed on the "The 1983 Bean Rust Workshop" (Stavely et al., 1983).

Our analyses showed that a total of 39 pathotypes of *U. appendiculatus* were identified in the States of Santa Catarina, Rio Grande do Sul, Goiás and Minas Gerais (Table 1). These pathotypes were named from 1 to 39. Only the pathotypes 16 and 19 were identified from more than one isolate. The cultivars with the widest resistance spectra were Redlands Pioneer (resistant to all pathotypes), California Small White 643 and Brown Beauty (resistant to 38 pathotypes), AxS 37 (resistant to 37 pathotypes) and Compuesto Negro Chimaltenango (resistant to 34 pathotypes). In despite of the fact that the cultivars California Small White 643, AxS 37 and Brown Beauty have been excluded from the new differential series proposed in 2002, in South African, we strongly recommend that these three cultivars need to be maintained for future pathogenic variability studies in Brazil. Otherwise, the information on resistance durability in these cultivars will be missed. The cultivars Ecuador 299, Mexico 235 and Mexico 309 showed high resistance spectra only in Goiás and Minas Gerais States. While, the cultivar Kentucky Wonder 814 showed high resistance spectra only in Rio Grande do Sul and Santa Catarina States (Table 1).

Table 1. Comparative analyses of pathogenic variability of *Uromyces appendiculatus* isolates collected in four Brazilian States.

Cultivars\Isolates	Goiás State[a]											Rio Grande do Sul and Santa Catarina States[a]																									Minas Gerais State[a]								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	1	4	6	9	10	11	12	R	S
1 US 3	+	-	+	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	4	35
2 *California Small White 643*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	38	1
3 Pinto 650	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	1	38
4 Kentucky Wonder 765	-	-	-	-	-	-	-	+	+	-	-	+	+	+	-	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	15	24
5 Kentucky Wonder 780	+	-	-	+	-	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	13	26
6 Kentucky Wonder 814	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	20	19
7 Golden Gate Wax	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	1	38
8 Early Gallatin	-	-	-	+	-	+	-	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	9	30
9 Redlands Pioneer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	39	0
10 Ecuador 299	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	+	-	+	+	+	-	+	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-	28	11
11 Mexico 235	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	+	+	+	+	+	-	+	-	-	-	+	-	+	-	-	+	+	-	-	-	-	-	-	-	28	11
12 Mexico 309	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	19	20
13 Brown Beauty	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	38	1
14 Pinto Olathe	+	-	-	-	-	+	-	-	-	-	+	+	-	-	-	+	+	-	+	-	-	+	-	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	-	18	21
15 AxS 37	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	37	2
16 NEP-2	+	-	-	+	-	+	+	-	+	-	-	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-	+	-	+	+	-	12	27
17 Aurora	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	3	36
18 51051	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	+	-	17	22
19 Compuesto Negro Chimaltenango	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+	-	-	-	-	-	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	34	5
Pathotypes	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**19**	**30**	**31**	**16**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**		

[a] In this analysis it was considered resistant the cultivars with reaction grade between 1 and 3 (-) and susceptible those with reaction grades between 4 and 6 (Santos & Rios, 2000; Souza et al., 2002) according to the evaluation scale adopted in both works (Stavely, 1985).

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PATHOTYPE CHARACTERIZATION OF BEAN RUST AND ANTHRACNOSE IN BULGARIA

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Rust and anthracnose are some of the most damaging diseases on bean in the regions with moderate climate. In Bulgaria, these diseases have insignificant effect on dry bean production due to their sporadic occurrence. However, these diseases cause considerable yield loss in years with favorable conditions for their development. Both rust and anthracnose fungi are characterized with considerable pathogenic diversity in their populations. This publication presents results from the investigation on pathogenic diversity in the Bulgarian rust and anthracnose populations.

Due to its late manifestation during the vegetation period, the rust caused by the obligate fungus *Uromyces appendiculatus* has a low effect on dry bean production in Bulgaria. Nevertheless, in years with cold and moist weather during May and June, the disease causes serious damages. Up to now, 7 physiological races have been determined in Bulgaria (Kiryakov and Genchev, 2003). The established races were identified by inoculation of a set of 19 standard differential bean cultivars (Stavely *et al.,* 1983). Races BG 1 and 2 were established in 1991, and races from 3 to 7 - during 2001 (Table 1). The isolates from race BG 7 were divided into two pathotypes (202 and 203) using a new set of 12 differential cultivars (Steadman et al., 2002). The isolates from BG 5 were referred to race 202, and those from BG 6 - to race 20, using the same differential set. Among the commercial cultivars included in dry bean production in Bulgaria, resistance to the established pathotypes was demonstrated by Abritus and Prelom.

Anthracnose caused by the fungus *Colletotrichum lindemuthianum* can cause the full destruction of the crops in years with cold and moist conditions during vegetation. This disease presents serious problems to bean production in the mountainous and semi-mountainous regions in Bulgaria. Investigating the race variability of the pathogen in the country, Kiryakov (2000) identified two races by using a standard set of 12 differential cultivars (Pastor-Corrales, 1991) - races 2 and 81 (Table 1). Race 81 is typical for the plain regions, and race 2 - for the mountainous and semi-mountainous areas. In 2003 race 6 was identified in the region of the

mountains Rhodoppi and Rila. Most of the commercial dry bean varieties in Bulgaria are susceptible to race 81 and resistant to races 2 and 6.

Table 1. Pathotypes of *U. appendiculatus* and *C. lindemuthianum* in Bulgaria

Race	Differential cultivars																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
U. appendiculatus**																			
BG1 (4200000)	-*	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BG2 (5010000)	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
BG3 (4010000)	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
BG4 (4210000)	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
BG5 (2010020)	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-
BG6 (5210000)	+	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
BG7 (5210020)	+	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	+	-	-
C. lindemuthianum***																			
81	+	-	-	-	+	-	+	-	-	-	-	-							
2	-	+	-	-	-	-	-	-	-	-	-	-							
6	-	+	+	-	-	-	-	-	-	-	-	-							

* - Resistant; + Susceptible

** Rust differentials: **1**) U.S.#3; **2**) C.S.W. 643; **3**) Pinto 650; **4**) K.W. 765; **5**) KW 780; **6**) K.W 814; **7**) G.G.Wax;
8) Early Gallatin; **9**) Redlands Pioneer; **10**) Ecuador 299; **11**) Mexico 235; **12**) Mexico 309; **13**) Brown Beauty; **14**) Olathe; **15**) A x S 37; **16**) NEP 2; **17**) Aurora; **18**) 51051; **19**) CNC

*** Anthracnose differentials: **1**) Michelite; **2**) Michigan Dark Red Kidney; **3**) Perry Marrow; **4**) Cornell 49-241
5) Widusa; **6**) Kaboon; **7**) Mexico 222; **8**) PI 207 262; **9**) TO; **10**)TU; **11**) AB 136; **12**) G 2333

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Proposal for designation of a rust resistance gene in the large-seeded cultivar Kranskop

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Approximately 90% of the area planted to large-seeded beans in South Africa is devoted to the high yielding South African red speckled sugar cultivar Kranskop and related cultivars, all with similar levels of moderate rust resistance (RR). In local pathogenicity and field trials (Liebenberg, 2003), the resistance of Kranskop was observed to be similar to that of the rust differential cultivars Redlands Pioneer and Pompadour Checa 50 (PC-50). PC-50, a new differential cultivar (Saladin *et al.,* 2000), has been reported to contain two single dominant genes conferring race-specific resistance to rust, namely *Ur-9* (seedling resistance), and *Ur-12* (adult plant resistance) (Finke *et al.,* 1986, Bokosi *et al.,* 1994; Kelly *et al.,* 1996; Jung *et al.,* 1998). The RR of Redlands Pioneer has not been characterized but the Redlands group was studied by Ballantyne (1978). Kranskop was developed from Bonus (SA), Redlands Autumn Crop, Redlands Greenleaf C, UI 50 and UI 51 (A.J. Liebenberg, personal communication, May, 1995). Superior resistance is being introgressed into the Kranskop group, but it is desirable to retain the hypostatic RR already in these cultivars. This study was undertaken to facilitate the continued use of the RR in Kranskop in the local breeding programs, and to determine the relationship of RR between Kranskop, Redlands Pioneer and PC-50.

Materials and Methods The F_1 population from the cross Bonus (SA) x Kranskop was advanced to F_2, as well as backcrossed to Bonus (SA) to produce BC_1F_1 and BC_1F_2 populations. Fifty one resistant and 20 susceptible plants from the F_2 population were selfed for F_3 tests. The crosses Redlands Pioneer x Kranskop, PC-50 x Kranskop and their reciprocals were also made. Plants were grown in a rust-free greenhouse at 20/28 EC (night/day) in commercial potting soil (pH_{H2O} 6.9), and inoculated when primary leaves were one- to two-thirds expanded. Inoculum concentration, spray-inoculation and treatment of plants were as described by Harter *et al. (*1935) and Stavely (1983). Rust reactions were determined using race RSA-Ua7, the only local race then available which differentiated between Kranskop (resistant) and Bonus (SA) (susceptible). Pustule size and type were evaluated 15 days after inoculation, using the standard rating scale for the evaluation of rust (Stavely *et al.,* 1983).

Results and Discussion Results for segregation of the Bonus (SA) x Kranskop cross are given in Table 1, and those for the allelic crosses in Table 2. Segregation ratios suggest that inheritance of resistance to race RSA-Ua7 in Kranskop is conditioned by a single dominant gene, provisionally designated *Ur-13*. This appears to be different to the RR in PC-50, as segregation of the F_2 plants resulting from both the PC-50 x Kranskop and its reciprocal cross indicated the presence of two independent genes.

Redlands Pioneer x Kranskop F_1 and F_2 plants and the reciprocal progenies all gave a reaction similar to that of Kranskop and Redlands Pioneer, with no segregation, suggesting that resistance to race RSA-Ua7 is conditioned by the same gene in these two cultivars. Kranskop and Redlands Pioneer also reacted in a similar manner to 11 Southern African races and numerous

isolates (Liebenberg, 2003). The possible similarity of rust resistance in these two cultivars is further supported by the fact that they share a common ancestor, Redlands Greenleaf A (Ballantyne, 1978).

Ur-13 maps to linkage group 8 of the Bat 93/Jalo 558 core map, near the *Bng-73* anchor marker (PN Miklas, personal communication, October 2003; Liebenberg, 2003). No other RR gene maps to this linkage group (Miklas *et al.*, 2002). Results of race identification obtained worldwide by various authors, suggest that the resistance in Redlands Pioneer is different from that in other differential cultivars (including *US#3* with *Ur-8* and Resisto with *Ur-10* which have not yet been mapped) and also other known resistance sources (Stavely, 1984; Stavely *et al*., 1989; Abd-Alla, 1996; Stavely 1999; Pastor-Corrales, 2001; Mmbaga and Stavely 1988; Balcita and Hartmann, 1993; Sandlin *et al.*, 1995; Bokosi, *et al*., 1997; Liebenberg 2003). *Ur-13* may be the same as *Ur*-Red, a gene hypothesized to be present in some of the Redlands cultivars (Ballantyne, 1978), but which can no longer be traced due to loss of the rust races concerned.

Table 1 Segregation for phenotypic reactions of Kranskop x Bonus (SA) F_1, F_2, F_3, BC_1F_1 and BC_1F_2 populations to bean rust race RSA-Ua7

Parent, cross and generation	Resistant	Segregating	Susceptible	Expected ratio	Chi-square	p
Bonus (SA)	0	-	>20	-	-	-
Kranskop	>20	-	0	-	-	-
Bonus(SA) x Kranskop F_1	39	-	0	All R	-	-
F_2	346	-	121	3:1	0.21	0.65
F_3	22	29	20	1:2:1	2.493	0.29
F_3 plants of segregating families	697	-	239	3:1	0.14	0.71
BC_1 F_1	52	-	54	1:1	0.038	0.85
BC_1 F_2 (families)	18	-	15	1:1	0.273	0.6
BC_1 F_2 (plants of segregating families)	487	-	172	3:1	0.268	0.6

Table 2 Segregation for phenotypic reactions of F_2 populations derived from the crosses Kranskop and Redlands Pioneer, and Kranskop and PC-50, to bean rust race RSA-Ua7

Cross and Generation	Very Resistant	Resistant	Intermediate	Susceptible	Expected Ratio	Chi-square	p
Kranskop	0	>20	0	0	-	-	-
Redlands Pioneer	0	>20	0	0	-	-	-
PC-50	0	0	>20	0			
Kranskop x Redlands Pioneer F_2	0	227	0	0	All R	-	-
Redlands Pioneer x Kranskop F_2	0	253	0	0	All R	-	-
Kranskop x PC-50 F_2 (VR:R:I:S)	102	45	35	13	9:3:3:1	2.61	0.46
Kranskop x PC-50 F_2 (R:S)		182		13	15:1	0.058	0.81
PC-50 x Kranskop F_2 (VR:R:I:S)	100	32	24	12	9:3:3:1	2.33	0.51
PC-50 x Kranskop F_2 (R:S)		156		12	15:1	0.23	0.63

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Evidence for Middle-American Origin of Rust Resistance in the Differential Cultivar Redlands Pioneer

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A genetic study indicated that the red speckled sugar (cranberry) bean cultivar Kranskop contains at least one major rust resistance (RR) gene, proposed as *Ur-13*, which also occurs in Redlands Pioneer (Liebenberg and Pretorius, 2004). Two SCAR markers, namely $SE_{AAC}M_{ACC384/405}$ (KB126), $SE_{ACA}M_{CTT320/396}$ (KB85) and one cleaved amplified polymorphic sequence (CAPS) marker $SE_{AAG}M_{CGT436}$*Hha* $I_{188/250}$ (KB4*Hha* I) (all co-dominant) developed from AFLP markers for *Ur-13*, showed potential for use in marker assisted selection (MAS). KB126 mapped 1.6 cM from the gene, KB85, 9.2 cM on the same side, and KB4*Hha* I, 14.5 cM on the opposite side (Liebenberg, 2003). The purpose of the present study was to use these markers, in conjunction with available southern African rust races, to trace the probable source of *Ur-13*, to verify the application potential of the markers and, so doing, to enable a better understanding of the origin and contribution of *Ur-13* in the germplasm involved.

Materials and Methods. Seventy-one accessions, including the rust differential cultivars (old and new international sets) (Stavely *et al.*, 1983; Stavely, 1984; Steadman *et al.,* 2002), sources of already characterized RR genes (summarized in Miklas *et al.* 2002), the Kranskop-group of cultivars, eight "Redlands" cultivars, the available parents of both groups (Ogle and Johnson, 1974; Ballantyne, 1978; A.J. Liebenberg, personal communication, May, 1995) and other local cultivars, as well as 78 breeding lines (mostly Kranskop-related) were tested for the above markers. Where results were critical, a second seed lot was obtained from an independent source. Between one and eleven southern African rust races (Liebenberg, 2003) were used to inoculate the germplasm studied.

Results and Discussion KB85(+) alone was in 21 accessions and five breeding lines of Middle-American origin (small seeded beans). Most gave a hypersensitive (HS) reaction or were resistant to race RSA-Ua7. Both KB85(+) and KB126(+) were in 14 of the small seeded accessions, including California Small White (CSW) 643, one of the parents of the Redlands group. All were HS or resistant to race RSA-Ua7. Kentucky Wonder (KW) 780 (of mixed origin), also had both these markers, but was susceptible to RSA-Ua7. KB126(+) alone was present only in the related cultivars Mexico 235, Ecuador 299 and MAR 2. KB4*Hha* I(+) alone was only in material of Andean origin, including Brown Beauty, one of the parents of the Redlands group. All were susceptible to race RSA-Ua7, with the exception of some breeding lines into which additional RR genes had been introduced. The combination KB85(+) and KB4*Hha* I(+) was only in PI 151385 and one breeding line. All three positive alleles together were present only in the Redlands and Kranskop groups (10 cultivars), and in 21 related breeding lines. All were resistant or gave a hypersensitive reaction to race RSA-Ua7 (Table 1). Five Andean cultivars and 21 breeding lines had all three negative alleles.

It appears, therefore, that KB 85(+), KB126(+) and *Ur-13,* are of Middle-American origin, and KB4*Hha* I(+) of Andean origin. CSW 643 is the only small seeded parent of the Redlands

group, and the only parent of Redlands Greenleaf C (the latter was used in the development of Kranskop (A.J. Liebenberg, personal communication, May, 1995)) which, in common with the latter two cultivars, has both positive alleles and is resistant to RSA-Ua7 (Table 1). Kranskop, Redlands Greenleaf C, CSW 643 and Redlands Pioneer reacted in a similar manner to the eleven races reported by Liebenberg (2003). The absence of KB85(+) and KB126(+) in the large seeded cultivars tested which are not related to the Redlands- or Kranskop groups is conspicuous (Table 1). This confirms the opinion of Ballantyne (1978) that a postulated, uncharacterized RR gene, termed *"Ur*-Red*"* (probably the same as *Ur-13*) was present in Redlands Greenleaf B and C and in Redlands Pioneer, and had originated from CSW 643.

Table 1 Survey of SCAR markers KB85, KB126 and CAPS KB4*Hha* 1 in 71 common bean accessions and 78 breeding lines, and their reaction to rust race RSA-Ua-7 (HS = hypersensitive; R = resistant and S = susceptible; + = positive allele; - = negative allele)

SCAR KB85	SCAR KB126	Reaction to rust race RSA-Ua7	CAPS KB4*Hha* 1	Accessions
+	-	HS, R or S	-	Small seeded (Middle-American), some with Andean elements
+	+	HS or R	-	Small seeded (including California Small White 643), some with Andean elements
+	+	R or HS	+	Members of the Redlands and Kranskop groups only (10 cultivars and 21 breeding lines)
-	+	H	-	Mexico 235, Ecuador 299 and MAR 2 (small seeded)
-	-	S	+	Large seeded (Andean), including Brown Beauty, some with Middle-American elements. Some lines with additional RR genes resistant or segregating

Although the majority of the genome of Redlands Pioneer is undoubtedly of Andean origin (Sandlin and Steadman, 1994), as is that of Kranskop, the RR gene itself appears to be of Middle-American origin. This observation explains the finding of Sandlin *et al.* (1999) that Redlands Pioneer, in common with small seeded cultivars (such as Mexico 235 and CNC), was resistant to Andean-specific isolates, but susceptible to non-specific isolates. This has important implications for the international bean community as Redlands Pioneer has been included in the new international differential set of cultivars as a source of Andean RR (Steadman, 2002). The implications are that this cultivar should be replaced by a true Andean source, or transferred to the Middle-American group.

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Some Applications of Co-Dominant SCAR Marker $SA14_{1079/800}$ for *Ur-4*

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The pyramiding of desirable genes in the common bean is becoming increasingly feasible with the advent of reliable molecular markers. However, some linked markers have limited application due to their frequent presence in either the Andean or Middle-American gene pools, depending on their origin. The RAPD marker $OPA14_{1100}$ (Miklas *et al.,* 1993) was, for instance, reported to occur in all Andean material tested, and RAPD marker $OA4_{1050}$ for *Ur-9* (Park *et al.,* 1999) occurred in the majority of the Andean accessions tested by Liebenberg (2003). This phenomenon restricts the combined use of markers from different gene pools, as well as the introduction of the gene into the gene pool containing the marker.

Although *Ur-4* is not of particular value in Africa, it is important in the New World as it gives protection against Beltsville race 108, which overcomes both *Ur-11* (as in PI 181996) and the RR genes present in CNC (J.R. Stavely, personal communication, November, 1999). *Ur-4* also gives protection against other races which overcomes CNC, and against Beltsville races 49, 50 and 51 from the USA and 65 from Puerto Rico, which overcome *Ur-5* (Stavely, 1984; Stavely *et al.*, 1989). In Puerto Rico, Beaver *et al.* (2002) reported that Early Gallatin (which contains *Ur-4*) has been resistant for the past 19 years.

Ur-4 is hypostatic to *Ur-5* and *Ur-11* for available southern African races. *Ur-5* and *Ur-11* are being introduced into South African breeding material, and in some cases, *Ur-4* may also have been transferred from BelMiDak-RR-9 or -RMR-11. The purpose of the present study was to determine the applicability of SCAR marker $SA14_{1079/800}$ (Mienie *et al*., 2004) in important *Phaseolus vulgaris* accessions.

Materials and Methods

Sixty eight *Phaseolus vulgaris*, two *P. coccineus* and one *P. acutifolius* (tepary) accessions were screened for the presence of the co-dominant SCAR marker $SA14_{1079/800}$. These included sources of already characterized rust resistance genes, among others *Ur-3*, *Ur-4*, *Ur-5* and *Ur-11* (summarized in Kelly *et al.* 1996), the standard differential cultivars (Stavely *et al.*, 1983; Stavely, 1984b; Steadman *et al.,* 2002b), as well as some relevant South African cultivars and breeding lines.

Results and Discussion

The 1079 bp "resistant" (+) allele was present in all material of Andean origin tested, regardless of the presence or absence of the gene, a finding consistent with that of Miklas *et al.* (1993) for the RAPD marker. These results confirm their opinion that this marker will be more useful as a marker in beans of Middle-American origin, where it appears to be generally absent, except where introduced together with *Ur-4*. The + allele was present in those Beltsville lines tested (BelDakMi-RMR-18, BelMiDak-RR-9 and -RMR-11 and BelMiNeb-RMR-7) which have been reported to have *Ur-4* (Stavely *et al*., 1994a and b; 1999a and b). It was in Early Gallatin (the source of *Ur-4* for the above) as was reported by Miklas *et al.* (1993) for the RAPD marker, and in Brown Beauty, a large seeded (Andean) cultivar reported to have *Ur-4* (Ballantyne, 1978; Stavely, 1986; Steadman, 1995).

The only small seeded (Middle-American) cultivar to have the + allele was Compuesto Negro Chimaltenango (CNC). This cultivar does not have *Ur-4*, as Beltsville races 78 (from the USA) and 85 (from Guatemala), for instance, attack CNC but not Early Gallatin or Brown Beauty (Stavely, 1992 and J.R. Stavely, personal communication, November, 1999). The presence of the + allele in CNC will prohibit its use for tracing *Ur-4* where CNC is involved

The 800bp "susceptible" (-) allele was present in the small seeded South African cultivars Teebus and Helderberg, both used as recurrent parents in the local backcross breeding programme. This will enable the tracing of the + allele in breeding lines derived from donor parents containing both *Ur-4* and the + allele. The latter was present in three small seeded breeding lines with BelMiDak-RR-9 as donor parent.

Indications have been found that KW 780 also carries *Ur-4* (Stavely, 1986; Steadman, 1995). However, the - allele was present in KW 780. This finding was confirmed using a separate DNA sample extracted from 20 seeds. Miklas *et al.* (1993) reported a tight linkage between the *Ur-4* gene and the RAPD marker $OA14_{1100}$. This was confirmed in the present study. However, the fact that the marker is present in Andean germplasm which lacks *Ur-4*, indicates that the linkage is not 100%. If KW 780 does contain *Ur-4*, the presence of the - allele in this cultivar indicates recombination between *Ur-4* and the marker in KW 780 (which is of Andean origin [Voysest *et al.,* 1994], with Middle-American elements [Sandlin and Steadman, 1994; Sandlin *et al*., 1999]). This cultivar, therefore, should be a good source of *Ur-4*, with the marker linked in repulsion, for large seeded (Andean) material (and CNC) lacking *Ur-4* but containing the $SA14_{1079/800}$ marker.

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CONVERSION OF THE RAPD MARKER FOR UR-4 TO A CO-DOMINANT SCAR MARKER $SA14_{1079/800}$

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Rust is an important disease of dry bean, and efforts are being made to improve the rust resistance (RR) of South African cultivars. Due to epistasis, it is sometimes difficult to detect the presence of multiple genes in breeding lines. Marker assisted selection makes this possible and the $OPA14_{1100}$ marker has been used for the detection of the dominant RR gene *Ur-4* in the presence of *Ur-11* (Kelly *et al.* 1993; Stavely *et al.* 1994a). *Ur-4*, which appears to be of Andean origin, as it occurs in all Andean material tested (Miklas *et al.*, 1993) was tagged by Miklas *et al.* (1993) with the RAPD marker $OA14_{1100}$ and has since been mapped to linkage group B6 (Miklas *et al*., 2002). No recombination was observed between the marker and the resistant allele in the mapping population. Although the marker was reported to be repeatable and easy to score (Miklas *et al*. 1993), it proved difficult to work with under local conditions. It was therefore decided to develop a SCAR marker from the RAPD fragment.

Materials and Methods

The fragment was isolated, cloned using a pGEM T-easy kit (Promega), transformed into bacterial cells (JM109) and sequenced by the central sequencing facility at Stellenbosch University, South Africa. The sequence of the 1127bp fragment is given in Figure 1. New primers (SA14-F: 5'-CTA TCT GCC ATT ATC AAC TCA AAC-3' and SA14-R: 5'-GTG CTG GGA AAC ATT ACC TAT T-3') were deducted from this sequence and the new reaction was optimized. PCR was conducted in a final volume of 20 µl containing approx. 100 ng genomic DNA, 30 ng each of forward and reverse primers, 2 mM $MgCl_2$, 200 µM each of dATP, dCTP, dGTP and dTTP, 1 x reaction buffer (10 mM Tris-HCl (pH 9), 50 mM KCl, 0.1% Triton® X-100) and 1 unit *Taq* polymerase (Promega, Madison, WI). Amplification was performed in a Hybaid thermal cycler or Hybaid Omnigene for one cycle at 94°C for 5 minutes; followed by 35 cycles of 94°C for 1 minute, 55°C for 1 minute and 72°C for 1.5 minutes. A final elongation step of 72°C for 5 minutes was included. Amplification products could be separated on 2% agarose (Seakem LE) for 1 hour at 80V.

Results

The SCAR marker acted co-dominantly, amplifying two different bands for the two alleles present at this locus (Figure 2), with a fragment of 1079 bp linked to the resistant allele (in BelMiDak-RR-9 and -RMR-11) and 800 bp linked to the susceptible allele (in Teebus and BelNeb-RR-1). The results were repeatable and easy to score. Our results confirmed the finding of Miklas *et al*. (1993) that the RAPD marker was only useful in a Middle-American genetic background. All cultivars of Andean origin were positive for the 1079 bp allele (resistance allele), except for KW780 (Liebenberg *et al.*, 2004). The SCAR marker mapped to exactly the same position as the RAPD marker (Miklas, personal communication, April 2002) on linkage group B6.

5'-**TCTGTGCTGG**TAGTTTTCCTTTAGAAGCTAAGATCTGGAATTTGGCTATCTGCCATTATCAACTCAAACGCAT
CATAAATTTTTTGAGTCAATTGTGTGTTTTGCTAAGAATTTTGGTATTCAATGTTGCAGACTAGAAATCTTCTTTT
GCTGGTGTTTTCTTCTCATTAATGGTGAATTTTTTTTATTATTGTTATTGTTATTTAGATATTTAACAACAATTACT
TTACTTTGTGTAGGTACTTGATAATCTTCCACATGATCTTATCTATGCAGAGAATCAAATTTCCTCATGGATGGAA
GTCTGGGTTGAGAAGCAGCATGACCAGTAAGTCTAAGCTTTATGTGTGGATGCTAAATATACTGGTACATATTCC
AGTGCCTCATTGGGAGTAGCTGTTAGAAAATCTGACAAATATCTCATGATATTTTTTTATATATCTTAGAATATTT
TAGAATATTCTAGATGATATTTTTTATTTTATGATTTTTGCTTTCTTATTTTTTATGGGGTTGAGTTAGGCTTAAAG
TCCACTTTGTAATATGGTATCAGAGCCCATTTCGAGTCTATCCTAGCGAGTATTTGTGTTGGGCCTATCGTGCCAC
CCGCTATCGGATCACCCATAATATATAGTCTCACGCACGAGTTGGCAGTCTCGGCGTGAGGGGGGTGTGTTGGAG
ATCCCACATCGACTAGAGATTAGAGTCTTTCATTGTATATAAGTGGGTGCAAATCTCAACTCTATGAGCCGGTTTT
ATGGGGTTGAGTTAGGCTTAAAGTCCACTTTGTAATATATTTAATCAAGATCTTTGTAATGATAGGTCTAATCATA
TCATATGAATAGGAATATTATTTCGTATATTTATTTGTATTTGTTTCATTAACTCTATATAAAACGCACTGAAATAT
TGTGTACTCAGTTTCAATATCTCTTGCATCTATTTTTCTCTTTAAAAACATCATATTAGAGCTGTACCAATTAGTCTC
CTCTTCCATAGTGTCTCTTCTTTTCAATTTGACTATCATTTTTCGGCTATGTCCTAATTTGGTTTACACTGGAAAAAT
CCATGGCAAATGTTGTTCACCAAGATTGACTTTCCCAATAGGTAATGTTTC**CCAGCACAGA**-3'

Figure 1 Sequences of the 1127 bp fragment of the RAPD product of OPA-14. The RAPD primers are indicated in bold type and the location of the SCAR primers is underlined.

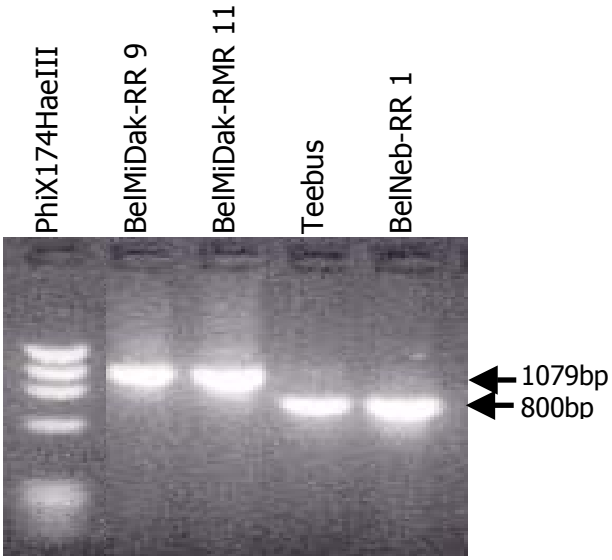

Figure 2 PCR amplification patterns of negative controls Teebus and BelNeb-RR-1 and positive controls BelMiDak-RR-9 and BelMiDak-RMR-11 with SCAR primers $SA14_{1079/800}$. PCR products were separated on a 2% agarose gel at 80 V for 2 hours and visualized with ethidium bromide.

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PUBESCENCE AND STOMATA TRAITS IN RELATION TO RUST RESISTANCE IN COMMON BEAN CULTIVARS

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Introduction

Rust caused by *Uromyces appendiculatus* var. *appendiculatus* causes economically important yield losses in dry bean producing areas of Mexico. Yield losses could reach devastating proportions according to germplasm susceptibility, environmental conditions and plant developmental stage of disease incidence and its severity. Studies in leaf morphological variations allow in the understanding of rust resistance. Rust resistance sources, other than the differentials, are constantly needed in common bean breeding programs (Araya *et al*., 1996). The objective was to asses the influence of stomata traits and foliar pubescence on rust resistance of common bean bred cultivars from different genetic and geographical origin.

Materials and Methods

In 2002, a field experiment was carried out at Chapingo, Edo. de México under a randomized complete block design with two replications. In Chapingo, State of Mexico (19° 31' N, 2240 masl) the soil type is a Mollisol and the climate is sub-humid tropical consisting of a summer rainfall regime (average annual rainfall of 692 mm) with small temperature oscillations, followed by a dry winter period and mean annual temperature of 16 °C (García, 1988). Experimental plots consisted of two 5 m rows, 0.76 m apart and a stand of 20 plants m^{-2}. Eight common bean cultivars from different genetic races were planted, two of them from Durango race exhibit indeterminate prostrate (Type III) growth habit: Pinto Bayacora and Chivá Busera; other two with similar growth habit from Jalisco race: Flor de Mayo Sol and Flor de Mayo RMC. Two indeterminate bush (Type II) cultivars from Mesoamerica race were also included: Negro Cotaxtla 91 and Negro INIFAP. Cultivars used as checks were Pinto UI114 from Durango race (rust susceptible) and Rayado Rojo from Nueva Granada race (rust resistant). Data on disease severity were recorded and scored using a 1 to 9 visual scale (CIAT, 1987).

After the plants were rated for disease reaction four leaf samples were collected in each replication. Two of them were cut into smaller pieces (0.5 cm^2) and immersed in the killing and fixing agent (FAA). Inocules were infiltrated in Paraplast® and then dehydrated, through a series of alcohol that involved a graded series of ethyl alcohol: Tertiary butyl alcohol (TBA), and the substitution of TBA by liquid paraffin. To finish the infiltration, a new liquid Paraplast volume was used. Liquid paraffin was then substituted with paraffin wax (in oven) and after a suitable infiltration period, the infiltrated specimens were poured into moulds, which, when set, were affixed to microtome chucks. Leaf tissues were sectioned into very thin slices (13 µm) with a microtome and then de-waxed, carefully rehydrated, stained with fast green and safranin, mounted and covered with a cover glass. The other two leaves were sun-dried and prepared to its observation in a stereoscope. In the electron microscope three random readings for stomata and trichomes number were registered in each leaf side. Each reading consisted in a 395 x 292.5 µm area. In the stereoscope average pustule diameter and density were recorded.

Results and Discussion

Field readings for rust reaction varied from 1 to 8 and cultivars Pinto UI114, Chivá Busera and Flor de Mayo RMC showed rust susceptibility (values close to 8) while the others exhibited rust resistance (values ≤3). Significant and negative correlation was observed between rust reaction and yield (r=-0.76**) and days to maturity (r=-0.78**). Results suggest that increments in rust severity could reduce drastically grain yields. Higher frequency for rust susceptible cultivars was observed among early

cultivars from Durango race. In contrast, late-maturing cultivars from Nueva Granada, Jalisco and Mesoamerica races showed rust resistance. Susceptible cultivars showed higher pustule size and density in comparison to resistant cultivars. An exception was Negro INIFAP, classified as rust resistant according to field readings, which showed high pustule density in both lamina sides. Pustules in Negro INIFAP showed lower size and a necrotic halo related with hypersensitivity response. Disease severity, scored using a visual scale, is a relatively effective method for cultivar classification therefore a positive correlation was detected between field readings and adaxial pustule diameter (r= 0.79**) and density (r=0.55*). Significant differences (P<0.05) were found among cultivars for stomata and trichome number. Significant and positive correlation was observed between stomata number and pustule density in adaxial (r=-0.63*) and abaxial (r=-0.66*) lamina surfaces. Rust susceptible cultivars showed the highest stomata number but similar values were also observed among resistant cultivars. Negro INIFAP stomata number and morphology could favor rust infection, however no disease development was observed probably due to biochemical changes in infected area (Wanjiru *et al*., 2003). Differences in trichome shape and length were observed. Short and curved trichomes were mainly observed in susceptible cultivars. According to Zaiter *et al*., (1990) long and straight trichomes were predominant in both leaf surfaces in resistant cultivars as Rayado Rojo. Positive and significant correlation was observed between trichome and pustule number in adaxial (r= 0.53*) and abaxial (r= 0.55*) leaf surfaces. Short and curved trichomes retained uredospores near foliar surface and stomata (Figure 1), so this trait could be related with greater rust infection probability. Stomata and trichome interacted with environmental factors (light, temperature, moisture, etc.), which modulated rust reaction. Relative importance of different foliar traits on rust race nonspecific resistance was achieved. Genetic variability was observed for traits related with rust resistance and resistant cultivars were identified, which could be used as gene source for common bean breeding.

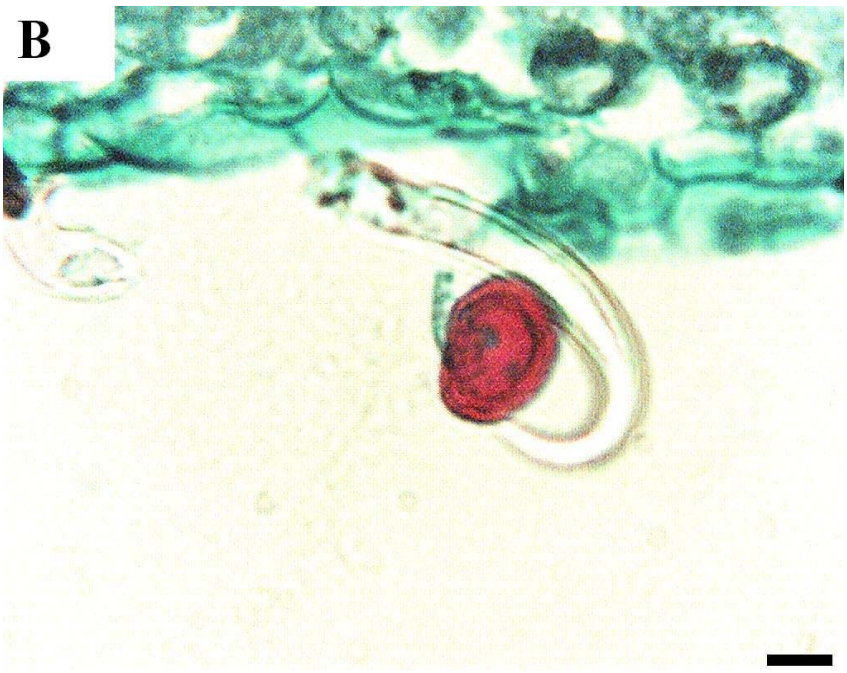

Figure 1. Short and curved trichomes retained uredospores near foliar surface and stomata, in cultivars Pinto UI114, susceptible drought genotype.

Confirmation of the *Ur-6* Location in *Phaseolus vulgaris* L.

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McClean et al. (1994) initially identified RAPD marker OV12.950 linked to the *Ur-6* gene for resistance to race 47 at 10.4 cM in a pinto bean cross Sierra x Olathe. Park et al. (2003a) subsequently reported eleven RAPD markers linked to the *Ur-6* gene for specific rust resistance using bulked segregant analysis in an F_2 population from the Middle American (MA) common bean cross Olathe (resistant) x Nebr. #1 sel. 27 (susceptible). Of these markers, six that displayed an amplified DNA fragment in the resistant DNA bulk were detected in a coupling phase linkage with the *Ur-6* gene, while five that displayed an amplified DNA fragment in the susceptible DNA bulk were identified in a repulsion phase linkage with the gene. Two coupling-phase markers OBC06.300 and OAG15.300 were detected as flanking markers for the *Ur-6* gene at 1.3 cM and 2.0 cM, respectively. Repulsion-phase marker OAY15.200 was the most closely linked to the *Ur-6* gene among the five markers at 7.7 cM. Park et al. (2003b) also reported that SCAR marker SOBC06.308 was developed based on the specific forward and reverse primer pair designed from the sequence of the RAPD marker OBC06.300.

The objective of this study was to confirm the *Ur-6* location on a RAPD marker-based linkage map previously constructed from a recombinant inbred line (RIL) population of the MA cross BelNeb-RR-1 x A 55.

Of the five repulsion-phase RAPD markers linked to the *Ur-6* gene identified in the F_2 population from the cross of Olathe x Nebr. #1 sel. 27, two markers OAB18.650 and G5.500 were also polymorphic between the parents BelNeb-RR-1 and A 55 that were utilized to create the RIL population from which the RAPD marker-based linkage map was constructed (Ariyarathne et al., 1999). These two repulsion-phase markers OAB18.650 and G5.500, very loosely linked to the Andean gene in the F_2 population, displayed an amplified DNA fragment in the A 55 parent susceptible to rust, whereas they were absent in the BelNeb-RR-1 parent resistant to rust. The two markers also segregated in the RIL population of the cross BelNeb-RR-1 x A 55. A goodness-of-fit to a 1:1 ratio for band presence to band absence for each of the markers was observed in the RIL population. The two markers OAB18.650 and G5.500 were observed to be located within a distance of 4 cM on linkage group 11 of the RAPD marker-based linkage map constructed using the RIL population from the cross BelNeb-RR-1 x A 55 (Figure 1), suggesting that the *Ur-6* gene was positioned on linkage group 11 of the map. However, seven coupling-phase RAPD and SCAR markers linked to the *Ur-6* gene found in the F_2 population were not noted on the RAPD map.

The *Ur-6* location found here confirms the finding of Miklas et al. (2002), who reported that the Andean gene was mapped on linkage group 11 of the core bean linkage map, based on the result of McClean et al. (1994). Important rust resistance genes of MA origin such as *Ur-3*, *Ur-7*, and *Ur-11* are located on linkage group 11 of the core bean map, while other rust genes *Ur-9* of Andean origin, *Ur-5* of MA origin, *Ur-4* of Andean origin, and *Ur-12* are located on LGs 1, 4, 6 and 7, respectively (Miklas et al., 2002; Park et al., 2003c). However, the *Ur-6* gene is located in a different region on linkage group 11 with respect to the other important rust resistance genes, based on the results of Miklas et al. (2002) and Park et. al. (2003c). Thus, a total of five rust resistance genes including four of MA origin and one of Andean origin have

been mapped on linkage group 11 of the core bean map.

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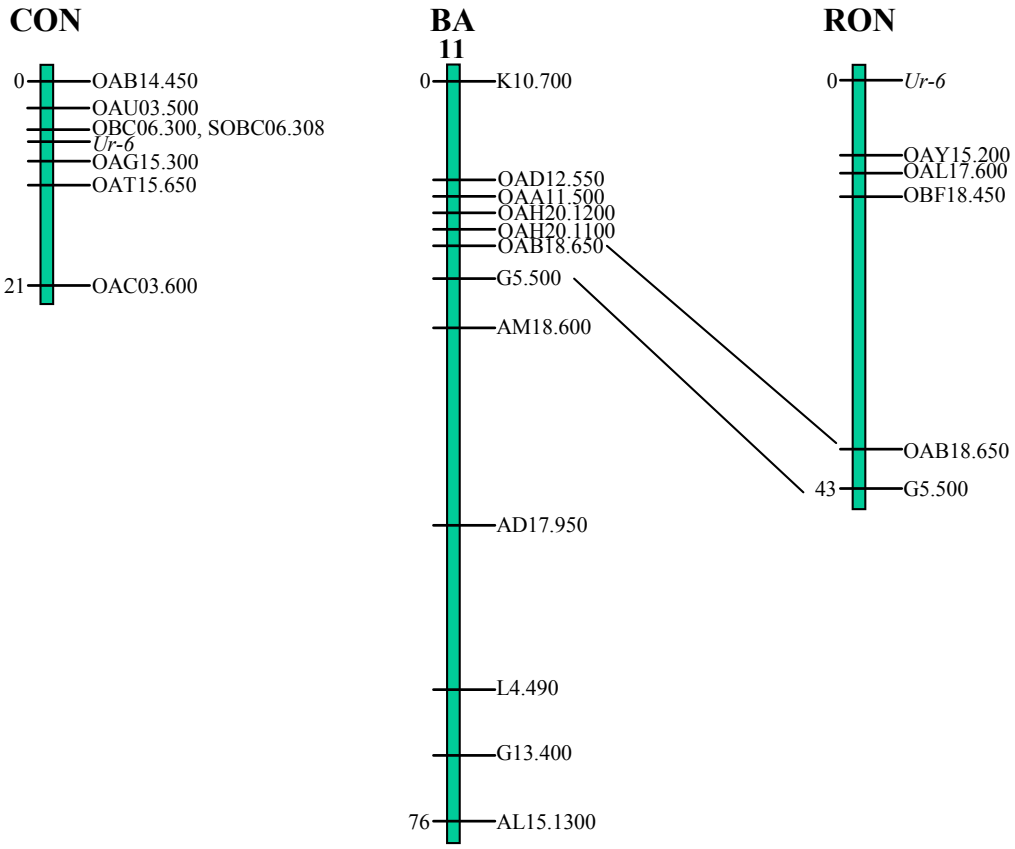

Figure 1. Linkage group 11 including the *Ur-6* gene controlling specific rust resistance and seven coupling-phase RAPD and SCAR markers developed using an F_2 population of the common bean cross Olathe x Nebr. #1 sel. 27 (CON), the *Ur-6* gene and five repulsion-phase RAPD markers developed using the F_2 population (RON), and RAPD markers developed using a RIL population of the cross BelNeb-RR-1 x A 55 (BA). The gene and marker names are given on the right and the length in cM is indicated on the left of linkage group 11. Markers OAB18.650 and G5.500 in linkage group 11 are connected between the F_2 and RIL populations by lines.

Survey of RAPD and SCAR Markers Linked to the *Ur-6* Gene in Pinto Beans

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Park et al. (2003a) reported eleven RAPD markers linked to the *Ur-6* gene for specific rust resistance using bulked segregant analysis in an F_2 population from the Middle American (MA) common bean cross Olathe (resistant) x GN Nebr. #1 sel. 27 (susceptible). Of these markers, six that displayed an amplified DNA fragment in the resistant DNA bulk were detected in a coupling phase linkage with the *Ur-6* gene, while five that displayed an amplified DNA fragment in the susceptible DNA bulk were identified in a repulsion phase linkage with the gene. Two coupling-phase RAPD markers OBC06.300 and OAG15.300 were detected as flanking markers that were tightly linked to the *Ur-6* gene at distances of 1.3 cM and 2.0 cM, respectively. Repulsion-phase RAPD marker OAY15.200 was the most closely linked to the *Ur-6* gene among the five markers at a distance of 7.7 cM.

Coupling-phase SCAR marker SOBC06.308 was developed based on the specific forward (GAAGGCGAGAAGAAAAAGAAAAAT) and reverse (GAAGGCGAGAGCACCTAGCTGAAG) primer pair designed from the sequence of the most tightly linked RAPD marker OBC06.300 to the *Ur-6* gene (Park et al., 2003b). The SCAR marker SOBC06.308 showed no recombination with the RAPD marker OBC06.300 in the F_2 population from the MA cross of Olathe x Nebr. #1 sel. 27, and thus, the SCAR and RAPD markers were observed at the same locus on the linkage group. The SCAR marker SOBC06.308 was also closely linked to the *Ur-6* gene at a distance of 1.3 cM.

Our goal was to determine the presence or absence of the SCAR marker SOBC06.308 as well as the coupling-phase RAPD markers OBC06.300 and OAG15.300 in 33 MA pinto bean cultivars and breeding lines with or without the *Ur-6* gene.

In combination with *Ur-3* or *Ur-11* of MA origin, the *Ur-6* gene was widely utilized in developing rust resistant pinto bean breeding lines including two BelDak (Stavely and Grafton, 1989) and six BelDakMi breeding lines (Stavely et al., 1992). All BelDak and BellDakMi bean lines were observed to be resistant to rust race 51. The presence of the two RAPD and one SCAR markers was associated with BelDak-RR-1 and 2 possessing the *Ur-6* gene (Table 1). Also, the presence of these three markers was associated with BelDakMi-RR-1, 2, 5, 10, 14 and 18 having the *Ur-6* gene, while the absence of the markers was associated with BelDakMi-RR-4 lacking the gene.

Along with either *Ur-5* or *Ur-11*, the Andean gene is currently being used in developing rust resistant pinto bean breeding lines in Colorado bean breeding programs. The presence of the markers was consistently associated with all ten Colorado lines mostly resistant to race 51 (Table 1). The gene was incorporated into several elite pinto bean cultivars such as Apache, Bill Z, Burke, Topaz, and Kodiak. The RAPD and SCAR markers were present in the five bean cultivars resistant to race 51. Other susceptible pinto cultivars or lines without the gene except UI-111 lacked the marker fragments. These results would be expected due to the tight linkage of the markers with the gene. Thus, the RAPD and SCAR markers could be useful in breeding and selecting the Andean gene for rust resistance in MA pinto bean germplasm.

Table 1. Presence (+) or absence (!) of two coupling-phase RAPD markers OBC06.300 and OAG15.300, and the SCAR marker SOBC06.308 tightly linked to the *Ur-6* gene in 33 Middle American pinto bean cultivars/breeding lines resistant (R) or susceptible (S) to rust race 51.

Pinto bean	Resistance	Rust	Coupling-phase markers		
cultivar/breeding line	gene (*Ur*)	race 51	OBC06.300	OAG15.300	SOBC06.308
Olathe	6	R	+	+	+
Apache	3 & 6	R	+	+	+
Bill Z	6	R	+	+	+
Burke	3 & 6	R	+	+	+
Kodiak	3 & 6	R	+	+	+
Topaz	6	R	+	+	+
Chase	3	S	-	-	-
Fiesta		S	-	-	-
Othello		S	-	-	-
P114		S	-	-	-
P650		S	-	-	-
UI-111		S	+	+	+
US-5	7	R	-	-	-
US-14	7	R	-	-	-
BelDak-RR-1	3 & 6	R	+	+	+
BelDak-RR-2	3 & 6	R	+	+	+
BelDakMi-RR-1	6 & 11	R	+	+	+
BelDakMi-RR-2	6 & 11	R	+	+	+
BelDakMi-RR-4	11	R	-	-	-
BelDakMi-RR-5	6 & 11	R	+	+	+
BelDakMi-RMR-10	6 & 11	R	+	+	+
BelDakMi-RMR-14	3, 6 & 11	R	+	+	+
BelDakMi-RMR-18	3, 4, 6 & 11	R	+	+	+
CO 12512	6 & 11	R	+	+	+
CO 12515	6 & 11	R	+	+	+
CO 12518	6 & 11	R	+	+	+
CO 12555	6 & 11	R	+	+	+
CO 12650	5 & 6	R	+	+	+
CO 12652	5 & 6	R	+	+	+
CO 12653	5 & 6	R	+	+	+
CO 12655	5 & 6	R	+	+	+
CO 12663	5 & 6	R	+	+	+
CO 12783	6 & 11	S	+	+	+

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Development of a SCAR Marker Linked to the *Ur-7* Gene in Common Bean

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Park et al. (2003) reported nine RAPD markers linked to the *Ur-7* gene for specific resistance to rust using bulked segregant analysis in an F_2 population from the Middle American common bean cross GN1140 (resistant) x Nebr. #1 (susceptible). Six markers were detected in a coupling phase linkage with the *Ur-7* gene, whereas three markers were identified in a repulsion phase linkage with the gene. Coupling-phase markers OAD12.550 and OAF17.900 were found with no recombination to the *Ur-7* gene. Repulsion-phase marker OAB18.650 was the most closely linked to the *Ur-7* gene among the three markers at 7.6 cM. All linked markers detected in the F_2 population also segregated in recombinant inbred lines from the cross BelNeb-RR-1 x A 55, and were located on linkage group 11 of the existing linkage map (Park et al., 2003).

Merits of SCAR markers over RAPDs have been reported and well discussed (Paran and Michelmore, 1993; Melotto et al., 1996). Therefore, our goal was to convert the most tightly linked RAPD marker OAD12.550 to the *Ur-7* gene into a SCAR marker on the basis of a specific forward and reverse 20-mer primer pair.

Converting RAPD to SCAR Markers: To develop a SCAR marker for the RAPD marker OAD12.550, the DNA fragment of the RAPD marker was excised and purified using the GENECLEAN II Kit (Q-BIO gene, Carlsbad, California). Insertion of the purified RAPD fragment into the pCR 2.1- TOPO and cloning of the transformed plasmid were conducted using the TOPO TA Cloning Kit (Invitrogen, Carlsbad, California). The cloned plasmid was harvested using the GenElute Plasmid Miniprep Kit (Sigma, St. Louis, Missouri). The RAPD fragment was sequenced using the M13 reverse and forward primers at the DNA sequencing & synthesis facility of the Iowa State University Office of Biotechnology (Ames, Iowa). A specific forward and reverse 20-mer primer pair was designed on the basis of the forward and reverse sequences of the RAPD fragment. The forward and reverse primer pair was synthesized at the Operon Technologies (Alameda, California). PCR was performed on 96-well plates in a MJ Research thermalcycler. The composition of the final volume of reactants was similar to those described by Rubio et al. (2001).

Developing a SCAR Marker: The coupling-phase RAPD marker OAD12.550 tightly linked to the *Ur-7* gene identified in the F_2 population from the cross GN1140 x Nebr. #1 was converted into a SCAR marker on the basis of the specific forward and reverse 20-mer primer pair. The exact length of the RAPD fragment OAD12.550 was 537 bp based on the sequence data (Figure 1). The sequence of the forward 20-mer primer was 5'-AAGAGGGCGTGAGATCGTCG-3', while that of the reverse 20-mer primer was 5'-AAGAGGGCGTCTTGAAGGTT-3'. The underlined sequences were the original 10-mer sequence of the OAD12 primer. Melting temperatures of the forward and reverse primers were 65EC and 61EC, respectively. We used 71EC as an annealing temperature.

The marker SOAD12.537, the name of the SCAR marker amplified with the specific primer pair, is shown in Figure 2. The marker SOAD12.537 was present in the resistant parent GN1140 and the DNA bulk from resistant F_2 plants, whereas it was absent in the susceptible parent Nebr.#1 and the DNA bulk from susceptible F_2 plants. The SCAR marker fragment segregated in the F_2 population of the cross GN1140 x Nebr. #1. A goodness-of-fit to a 3:1 ratio

for band presence to band absence for the marker SOAD12.537 was observed. The marker SOAD12.537 showed no recombination with the RAPD marker OAD12.550 in the F_2 population, and thus, the SCAR and RAPD markers were observed at the same locus on the linkage group. The marker SOAD12.537 also showed no recombination with the *Ur-7* gene. The results confirm that the SCAR marker SOAD12.537 was derived from the RAPD marker OAD12.550. This is the first report on development of a SCAR marker linked the *Ur-7* gene in common bean.

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5'**AAGAGGGCGTGAGATCGTCG**TTGGAGTCAACCGGCAAAAACAGTGTTAGAATCC
AAGTGACAATGAGAGCCAACAACCTTTCATTCAACTTCAACCCTTCAACAGAACAA
GTTCTTACTTTGTGCTTGAGGATTCTTCAAACAACTTTTATAATATTGAATCTTATTA
AAATCTTCCACCACACCAATATTCCCTTAGTTGATTCTCAATCCAACATACTTTAAG
CCAGTTACGGCAGCCCATACCTCATGAGTAATCTCAATATCCACACCCTTGACATG
AGAAACAAGATAACCTTCAAACTTTAAATTGGTGCAGAAAACCCAAATAAGATCCAG
ATAGATGTTCCCTTTCATCTCTAAGAACCTCTATAGCAGTTGTTCTTTGAGTAGATTT
CTCACATCATCCCGCTTTTGACTCTTCACCCAGCTAAAAGAGACAACTTTTGGATTG
TTTATCACTTTTCTACTTGTTTCAAAGCGATATTTCTCAATCAATTCATTGTCTCCTG
AAAACC**AACCTTCAAGACGCCCTCTT**3'

Figure 1. The full DNA sequence (537 bp) of the most tightly linked RAPD marker OAD12.550 to the *Ur-7* gene. The two underlined sequences above were the 20-mer sequences of the specific forward and reverse primers, respectively, used to develop the SCAR marker.

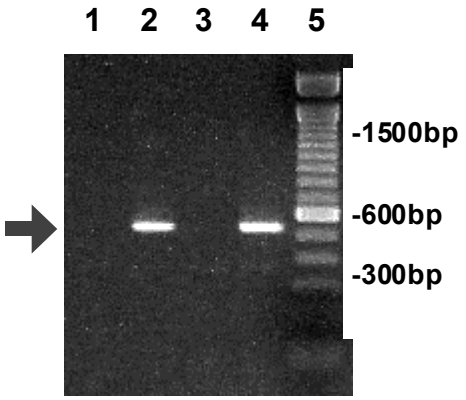

Figure 2. Coupling-phase SCAR marker SOAD12.537, amplified with the specific primer pair designed from the sequence of the RAPD marker OAD12.550, expressing polymorphism between two DNA bulks from susceptible and resistant F_2 plants, and between the susceptible parent Nebr.#1 and the resistant parent GN1140. 1=Nebr.#1, 2=GN1140, 3=DNA bulk from susceptible F_2 plants, 4=DNA bulk from resistant F_2 plants, and 5=molecular size marker.

SCAR MARKER LINKED TO THE COMMON BEAN RUST RESISTANCE GENE *Ur-11*

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Common bean (*Phaseolus vulgaris* L.) rust, caused by the fungus *Uromyces appendiculatus*, may lead to serious losses to the culture mainly in regions with mild temperatures and high humidity. The use of resistant cultivars is an alternative strategy to control the disease. In the gene pyramiding bean breeding program of BIOAGRO/UFV it was observed that the *Ur-11* gene present in cultivar Belmidak RR-3 is an important rust resistance source to be used in Brazil. This program presently uses the RAPD marker $OAE19_{890}$ linked in repulsion phase to *Ur-11* (Johnson et al., 1995) to assist the introgression of this gene into "carioca-type" cultivars. This marker was validated by Oliveira et al. (2002) in the population derived from the cross between Rudá (susceptible) and Belmidak RR-3 (resistant). In the present work the RAPD marker was converted into a SCAR in order to make its amplification more reproducible and accurate.

DNA from cultivar Rudá was amplified with RAPD primer OPAE19, and the products were fractionated in an agarose gel. The band of interest (890 bp) was excised from the gel and inserted into the pGEM-T Easy vector (Promega, Madison, WI). After sequencing the fragment, SCAR primers were designed and synthesized. The primer sequences were:

5'-CAGTCCCTGACAACATAACACC-3' (sAE19F) and

5'-CAGTCCCTAAAGTAGTTTGTCCCTA-3' (sAE19R), and the marker was designated $sAE19_{890}$. The primers were then tested in six resistant and six susceptible F_2 plants (Rudá x Belmidak RR-3). The PCR reactions (25 μL) contained 30 ng of genomic DNA, 0.2 μM of each SCAR primer, 10 mM/50mM Tris/KCl (pH 8.0), 2 mM $MgCl_2$, 0.48 mM of total dNTP, and 1 U of Taq DNA polymerase. The amplification program included a initial step of 5 min at 94 °C, 35 cycles (94 °C/15 s, 58 °C/1 min, 72 °C/1 min 30 s) and one final step at 72 °C for 7 min. Only the susceptible plants and the progenitor Rudá harbored the marker band (Figure 1).

To determine the genetic distance between the marker and the resistance gene, the reactions of 53 F_2 plants (Rudá x Belmidak RR-3) to *U. appendiculatus* pathotype 10 (Faleiro et al., 1999) were determined and they were also tested with RAPD marker $OAE19_{890}$ and SCAR $sAE19_{890}$. The plants were scored visually for the disease symptoms using a 1 to 6 scale (Stavely et al., 1983). The genetic distances were determined with the aid of MAPMAKER (Lander et al., 1987) using a LOD score of 3.0. The segregation analyses showed that $OAE19_{890}$ and $sAE19_{890}$ were located at 1.0 cM of the resistance gene *Ur-11* (Table 1). To confirm the results obtained with the F_2 population the corresponding $F_{2:3}$ families were also evaluated for resistance/susceptibility to *U. appendiculatus*. This analysis allowed us to determine the specific

genotypes of each F_2 plant. The plants harboring the marker could divided into susceptible (rr) and resistant (Rr), and the plants with no marker were resistant (RR).

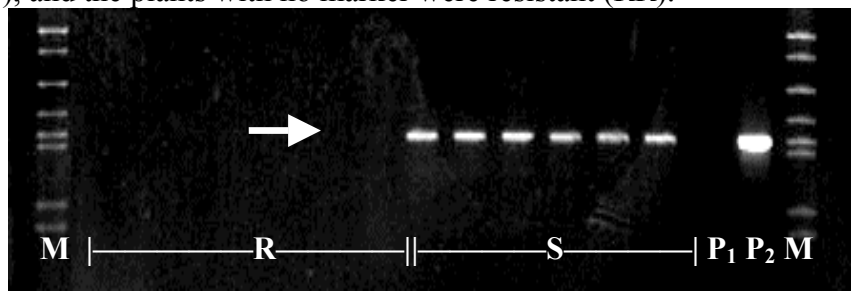

Figure 1 - Electrophoretic analyses of DNA amplification products produced with SCAR $sAE19_{890}$. Lanes are as follows: P_1, Belmidak RR-3; P_2, Rudá; R, F2 resistant plants; S, F2 susceptible plants. M refers to lambda phage DNA digested with *Eco*RI, *Bam*HI and *Hin*dIII (size markers). The arrow indicates the SCAR marker.

Table 1. Segregation for resistance and linkage analysis between molecular markers $OAE19_{890}$ and $sAE19_{890}$, and the rust resistance gene *Ur-11* in an F_2 population derived from a cross between cultivars Rudá and Belmidak RR-3.

Locus tested	Generation	Expected ratio	Observed ratio	χ^2	Probability (%)	cM[a]
Ur-11	$F_{2:3}$	1:2:1	12RR:28Rr:13rr	0.578	74.87	
$OAE19_{890}$	F_2	3:1	13(-):40(+)	0.188	99.06	1.0
$sAE19_{890}$	F_2	3:1	13(-):40(+)	0.188	99.06	1.0

[a]**Genetic distance in centiMorgan**

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Halo Blight Disease in Bean Landraces and Genotypes From a Farmers' Mixture

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INTRODUCTION

Halo blight disease of beans caused by *Pseudomonas syringae* pv. *phaseolicola* is among the important factors limiting the production of beans in Tanzania. Availability of acceptable bean cultivars with resistance to halo blight pathogen is one of the approaches to management of the disease. This study examines the potential of landraces and components from bean mixtures to management of halo blight.

MATERIALS AND METHODS

This study was conducted at the Agricultural Research Institute (ARI) Uyole research fields during the 2000 – 2002 growing seasons. Nine components separated from a local mixture from Iringa and three landraces, one (Sam-1) from Njombe and two (Kablanketi and Kigoma) from Mbeya were used. The bean genotypes were evaluated in single rows each 3 meters long replicated 3 times. Beans were planted at a between and within row spacing of 75cm and 10cm respectively. Disease infection was natural. Halo blight disease severity was scored at 21, 28, 35, 42 and 48 days after planting. A 1 – 9 scale was used, where 1.0 indicate no to very little disease symptoms and 9.0 = severe symptoms to death of plant

RESULTS AND DISCUSSION

Mean halo blight disease severity scores recorded at 21, 35 and 48 days after planting are presented in Table 1. The 8 bean components from a local mixture collected from Iringa (Iramix series) had an intermediate to low susceptibility to halo blight. The results conform to the findings of Gondwe (1998) who found resistance to race 7b of *P.s. phaseolicola* in Nyamuhanga – a landrace from Iringa. Beebe and Pastor-Corrales (1991), suggested that genetic diversity such as exists in landraces is the surest strategy for stable resistance to certain diseases and is a traditional disease control mechanism that should not be abandoned.

Halo blight disease was comparably more severe in year 2000. In 2001 halo blight disease on the tested genotypes was negligible except for Kigoma. The results of Gondwe (1998) showed that Kigoma was susceptible to all the *P.s. phaseolicola* strains occurring in the southern highlands of Tanzania. In 2002 halo blight disease severities were higher than those recorded in 2001 but slightly lower than those recorded in 2000. This trend requires further investigations. All the bean genotypes showed a declining trend in disease severity in successive evaluations. The observation is of practical importance because in informal seed supply systems farmers are normally advised to use seed from health plants. Bean plants with lost symptoms of halo blight may be mistaken for health plants. Therefore, farmers need to understand the disease symptoms and progress at different stages of growth of the bean plants. The knowledge may minimize the risk of perpetuating the disease through the use of seed from symptom less plants.

Table 1. Halo blight disease scores for twelve bean genotypes evaluated under field conditions at ARI Uyole.

		Mean halo blight disease severity scores (1 – 9)								
Bean genotype	Origin	Year 2000			Year 2001			Year 2002		
		28DAP	35DAP	48DAP	28DAP	35DAP	48DAP	28DAP	35DAP	48DAP
Iramix Ib	Iringa	2.33	4.33	1.67	1.00	1.00	1.00	1.67	2.00	2.00
Iramix 2	Iringa	4.33	4.33	1.67	1.00	1.00	1.00	1.67	2.33	1.00
Iramix 3	Iringa	3.00	2.33	1.00	1.00	1.00	1.00	2.33	3.67	1.00
Iramix 4a	Iringa	3.00	3.00	1.00	1.00	1.00	1.00	2.33	3.00	1.00
Iramix 5	Iringa	2.33	3.67	1.00	1.00	1.00	1.00	1.67	2.33	1.00
Iramix 6	Iringa	4.33	5.00	1.0	1.00	1.00	1.00	2.33	2.00	1.00
Iramix 9b	Iringa	2.33	3.00	1.00	1.00	1.00	1.00	1.67	1.00	1.00
Iramix12	Iringa	4.33	5.00	1.00	1.00	1.00	1.00	4.33	2.00	1.00
Sam-1	Njombe	5.00	6.33	2.33	1.00	1.00	1.00	3.33	6.33	1.00
Kigoma	Mbeya	6.00	8.67	7.00	1.00	2.00	6.00	4.67	6.67	1.67
Nyamuhanga	Iringa	6.33	8.67	5.00	1.00	1.00	1.00	4.67	6.33	4.67
Kablanketi	Mbeya	5.67	8.33	5.67	1.00	1.00	1.00	3.00	3.67	1.00

Iringa, Njombe and Mbeya are districts in the southern highlands of Tanzania.
DAP = Days after Planting

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Methods For Determining Resistance of Edible Beans To Fusarium Root Rot

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Introduction: The fungus *Fusarium solani* f. sp. *phaseoli* causes Fusarium root rot of edible beans (*Phaseolus vulgaris*), a disease characterized by symptoms such as visible rotting of the stem and roots, and reduction of stand, shoot weight, and root weight. The purpose of this study was to develop quick, easy, and reliable methods for showing resistance of dry bean genotypes to Fusarium root rot.

Methods and Materials: Eight trials encompassing 11 colored dry bean entries and 9 white dry bean entries were conducted between October 2002 and September 2003. In the first four trials (trials 6, 11, 16, 17), seeds were sown in potting mix (PBX for trials 6, 11 and 16, PBXG for trial 17, Plant Products, Ontario) in 3.5-inch pots (trials 6, 11, 16) or 12-inch pots (trial 17), 4 seeds per pot, 4 replicate pots. To expose seeds and seedlings to *F. solani* f. sp. *phaseoli*, one plug removed from a culture of the fungus was placed on each seed at the time of seeding. Checks were not inoculated with the fungus. Plants were grown in a greenhouse and plant performance was measured 2 weeks (trial 16), 4 weeks (trials 6, 11), or 6 weeks (trial 17) after inoculation. In trials 18, 19, 21 and 22, seeds were sown in extra fine vermiculite in 4-inch pots (trials 18, 19) or 6-inch pots (trials 21, 22), 1 seed per pot. Nine days after seeding, 10 emerged plants per entry were inoculated by pouring 10 ml of a suspension of conidia of the fungus (10^6 conidia/ml) around the stem. Ten check plants remained uninoculated. Each plant was a replicate. Plant performance was measured 4 weeks (trials 18, 19), 6 (trial 21), or 8 weeks (trial 22) after seeding. Primary measures of plant performance were stand (plants per replicate), shoot fresh weight, shoot dry weight, root dry weight, and disease severity rated on a scale where 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75% and 4 = 76-100% necrotic discoloration of the hypocotyl surface. All trials were arranged as randomized complete blocks. Because of limitations in greenhouse space and labor, the number of entries per trial ranged from 5 to 20 and the number of performance attributes measured per trial ranged from 3 to 5. The attributes used to measure disease resistance were disease severity (DIS), stand (STD), fresh shoot weight (FSW), dry shoot weight (DSW) and dry root weight (DRW). STD, FSW, DSW, and DRW of inoculated plants were then expressed as a percentage of values for uninoculated plants, and labeled STD%, FSW%, DSW%, and DRW%, respectively. Pearson correlation coefficients were calculated between all available performance attributes within each trial.

Results: Data for individual trials are presented in Table 1. The highest correlations (0.95 to 0.99) were between per cent fresh shoot weight (FSW%) and per cent dry shoot weight (DSW%). Per cent dry root weight (DRW%) was consistently highly correlated with per cent fresh shoot weight (0.80 to 0.99) and per cent dry shoot weight (0.83 to 0.99). Disease severity (DIS) was moderately to highly correlated (-0.60 to -0.94) with per cent stand (STD%), and weakly to highly correlated with per cent fresh shoot weight (0.0 to 0.93) , per cent dry shoot weight (0.0 to 0.90) and per cent dry root weight (0.0 to 0.78). Across trials 16, 17, 18, 19, 21, and 22 (68 data pairs), the correlation between per cent fresh shoot weight and per cent dry root weight was 0.93.

Table 1. Correlation coefficients relating performance attributes of edible beans inoculated with *Fusarium solani* f. sp. *phaseoli*.

Comparison	Trial							
	6	11	16	17	18	19	21	22
STD% x FSW%	0.76	0.88	0.46	0.32				
STD% x DSW%	0.72	0.81	0.53					
STD% x DRW%			0.35	0.37				
STD% x DIS	-0.85	-0.94	-0.60	-0.71				
FSW% x DSW%	0.99	0.97	0.98				0.95	0.98
FSW% x DRW%			0.84	0.80	0.92	0.83	0.97	0.99
FSW% x DIS	-0.93	-0.86	-0.40	-0.44	-0.41	-0.41	-0.63	0.0*
DSW% x DRW%			0.83				0.87	0.99
DSW% x DIS	-0.90	-0.80	-0.41				-0.51	0.0*
DRW% x DIS			-0.17	-0.30	-0.63	-0.62	-0.78	0.0*

* All disease ratings 4

Conclusions: The existence of weak to strong correlations between attributes of plant performance in the presence of *F. solani* f. sp. *phaseoli* could guide the selection of disease resistant bean genotypes. Criteria for choosing attributes could include relationship to yield reduction, ease and objectivity of measurement, and cost of obtaining the measurement. Visual assessment of disease severity is subjective and was not consistently highly correlated with the other attributes measured. Use of stand reduction was compromised by variation among and within seed lots in emergence from inoculated or uninoculated seed. Measurements of shoot and root weights are objective. Reduction of root weight could be considered a primary effect of a root-invading pathogen, and maintenance of root weight in the presence of the pathogen a basic measure of resistance of herbaceous plants to root rot. However, reliable determination of root weight depends on complete removal of the root substrate, which requires more time than determination of shoot weight and may not be possible where the substrate clings tightly to the roots. On the other hand, fresh shoot weight can be determined quickly, easily and objectively. Further, percentage impact of the fungus on fresh or dry shoot weight is highly correlated with the percentage impact of the fungus on root dry weight. We therefore suggest that resistance of beans to Fusarium root rot can be easily, objectively and quantitatively expressed as the percentage reduction in fresh shoot weight of plants inoculated after emergence compared to uninoculated plants.

Dynamics of Resistance of Edible Beans to Fusarium Root Rot

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Introduction: Fresh shoot weight and dry root weight were reported to be suitable measures of resistance of edible beans (*Phaseolus vulgaris*) to Fusarium root rot, caused by *Fusarium solani* f. sp. *phaseoli* (Hall and Phillips 2004). The purpose of this study was to determine whether the duration of exposure of edible beans to *F. solani* f. sp. *phaseoli* affects the assessment of resistance to Fusarium root rot.

Methods and Materials: Resistance of cultivars Taylor (cranberry bean), California White (white kidney bean), and Vista (navy bean) to Fusarium root rot was determined from measurements of fresh shoot weight and dry root weight of 4-week-old (trial 18), 6-week-old (trial 21), and 8-week-old (trial 22) plants 19 days, 33 days, and 47 days after inoculation, respectively. Seeds were sown in extra fine vermiculite in 4-inch pots (trials 18) or 6-inch pots (trials 21, 22) at the rate of one seed per pot. Nine days after seeding, 10 emerged plants per entry were inoculated by pouring 10 ml of a suspension of conidia of *F. solani* f. sp. *phaseoli* (10^6 conidia/ml) around the stem. Ten check plants remained uninoculated. Each plant was a replicate. All trials were arranged as randomized complete blocks. Impact of inoculation was expressed as shoot weight of inoculated plants as a percentage of shoot weight of uninoculated plants (FSW%) or root weight of inoculated plants as a percentage of root weight of uninoculated plants (DRW%). FSW% and DRW% were used as measures of resistance.

Results and Discussion: The results are shown in Figures 1A to 1H. The three cultivars had high and similar levels of resistance at week 4, with FSW% and DRW% values near 80% (Figs. 1G, 1H). Taylor continued to show high levels of resistance at weeks 6 and 8, as shown by quantitatively similar trends in inoculated and uninoculated plants for both shoot weight and root weight (Figs. 1A, 1B). Shoot weight increased steadily at the same rate in inoculated and uninoculated plants throughout this period, whereas root weight peaked at week 6 in both groups of plants. California White showed intermediate and progressively declining levels of resistance at weeks 6 and 8, reaching resistance values near 50% at week 8 (Figs. 1G, 1H). Shoot weight and root weight increased steadily between week 4 and week 8 in both inoculated and uninoculated California White plants, but at a slower rate in inoculated plants (Figs. 1C, 1D). Resistance values for Vista declined from near 80% at week 4 to near 20% at weeks 6 and 8 (Figs. 1G, 1H). In this cultivar, shoot weight and root weight did not increase between week 4 and week 8 in inoculated plants (Figs. 1E, 1F). We conclude that time-course changes in plant performance must be considered in evaluating resistance of edible beans to Fusarium root rot. In this collection of beans, cultivars that appeared to have similar levels of resistance at week 4 differed dramatically at weeks 6 and 8. Resistance of seedlings may not reflect resistance of older plants.

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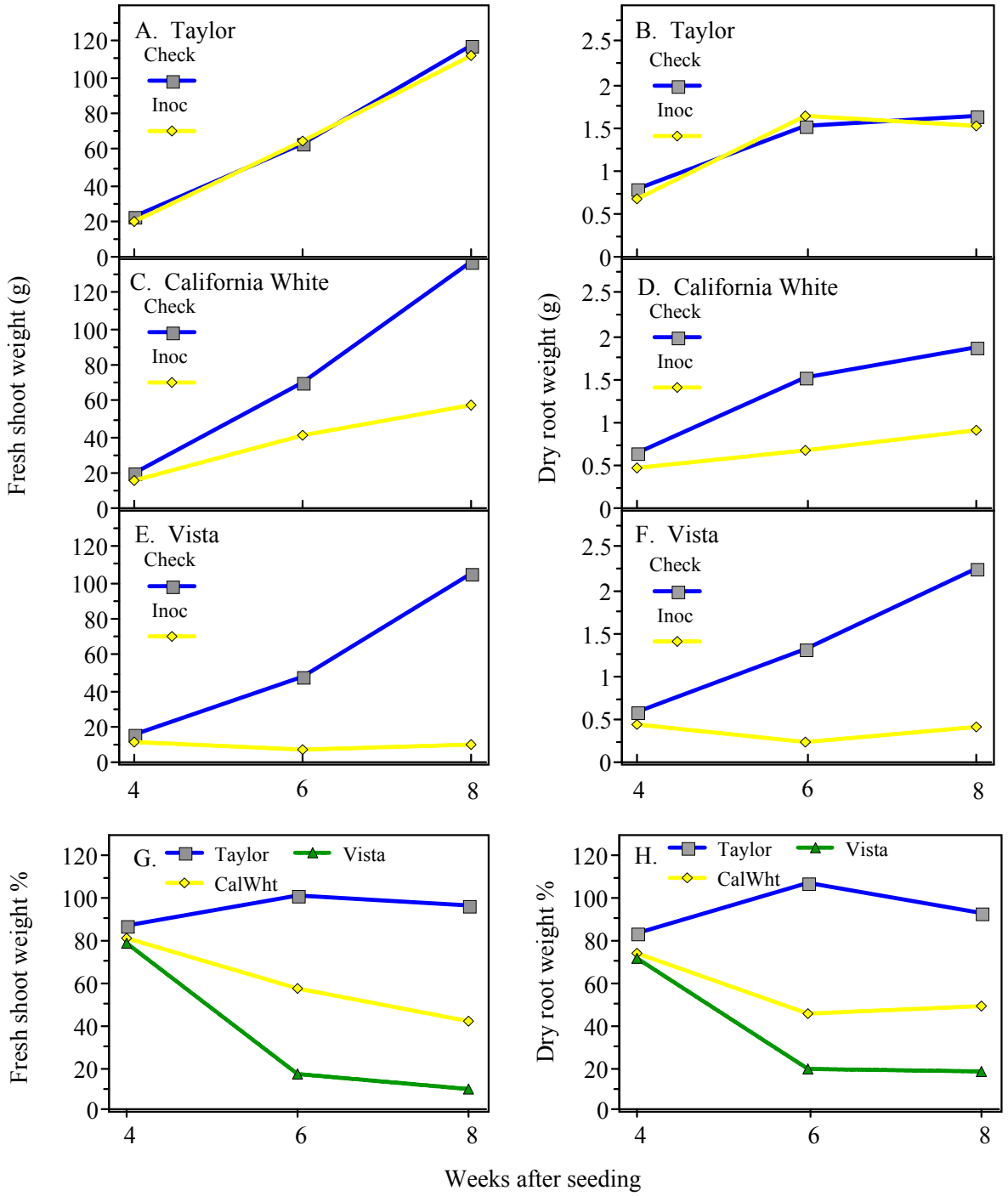
Figure 1. Effects of post-emergence inoculation of edible bean cultivars Taylor, California White, and Vista with *Fusarium solani* f. sp. *phaseoli* on fresh shoot weight and dry root weight 4,6,and 8 weeks after seeding. Weights are given in grams and as percentages (inoculated/uninoculated).



Inheritance of White Mold Resistance in the Interspecific Crosses of Pinto Cultivars Othello and UI 320 and *Phaseolus coccineus* L. Accessions PI 433246 and PI 439534

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Introduction

White mold (caused by *Sclerotinia sclerotiorum* Lib de Bary) is an endemic and one of the most devastating diseases of the common bean in the U.S. Chemical control is often inadequate, and it increases production costs for growers and minimizes their competitive edge in the national and international market. Use of resistant cultivars is pivotal to any effective and economical long-term strategy to control white mold. However, only low to moderate level of resistance to white mold exist in the common bean. On the other hand, some accessions of *Phaseolus coccineus* L. are known to possess much higher level of resistance than the common bean (Gilmore et al., 2002). White mold resistance from *P. coccineus* has been introgressed into common bean (Miklas et al., 1998). Although comparative data is not available, there is a general feeling that the level of white mold resistance introgressed in common bean, in general, is lower than that of *P. coccineus*. Our interest in *P. coccineus* resistance for white mold was rejuvenated because of the national *Sclerotinia* initiative. As part of our project we screened all *P. coccineus* accessions that were reported to be resistant to white mold by Gilmore et al. (2002). Moreover, we selected *P. coccineus* accessions PI 433246 and PI 439534 to study the inheritance and introgress their white mold resistance into the common bean. Our objective is to report the inheritance of resistance in this article.

Materials and Methods

Pinto bean cultivars Othello and UI 320 were selected for the inheritance study. Both Othello and UI 320 are early maturing in the Western U.S. Moreover, Othello is widely adapted despite its susceptibility to anthracnose, common bacterial blight, rust, and white mold. On the other hand, UI 320 is resistant (*I* gene) to *Bean common mosaic virus* (BCMV) and some races of the pathogen causing rust. Othello was crossed with PI 433246, and the resulting interspecific F_1 was backcrossed on to Othello and allowed to produce F_2 seed. Similarly, UI 320 was crossed with PI 439543. The F_1 was backcrossed on to UI 320 and allowed to produce F_2 seed. The four parents, two F_1, two F_2, and two backcrosses were grown in the greenhouse, and inoculated with *S. sclerotiorum* isolate using the straw-test (Myers et al., 1999; Petzoldt and Dickson, 1996). Individual plants were rated for their reaction to white mold. The frequency distribution and mean disease scores for each genotype were determined. Also, the frequencies were grouped into resistant (receiving scores of 1 to 5) and susceptible (6 to 9), and subjected to the χ^2 test.

Results and Discussion

Othello with a mean white mold score of 7.91 was the only susceptible parental genotype. To our pleasant surprise UI 320 with a mean disease score of 2.67 was resistant (Table 1). Nonetheless, both Othello and UI 320 had a wide range. For example, out of 33 plants scored for Othello, six showed a resistant reaction. Similarly, one out of 32 plants for UI 320 was susceptible. Both *P. coccineus* accessions were resistant to white mold. Similarly, the two interspecific F_1 hybrids had a resistance reaction suggesting that the resistance to white mold in the two accessions is controlled by dominant allele(s). Furthermore, the F_2 of Othello/PI 433246 segregated into 24

resistant to 7 susceptible, giving a good fit to a 3 resistant to 1 susceptible ratio (*P*=0.7558). This suggested that the white mold resistance in PI 433246 is controlled by a single dominant allele. However, in the backcross to Othello, one would have expected a 1 resistant to 1 susceptible ratio. Instead, there was an excess of susceptible plants. This might have occurred because of an excess of Othello gametes and/or the tendency of the interspecific hybrid to revert to the parental genotype, especially when backcrossed on to the common bean cultivar Othello.

Out of 26 F_2 plants of UI 320/PI 439534, six were susceptible to white mold, and all others had a resistant reaction. This gave a good fit to a 3 resistant to 1 susceptible ratio (*P*=0.8209), indicating that the resistance in PI 439534 was also controlled by a single dominant allele. But, only one out of 31 plants in the backcross to UI 320 was susceptible, whereas one would have either expected a 1 resistant to 1 susceptible ratio supporting the F_2 segregation, or else all resistant if the same dominant allele controlled white mold resistance in both UI 320 and PI 439534. The relatively small population size, and our inability to pure-line all four parents for their reaction to white mold before crossing them would not permit us to arrive at any definitive conclusions. Abawi et al. (1978) also reported a single dominant allele controlling resistance to white mold in a *P. vulgaris / P. coccineus* population.

Table 1. White mold reaction of pinto bean cultivars Othello and UI 320 and *Phaseolus coccineus* L. accessions PI 433246 and PI 439534, and their F_1, F_2, and backcrosses evaluated in the greenhouse at Fort Collins, Colorado in 2004 using the straw-test.

	White mold reaction†				
Genotype	Range	Mean	No. plants	Ratio	*P* value-
Othello	3-9	7.91	33	---	
PI 433246	1-9	3.38	21	---	
Othello/PI 433246					
F_1‡	1-7	4.00	16		
F_2	1-9	4.74	31	24R:7S	0.7558
Othello^2//PI433246	3-9	7.35	34	7R:27S	
UI 320	1-9	3.22	36	---	
PI 439534	1-5	2.05	38	---	
UI 320/PI 439534					
F_1	1-3	2.60	10	---	
F_2	1-9	4.23	26	20R:6S	0.8209
UI 320^2//PI 439534	1-9	3.13	31	27R: 1S	

†Scored on a 1 to 9 scale where 1= symptomless, and 9= severely diseased.
‡Three plants with a disease score of 7 probably were the female Othello self, and not hybrids!

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IDENTIFICATION OF PARTIAL RESISTANCE TO *SCLEROTINIA SCLEROTIORUM* IN COMMON BEAN AT MULTIPLE LOCATIONS

J. R. Steadman, coordinator and collaborator, L. K. Otto-Hanson and K. Powers, statistical analysis (University of Nebraska-Lincoln). Data from C. Kurowski (California), R. Mainz (Minnesota), J. Kelly (Michigan), P. Griffiths (New York), K.Grafton (North Dakota), J. Myers (Oregon), P. Miklas (Washington), and H. Schwartz (Colorado) .

No complete resistance to *Sclerotinia sclerotiorum*, cause of white mold, has been found in common bean. The development of bean cultivars with partial physiological resistance and architectural avoidance to white mold would reduce disease losses and require no input costs of producers. The objective of the study was to identify bean germplasm with broad partial resistance to white mold. To accomplish this, putative sources of resistance developed by bean breeders were evaluated by greenhouse and field screening methods in different US locations.

Field tests consisted of two rows of each entry and a common susceptible genotype, resulting in a three-row plot 4.6 m (15 ft.) long replicated three times in a randomized complete block design. The greenhouse tests were the straw (Petzoldt and Dickson, 1996), the oxalate (Kolkman and Kelly, 2000), and the detached leaf (Steadman et al, 1997). Twelve separate screening tests, six field and six greenhouse were rated on a scale ranging from most resistant (1) to most susceptible (12)(Table 1). Spearman and Pearson correlations were used to compare entry rankings in each test. Each test included eleven common bean lines, the cultivar Dwarf Bees was added for greenhouse screening, and CO75944 was added for field screening, totaling twelve lines .

The highest positive field correlations were ND and MI ($r=0.767$, $p=0.006$), ND and WA ($r=0.705$, $p=0.010$), MI and WA ($r=0.866$, $p=0.006$), MN and CA ($r=0.722$, $p=0.008$), and OR and WA ($r=0.809$, $p=0.001$). The highest positive greenhouse screening correlations were MI (straw test) and CO (straw test) ($r=0.708$, $p=0.010$), MI (straw test) and WA (straw test) ($r=0.711$, $p=0.010$), and CO (straw test) and WA (straw test) ($r=0.670$, $p=0.017$). The field and greenhouse significant correlation was the CO (oxalate test) and the ND field ($r=0.713$, $p=0.014$).

When an ANOVA was used on ranking, with each test as a block and bean line(entry) as a treatment, there were significant differences ($p=0.001$) among lines (Table 2). Dwarf Bees, Cornell 601, G122, Cornell 501, and AN 37 had the lowest mean rank (=most resistant). When greenhouse and field tests were analyzed separately, N02 302, G122, Cornell 50l, and AN 37 were ranked lowest in field tests, but Cornell 601 and Dwarf Bees replaced N02 302 in the lowest rankings from greenhouse tests, where N02 302 ranked the highest (=most susceptible). N02 302 probably has field avoidance to white mold.

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Table 1. Comparison of rankings* of 12 bean lines for white mold reaction at nine locations.

	Field						Greenhouse/Laboratory					
Cultivar	ND	WA	MN	OR	CA	MI	WA	NY	MI	CO[1]	CO[2]	NE
N02 302	3	3	5	3	5	2	13	12	10	6	9	8
G122	4	1	6	2	3	5	3	4	3	2	7	5
Cornell 501	2	4	4	1	11	3	6	2	5	6	4	11
AN 37	7	2	7	4	7	1	4	9	7	6	3	4
Cornell 601	1	6	8	10	4	4	5	1	6	1	2	1
Bunsi	8	7	1	6	1	7	8.5	5	11	4	10.5	6
IO1892-115M	6	8	3	11	6	8	10	6	2	10.5	5.5	10
Dwarf Bees	-	-	-	-	-	-	1	7	1	8	1	-
AN 69	10	10	9	9	8	10	2	11	4	9	5.5	3
CO75944	9	11	11	7	9	-	7	-	-	-	-	-
USWA 6	5	5	10	5	10	6	11	8	9	3	8	9
AN 1	11	9	2	8	2	9	12	3	12	10.5	10.5	7
Beryl	12	12	12	12	12	11	8.5	10	8	12	12	2

(-) = No Data; *Most resistant (1) to most susceptible (12).
[1]Colorado oxalate test and [2]Colorado straw test.

Table 2. Mean ranking of bean lines (1=most resistant) for white mold reaction in 12 separate greenhouse and field tests.

Entry	Mean Ranking	T Grouping	Seed Class	Source
Beryl	10.292	A	GN	*Check-Susceptible*
CO75944	9.000	B A	PTO	M. Brick
AN 1	8.042	B A C	GN	P. Miklas
AN 69	7.542	B D A C	PTO	P. Miklas
USWA 6	7.417	B D C	SR	P. Miklas
IO1892-115M	7.167	B D C	BLK	J. Kelly
N02 302	6.583	B D E C	NAVY	J. Kelly
Bunsi	6.208	F D E	NAVY	*Check-Intermediate*
AN 37	5.083	F D E	PTO	P. Miklas
Cornell 501	4.917	F D E	SNAP	P. Griffiths
G122	4.167	F E	CRAN	*Check-Resistant*
Cornell 601	4.083	F E	RK	P. Griffiths
Dwarf Bees	3.6	F	*P. coccineus*	Territorial Seeds

LSD=2.7514; Alpha=0.05

PLANT POPULATION AND GRAIN YIELD OF COMMON BEAN (CV. BRS-MG TALISMÃ) AS A RESULT OF NITROGEN AND PHOSPHORUS DOSAGES.

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Introduction: Aiming to study N and P_2O_5 dosages effect on plant population and grain yield of the common bean BRS-MG Talismã new cultivar, were conducted four experiments (winter-spring 2000, spring-summer 2000/2001, spring-summer 2001/2002, winter-spring 2002) in a typical dark red latossol at experimental area of the Departamento de Agricultura, Universidade Federal de Lavras (UFLA), Lavras-Minas Gerais State, Brazil.

Material and Methods: It was used randomized complete block design with three replications in a 4 x 4 factorial scheme using four phosphorus dosages (0, 100, 200 and 300 kg.ha^{-1} of P_2O_5, triple superphosphate source), and four N dosages (0, 70, 140 and 210 kg.ha^{-1} of N in two split dosages: the first ½ at the sowing and the other ½ as side dressing at the beginning of V4 stage of the common bean cycle). It was determined the initial and the final plant populations and the grain yield.

Results and Discussion: Increasing N dosages resulted low plants population (Figure 1), while in most of the situations the increasing P dosages diminished this N effect over plants population (Figure 2). The grain yield increased in the quadratic way when N or P_2O_5 dosages where enhanced, reaching the highest productivity peaks that changed with the sowing seasons (Figures 3 and 4).The yield response to N and P_2O_5 dosages calculated by the 90% criteria of maximum productivity were higher to those recommended officially in Minas Gerais State, Brazil (Table 1).

0 kg.ha^{-1} P_2O_5 oooo $\hat{Y} = 298733 - 180\ x - 3{,}47\ x^2$ (R^2= 99,9%)**
100 kg.ha^{-1} P_2O_5 - - $\hat{Y} = 303000 - 866{,}7\ x$ (R^2= 91,0%)**
200 kg.ha^{-1} P_2O_5 __ $\hat{Y} = 294667 - 28{,}6\ x - 4{,}1\ x^2$ (R^2= 99,9%)**
300 kg.ha^{-1} P_2O_5 ▬ $\hat{Y} = 302200 - 625{,}7\ x$ (R^2= 92,6%)**

0 kg.ha^{-1} N ooo $Y = 251200$
70 kg.ha^{-1} N - - $\hat{Y} = 208622 + 151{,}8\ x$ (R^2= 89,7%)**
140 kg.ha^{-1} N __ $\hat{Y} = 157822 + 228{,}2\ x$ (R^2= 97,6%)**
210 kg.ha^{-1} N ▬ $\hat{Y} = 108711 + 529{,}3\ x - 1{,}07\ x^2$ (R^2= 95,5%)*

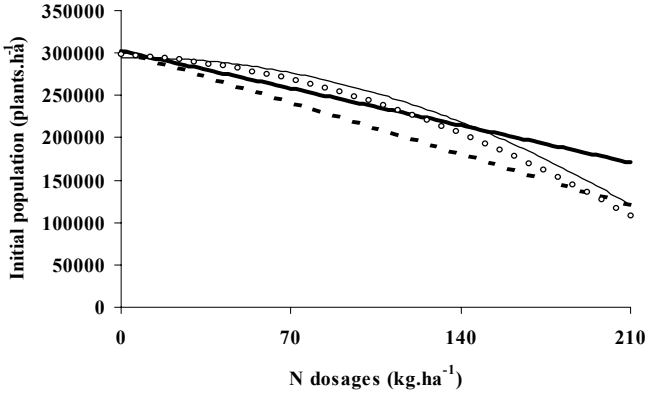

FIGURE 1. Initial populations of common bean (cv. BRS MG Talismã) in function of N and

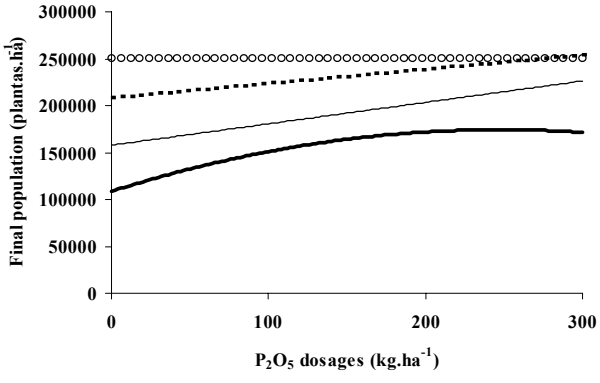

FIGURE 2. Final populations of common bean (cv. BRS MG Talismã) in function of P_2O_5 and

P_2O_5 doses. UFLA, Lavras - MG, winter 2002.

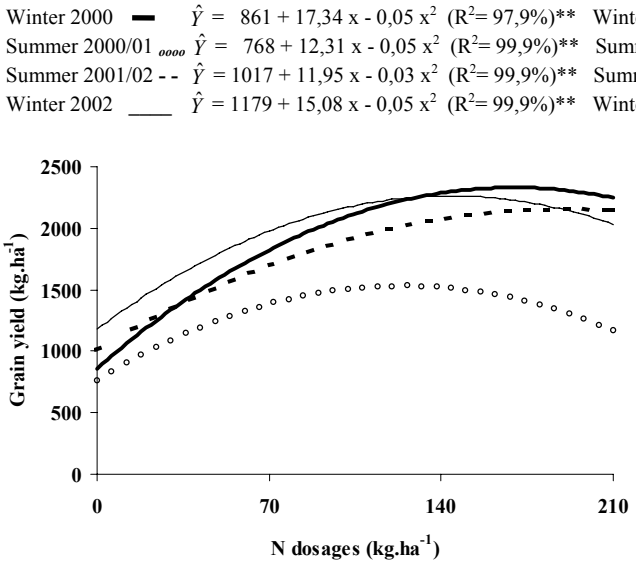

FIGURE 3. Grain yield of common bean (cv. BRS MG Talismã) in function of N doses and sowing seasons. UFLA, Lavras - MG, 2000/2002.

N doses. UFLA, Lavras - MG, summer 2001/2002.

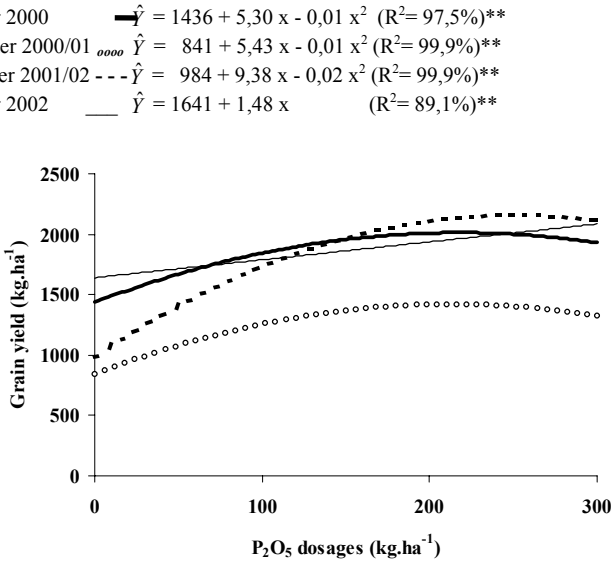

FIGURE 4. Grain yield of common bean (cv. BRS MG Talismã) in function of P_2O_5 doses and sowing seasons. UFLA, Lavras - MG, 2000/2002.

TABLE 1 Maximum (MY) and 90% of the maximum yield (0,9 MY) with respective doses. UFLA, Lavras - MG, 2000/2002.

Seasons	MY ($kg.ha^{-1}$)	Dose for MY	0,9 MY ($kg.ha^{-1}$)	Dose for 0,9 MY	Recomended dose
N:					
Winter 2000	2332	170	2099	102	70
Summer 2000/2001	1533	124	1380	69	50
Summer 2001/2002	2162	192	1946	108	70
Winter 2002	2264	144	2038	78	70
P_2O_5:					
Winter 2000	2013	218	1812	88	70
Summer 2000/2001	1422	214	1280	108	80
Summer 2001/2002	2161	251	1945	108	90
Winter 2002	-	-	-	-	-

Reference:

RIBEIRO, A.C.; GUIMARÃES, P.T.G.; ALVAREZ V., V.H.(eds.) Recomendações para o uso de corretivos e fertilizantes em Minas Gerais – 5ª aproximação. Viçosa-MG, 1999. 359p.

MANGANESE AND IRON CONTENTS OF BEAN LEAF INFLUENCED BY CROP RESIDUES IN NO TILL - SYSTEM[1]

I. P. Oliveira[2], E. V. Brandstetter[3], R. M. Oliveira[2], M. Thung[2]; H. Aidar[2] and J.Kluthcouski[2]

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It's worldwide known about the plant behavior when the same crop is cultivated year after year on the same area. In the first year, generally crop productivity is high but yield decreases about 50% by each year with continuos cultivation system. Nevertheless, this plant behavior is paradoxical with the necessity to produce according to the principles of sustainability – to get economical production preserving the environment.

No till system in Brazil is becoming the most popular practice of cropping system. In Brazilian Central Region, this practice is being profitable to maintain high grain productivity, partially preserving the soil chemical, physical and biological properties. Factors as crop management and fertilization, among many others, have been sponsored by temporary decline of grain production. From plant nutrition point of view, plant residue has influence on grain production in a long duration cultivation.

This paper is written to alert about the use of corn, rice, sorghum, soybean and brachiaria residues and discuss their effects on iron and manganese concentrations in bean leaf harvested in areas cultivated with common bean, cultivar Pérola, under 45, 90, 135 and 180 kg/ha de N, applied 45 days after planting, in no-till system.

In general, nitrogen fertilization mainly in no till system, constitutes as an important factor for increasing crop productivity. The nitrogen recommendation for irrigated bean crop vary form 40 to 120 kg/ha of N and crop generally absorb about 200 kg/ha of N in dry season.

The basic fertilization of 150 kg/ha of 8:20:20 ($N:P_2O_5:K_2O$) formulation was applied in soil at planting. Plants were irrigated with aspersion method. Leaf samples were collected at flowering growth stage, dried in oven during 72 hours at temperature about 65º to 70ºC, grind and analyzed.

The iron concentration in leaf of common bean is present in Figure 1.

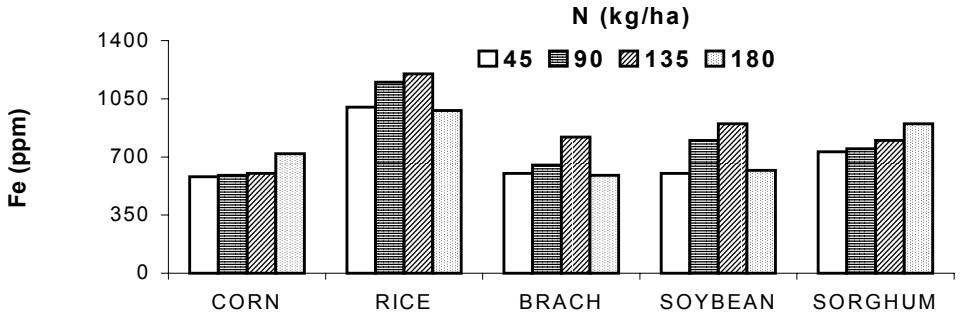

Figure 1. Fe content of dry bean leaf cultivated on crop residue.

The highest amounts of Fe were observed in bean leaves (1040 ppm of Fe) came from area in which rice crop was previously grown. According to Thung & Oliveira (1998), the toxic level of Fe for bean leaf is 500 ppm while double amounts were observed in present study. Oliveira et al. (2002) reported that rice is an exhausting crop of iron from soil. Sometimes, rice plants absorb iron in amounts higher than its own needs; then toxic concentrations can be obtained in plant residues. As a consequence, subsequent crops generally never grow well and low yield due two reasons: a) - high concentration of Fe in the organic matter produced by previously crop and b) - high absorption of Fe by the subsequent crop.

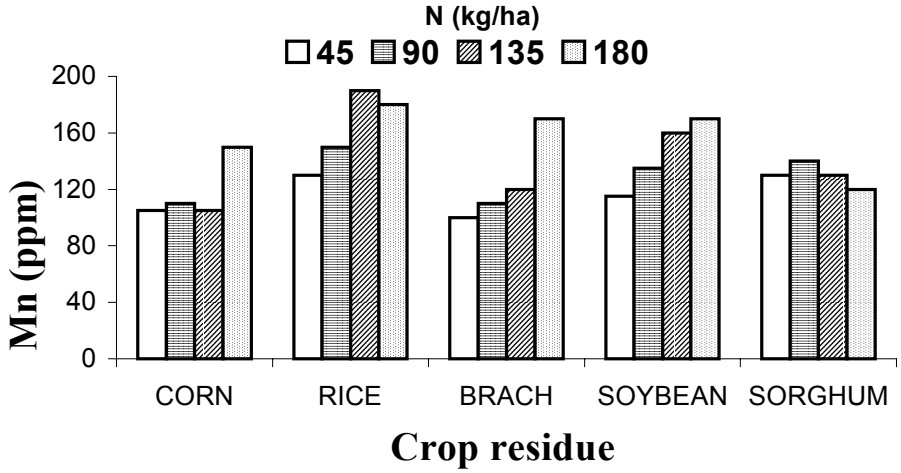

Figure 2. Mn content of dry bean leaf cultivated on crop residue

Similar to Fe, also higher amount of Mn was observed in bean leaves (165 ppm of Mn) and this Mn came from area in which residue of rice was used. According to Thung and Oliveira (1998), the toxic level of manganese for bean leaf is higher than 700 ppm while the level of Mn presented in bean leaf was lower than 300ppm, the critical level for bean crop. Oliveira et al. (2002) reported that the Mn toxicity is largely spread in Central Brazil but most of the time this toxicity would not cause plant mortality.

These results suggest that high Fe content in bean plants came from rice residue and this micronutrient requires more attention than Mn under field condition. The subsequent crops probably not only absorb toxic concentrations of manganese but in a few environmental condition would prejudice the grain production.

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PHOSPHORUS AND ZINC CONTENTS OF BEAN LEAF INFLUENCED BY CROP RESIDUES IN NO TILL - SYSTEM[1]

I. P. Oliveira[2], E. V. Brandstetter[3], R. M. Oliveira[2], M. Thung[2]; H. Aidar[2] and J.Kluthcouski[2]

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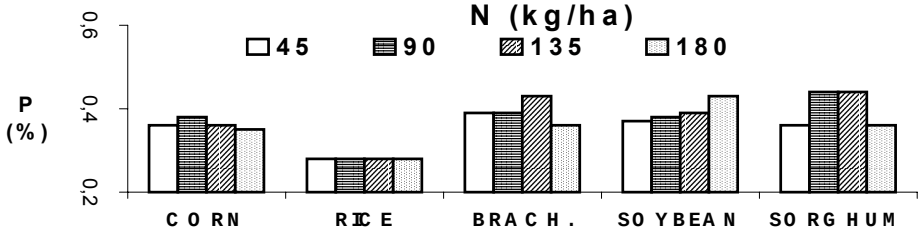
Agricultural systems refer to the use of a certain area with several crops in succession or rotation, inserting an area utilization by using succession and rotation of plant species intercalated objecting grain and or organic matter productions in different soil management forms and inputs (Silva 1998).

The universe, constituted by several species of plants with specific characteristics, besides the different forms of possible soil management to be accomplished, makes the system analysis of an agricultural system more complex than of an isolated crop. This complex one has as objective the optimization of land use.

In the last two years, objective to increase growing demands for foods, the research, is focused on agricultural systems, has been contemplated enough, above all in areas with irrigation facility, technique that makes possible the most intensive land use. In general those researches evaluate crop rotations, soil management crop rotations and fertilization (Pöttker & Roman, 1998; Klepker & Anghinoni 1995; Silveira et al 1994).

This research was carried out with the objective to know the effects of corn, rice, sorghum, soybean and brachiaria residues, on P and Zn concentrations of bean leaf harvested in areas cultivated with common bean, cultivar Pérola, under 45, 90, 135 and 180 kg/ha de N, applied 45 days after planting, in no-till system.



Crop residue

Figure 1. P content of dry bean leaf cultivated on crop residue.

In general, the nitrogen fertilization, mainly in no till system, constitutes an important factor for increasing productivity. The nitrogen recommendation for irrigated bean crop vary form 40 to 120 kg/ha of N, while the crop absorb for its complete development amount equivalent two times these values. The basic fertilization of 150 kg/ha of 8:20:20 ($N:P_2O_5:K_2O$) formulation was applied at planting. Plants were

irrigated with aspersion irrigation system. Leaf samples were collected at bean flowering growth stage, dried in over during 72 hours at temperature about 65º and 70ºC, grind and analyzed.

The phosphorus concentration in leaf of common bean is presented in Figure 1. The lowest concentration of phosphorus in the leaf bean was observed in bean cultivated on rice residue. In Brazil, decrease in yield has been observed when crops are cultivated year after year in the same area. According to plant nutrition philosophy, the crop residue can contain excessive concentration of toxic elements and sometimes low concentration of essential plant nutrients. According to these results, very low phosphorus concentrations can be observed in rice crop residue.

Zinc concentration in bean leaf is presented in Figure 2. Lower concentrations of zinc were observed in bean leaf when the crop was cultivated on rice residue. Based on the Zinc importance for bean production and the low zinc concentration found in savanna soils, the low productions generally observed after first year planting can be sponsored to the low

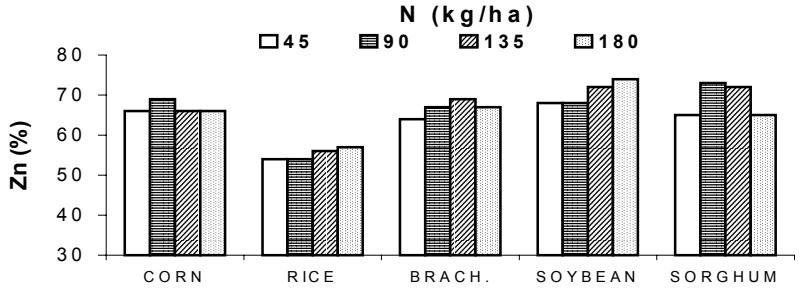


Figure 2. Zn content of dry bean leaf cultivated on rice crop residue.

concentrations of nutrients observed in any crop residues. Tisdall et al (1985) recommend to alternate groups of system crops that present differentiated root systems whose functions are to recycle nutrients of the deepest layers for the superficial soil layers and to care deeply the plant nutrition in relation to the lack of nutrients by using balanced nutrient formulation different from traditional ones to maintain top productions forever avoiding economic disturbance in agricultural activities.

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ECONOMICAL FEASIBILITY OF THE COMMON BEAN CROP (CV. BRS-MG TALISMÃ) IN FUNCTION OF NITROGEN AND PHOSPHORUS INCREASING DOSAGES

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Introduction:

Aiming to determine, from common beans yield obtained with N and P_2O_5 dosages, the yield cost variations and crude margin of the common bean activity in each situation, were set four field experiments (winter-spring 2000, wet season 2000/2001, and 2001/2002 and winter-spring 2002) in an experimental area of the Departamento de Agricultura, Universidade Federal de Lavras (UFLA), Lavras – Minas Gerais State, Brazil.

Material and Methods:

It was used randomized complete block design with three replications in a 4x4 factorial scheme using four phosphorus dosages (0, 100, 200 and 300 $kg.ha^{-1}$ of P_2O_5, triple superphosphate source) and four N dosages (0, 70, 140 and 210 $kg.ha^{-1}$ of N, urea source, in two split dosages: the first ½ at the sowing and the other ½ as side dressing at the beginning of V4 stage of the common bean cycle). For the economical studies of the effects that had showed to be significant by the analysis of variance, estimated the effective price and the crude profit in each given situation. The products and services prices were collected at the local market in Lavras, during the month of February/2004, and for the profit estimative were used three levels of prices given by the producers (R$ 0,50, R$ 1,00 and R$ $1,50.kg^{-1}$ of bean). From those estimated cost and crude profit, could be calculated by difference, a crude margin which did not include the machines, irrigation equipment depreciating and the financial costs and administrative remunerations.

Results and Discussion:

In most of the analyzed situations, the crude margin gained with bean had rose in a square way when N or P_2O_5 dosages were increased (Figures 1 and 2). These N and P_2O_5 dosages related to points of maximum crude margin overcame those calculated dosages by the 90% criteria of maximum yield and the recommended doses in Minas Gerais State, Brazil (Table 1). Putting analysis with differentiated prices showed to be efficient and realized that to sell the product by the lowest price of the season resulted in a negative crude margin, while, the highest season price always resulted in positive crude margin.

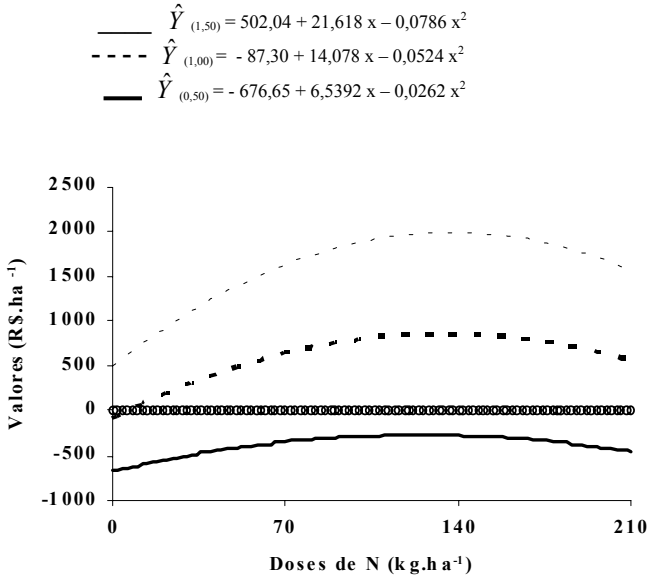

FIGURE 1. Crude margin (R$.ha^{-1}) of bean crop in function of N doses, winter 2002, for three price situations: R$ 0,50, R$ 1,00 and R$ 1,50.kg^{-1} of bean. N price = R$ 1,00.kg^{-1}.

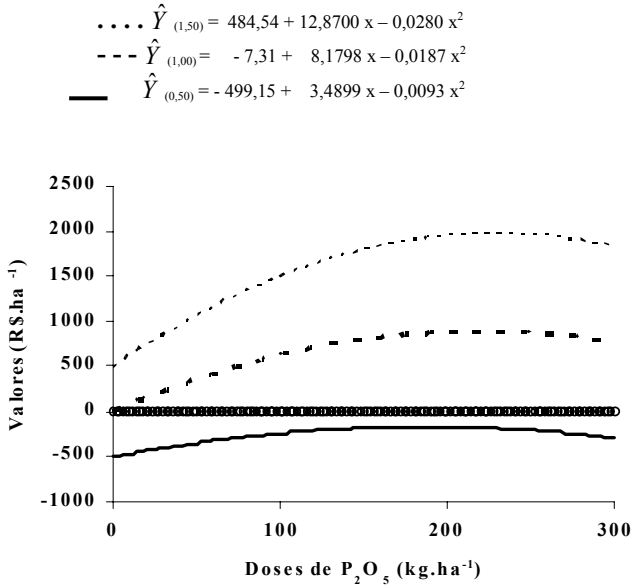

FIGURE 2. Crude margin (R$.ha^{-1}) of bean crop in function of P_2O_5, doses, Summer 2001/2002,for three price situations: R$ 0,50, R$ 1,00 and R$ 1,50.kg^{-1} of bean. P_2O_5 price = R$ 0,72.kg^{-1}.

TABLE 1. N and P_2O_5 dosages (kg.ha^{-1}) for maximum crude margin with differenciated prices of beans and for maximum (MY), 90% of the maximum yield (0,9 MY) and official recomended dose. UFLA, Lavras – MG, 2000/2002.

Searson	Price of bean (R$.kg^{-1})			Dose for MY	Dose for 0,9 MY	Recomended dose
	0,50	1,00	1,50			
	N (kg.ha^{-1})					
Winter 2000	150	160	164	170	102	70
Summer 2000/01	104	114	118	124	69	50
Summer 2001/02	160	176	180	192	108	70
Winter 2002	124	134	138	144	78	70
	P_2O_5 (kg.ha^{-1})					
Winter 2000	120	168	184	218	88	70
Summer 2000/2001	120	166	182	214	108	80
Summer 2001/2002	186	220	230	251	108	90
Winter 2002	-	-	-	-	-	-

Reference:

RIBEIRO, A.C.; GUIMARÃES, P.T.G.; ALVAREZ V., V.H.(eds.) Recomendações para o uso de corretivos e fertilizantes em Minas Gerais – 5ª aproximação. Viçosa-MG, 1999. 359p.

RAINFALL PATTERN AND SEED YIELD OF DRY BEAN IN THE SEMIARID HIGHLANDS OF MEXICO[1]

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Introduction. One of the main limiting factors affecting grain yield of dry beans in the semiarid highlands of Mexico is the uneven distribution patterns and the scarcity of rainfall. This region includes the states of Chihuahua, Durango, Zacatecas and Aguascalientes, where are planted annually 1.2 million hectares under rainfed conditions. The average amount of precipitation during the growing season (June-October) ranges from 250 to 400 mm. It is common that from this total amount of rainfall, a high percentage (>60%) occurs during the vegetative period, while during the reproductive period dry bean crop may be subjected to water stress. Thus, grain yield of dry bean may be reduced drastically. A strategy to face this problem is by using dry bean varieties adapted to this condition. It has been showed that early genotypes may have an advantage over late genotypes under this environment, since the formers may escape drought and reduce production risks (1). Therefore, the objective of this study was to analyze the relationship between rainfall distribution patterns and grain yield of dry beans

Materials and Methods. The study was conducted at the Research Station of Sandovales, Aguascalientes (22° 09' N, 102° 18' W, 2000 masl) from 2000 to 2003. Data was gathered from a total of six field trials, which were established on June 30 in 2000; June 27 in 2001; June 14 and July 8 in 2002 and July 14 and 29 in 2003. Three early (Pinto Villa, Pinto Zapata and Azufrado Tapatio) and three late (Tlaxcala-62, Flor de Mayo M-38 and Bayo Criollo del Llano) dry bean genotypes were tested.. The experimental design was a randomized complete block with four replications. The experimental unit consisted of four rows of 6 m long and 0.76 m apart. Daily precipitation was recorded from a near climatological station in each growing seasons. Days to flowering (DF) and days to maturity (DM) were registered on each plot, and at harvest, seed yield (SY) was recorded at all experiments. Average grain yield was estimated for both early and late genotypes in each year. For each trial rainfall was accumulated for the vegetative (VP), reproductive (RP) periods and for the whole growth cycle (WC). Limits were established such as, VP was considered to be 40 and 50 days after sowing (das) in early and late genotypes, respectively, whereas RP was considered from 41 to 85 and from 51 to 95 das in early an late genotypes. Linear regression analysis was realized between accumulated rainfall in VP, RP and WC vs SY average of both early and late dry bean genotypes.

Table 1. Accumulated rainfall in the vegetative (VP) or reproductive period (RP) and during the whole cycle (WC) and seed yield average (SY) of three early and three late dry bean cultivars.

	Early genotypes				Late genotypes			
Sowing date	VP	RP	WC	SY	VP	RP	WC	SY
	mm			-- g m^{-2} --	mm			-- g m^{-2} --
30/06/00	102.0	46.0	148.0	65.1	126.0	22.0	148.0	24.8
27/06/01	138.0	84.0	222.0	73.3	153.0	128.0	281.0	56.3
14/06/02	169.2	119.8	289.0	45.6	200.4	158.2	358.6	28.4
08/07/02	136.4	99.6	236.0	49.2	136.4	121.6	258.0	41.6
14/07/03	126.6	188.8	315.4	163.3	203.2	121.4	324.6	150.4

29/07/03	224.4	78.4	302.8	109.8	227.6	79.4	307.0	100.7

Results and Discussion. Taking into account all six environments, accumulated rainfall in the VP ranged from 100 to 230 mm, while in the RP accumulated rainfall varied from 22 to 190 mm. Rainfall during the whole cycle was from 148 to 360 mm. From the total rainfall, an average of 60 to 65% occurred during the vegetative period and only from 35 to 40% occurred during the reproductive period (Table 1). The relationship between accumulated rainfall and seed yield showed a close association (r=0.63*) in the RP for the early genotypes, while the narrowest association for the late genotypes was observed in VP (r=0.63*) (Figures 1A and 1B). Results indicated that it was most important the rainfall occurring at the reproductive stage for early genotypes, whose grain yield may be defined during this period. It was reported that seed production and pods/plant of dry beans were most affected when water stress was imposed during flowering and pod filling stages (2). For late genotypes it seems most important rainfall during the vegetative stage, which may be attributed to that late genotypes flowering at 50 das have more time to accumulate biomass when no water stress is present. This may contribute to sustain grain yield through carbohydrates remobilization, even if plants are exposed to drought, but display lower yields than early genotypes. Late cultivars such as Bayo Criollo del Llano were widely grown in the area around test site, it seems that local farmers gave a high price value to straw derived from those native late cultivars. However, currently the introduction of more yield efficient early cultivars is giving more benefits to farmers and reducing production risks.

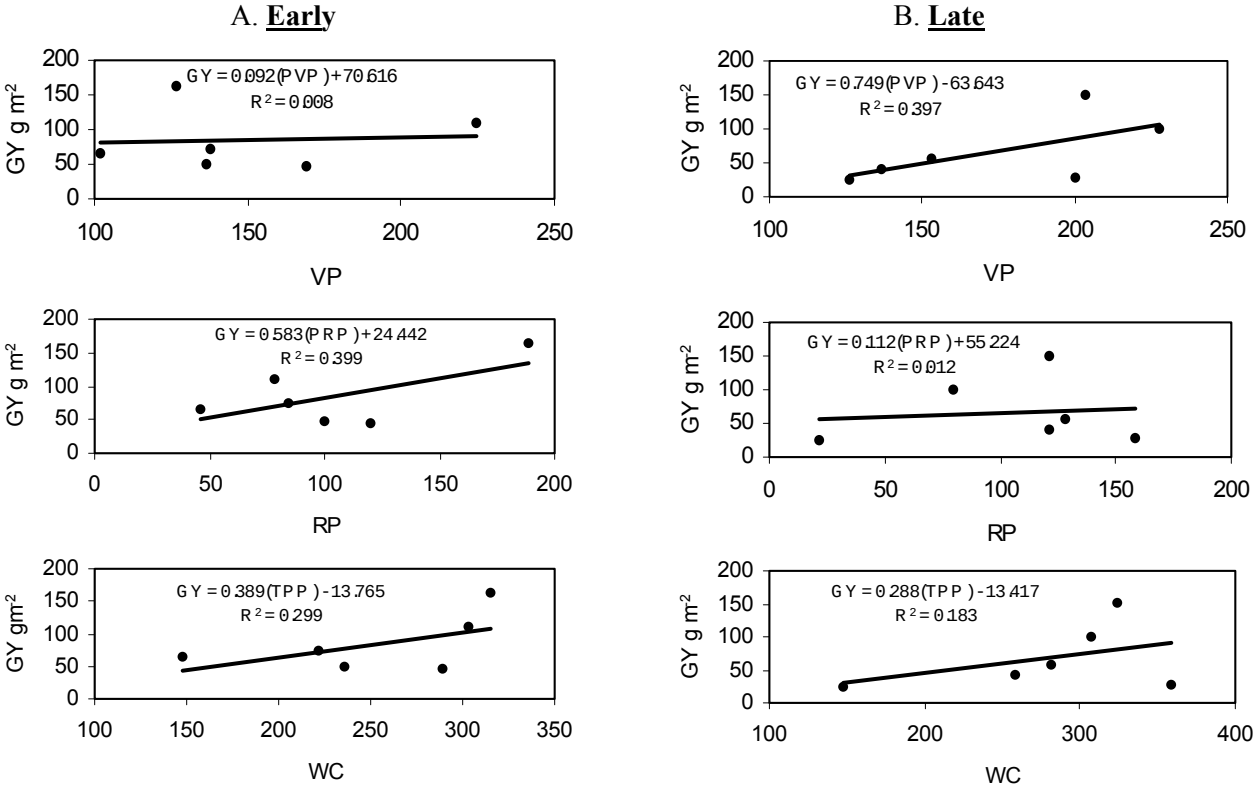

Figure 1. Relationship between accumulated rainfall in the vegetative (VP), reproductive (RP) or in the whole cycle (WC) and grain yield average (GY) of early (A) and late (B) dry bean genotypes.

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SEED YIELD OF DIVERSE BEAN CULTIVARS GROWN IN THREE REGIONS OF MEXICO[1]

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The growth of dry beans under limited rainfall conditions is a common production practice in Mexico where only around 12% of the total area is grown under irrigation. Seed yield of rainfed beans varies annually from region to region dependent on moisture availability during the season, and the occurrence of diseases. Lowest yields and rainfall patterns are observed in the semiarid highlands. In the semiarid (North) and humid highlands (Central), most of the commercial beans grown belong to the Durango and Jalisco races, respectively, whereas the most widely grown beans in the lowland tropics belong to the Mesoamerican race (Singh *et al*., 1991). Over the last two decades, the INIFAP breeding program has been systematically testing new materials in multisite trials in the search for sources of drought resistance. We report on the seed yield, from three contrasting sites, of a set of bean cultivars released as promising sources for drought in different regions of the world.

A set of 49 bean cultivars, mostly from the Durango and Mesoamerican races, was assembled with bred cultivars from different programs and with 'G' accessions from the CIAT bush core collection selected under rainfed conditions in the semiarid regions of Mexico. The trial was grown in three different locations from 2001 to 2003. Test locations were: Cotaxtla, Veracruz (lowland tropics at 70 masl) during 2001 under two moisture treatments, irrigated and terminal water stress); Celaya, Guanajuato (mid altitude Bajio region at 1675 masl) during 2002 and 2003 under rainfed conditions; and in Sandovales, Aguascalientes (semiarid highlands at 2010 masl) during 2002 and 2003 under rainfed conditions and rainfed plus supplemental irrigation. An experimental triple lattice design was utilized with two 6 m row plots and 0.6 to 0.76 m row widths. Several traits were recorded but only seed yield data in g m^2 is reported here.

As expected, 'G' accessions, mostly pinto landraces from the Durango race, performed poorly in the trials at the lowland tropics and in the Bajio region. Yields in Cotaxtla were lower mainly due to a short growing cycle (due to its photoperiod sensitivity coupled to short winter days), while lower yields at Celaya were mostly due to the inherent susceptibility to diseases (rust, anthracnose and common bacterial blight) and to leafhoppers (*Empoasca kraemeri*). On the contrary, some 'G' accessions exhibited outstanding performance under both, rainfed and irrigated conditions at Sandovales in the semiarid highlands (Table 1). In the lowland and mid-altitude sites, locally adapted and introduced bred cultivars were among the top 25% yielding entries and a few were among the top yielders in all four trials. Entries included: TLP 19 and SEA 10 improved CIAT lines from the Mesoamerican race and 97-RS-101 from the Durango race. 97-RS-101 was selected from the 1997 USDA-ARS rust resistance nursery grown in Saginaw, Michigan. In the four trials at Sandovales, superior cultivars included: improved cultivar Pinto Villa and landraces G 13637 (Apetito) and G 842 (PI 201331). In addition,

cultivars 97-RS-101 and SEA 10 (from the CIAT International Drought Nursery) were among the top yielding entries in six of the eight trials and Pinto Zapata and 97-RS-110 excelled in five of these trials. Superior cultivars at each site included genotypes with type II and type III growth habit, early to mid-season maturity combined with disease resistance. In addition, those cultivars that displayed broad adaptation exhibited a neutral photoperiod reaction.

Rank correlations were calculated among and within locations and the correlations within locations: two trials at Cotaxtla, r = 0.80**; two trials at Celaya, r = 0.75**; four trials at Sandovales, r = 045** to 0.65**. Correlations among locations showed intermediate values only between Veracruz and Celaya (r = 0.41** and 0.60**); correlations between Sandovales and the other locations displayed low r values from 0 to -0.32*. The same or related cultivars were identified in the mid-altitude and lowland tropical environment, whereas the semiarid highland site was different and is unique as a test site for cultivar selection for drought.

Table 1. Seed yield (g m^2) of 25% top yielding entries out of 49 cultivars grown at eight environments in three locations of Mexico

Cotaxtla 2001	**Irrigated**		**stressed**	**Celaya**	**2001**		**2002**
Cultivar	**g m^2**	**Cultivar**	**g m^2**	**Cultivar**	**g m^2**	**Cultivar**	**g m^2**
Ng. Veracruz	280	G 6762	212	97 RS 110	197	DON 35	210
Bibri	268	Pt Zapata	204	VAX 2	175	VAX 2	208
Ng. INIFAP	266	Ng. Veracruz	204	97 RS 101	158	SEA 10	203
VAX 2	266	97 RS 110	189	Ng. INIFAP	153	Ng. INIFAP	201
ICA Quimbay	259	97-RS-101	189	TLP 19	149	97 RS 110	197
Pt. Zapata	245	SEA 10	183	Ng. 8025	145	B 98311	189
TLP 19	240	TLP 19	177	Ng. Durango	145	97-RS-101	186
97-RS-101	232	G 17666	176	DON 35	137	Ng. 8025	185
SEA 10	232	G 801	175	Ng. Veracruz	135	SEA 5	184
G 3107	228	Bibri	175	Huasteco 81	134	Bibri	181
By. San Luis	227	G 1688	173	SEA 10	134	Pt. Zapata	174
Ng. 8025	226	By. San Luis	169	G 6762	131	TLP 19	172
Average n=49	178		135		75		131

Sandovales2001	**Irrigated**		**Rainfed**	**Sandovales2002**	**Irrigated**		**Rainfed**
Cultivar	**g m^2**	**Cultivar**	**g m^2**	**Cultivar**	**g m^2**	**Cultivar**	**g m^2**
G 13637	228.6	G 2846	116.8	G 13637	138.8	G 18147	84.7
G 22923	186.5	G 13637	111.7	Pt. Villa	122.9	SEA 10	63.0
Ng. Durango	175.7	Pt. Villa	105.6	SEA 10	119.2	Pt. Villa	61.2
Pt. Villa	160.9	Namiquipa	91.0	G 22923	107.1	G 13637	56.9
G 2846	158.1	G 1354	88.0	Namiquipa	100.8	Namiquipa	56.6
G 16054	155.4	DON 38	87.7	G 4523	94.9	G 842	50.5
By. S. Luis	153.0	97 RS 101	87.0	G 842	89.5	Black Jack	50.0
G 19953A	152.6	G 842	82.7	97 RS 101	89.3	G 16054	48.8
G 847	151.6	G 22923	79.1	G 847	87.7	Pt. Zapata	46.1
G 2774	150.3	G 17427	76.5	DON 35	86.7	G 17427	45.1
G 842	148.0	Bibri	74.2	Pt. Zapata	86.0	G 1977	44.4
G 18147	140.8	97 RS 110	73.0	G 18147	85.4	MC 6	43.8
Average n=49	114		62		68		36

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GENETIC ANALYSIS OF SOME METRIC TRAITS IN SMALL-SEEDED BEAN (*PHASEOLUS VULGARIS* L.) LINE CROSSES

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Introduction

Understanding the type of gene action and mode of inheritance of complex metric traits of the breeding materials is helpful for breeders in choice of suitable breeding procedure. The intrinsic genetic properties of breeding populations can be evaluated using genetic designs (Hallauer and Miranda, 1988). Therefore, the present study was undertaken to assess the relative magnitude of gene effects contained in the means of some metric traits in crosses of small seeded bean lines using factorial analysis of generation means.

Materials and Methods

The experimental material consists of six generations (P_1, P_2, F_1, F_2, BC_1 and BC_2) of two crosses, Roba x G-6 and DOR-794 x Red Wolayta. The materials were grown in compact family design with two replications during the 2002 main rainy season ('Meher') at Awassa agricultural research station, southern Ethiopia. The non-segregating generations (P_1, P_2 and F_1) and back cross generations (BC_1 and BC_2) of each cross were sown in three rows plot of 2 meters long. F_2 generations were sown in four rows of plot of 2 meters long. The spacing was maintained at 40 cm between rows and 10 cm within rows. The data on grain yield (gm/plant), plant height (cm), numbers of pods/plant and pod length (cm) were collected on individual plant basis (20 plants each in P_1, P_2 and F_1, 30 plants in F_2 and 24 plants each in BC_1 and BC_2). Hayman (1958) and Jinks and Jones (1958) model were used to determine the type of genetic information contained in generation means. The significance of scales and gene effects were tested by t-test as described by Sharma (1998).

Results and Discussion

The results of scaling tests applied to detect the presence of epistatic interaction and estimates of gene effects are presented in Table 1. The analysis showed that the dominance [h] gene effect was larger than additive [d] gene effects in magnitude in all the crosses for all the traits under study. DOR -794 x Red Wolayta cross would be realized in selection practiced in advanced generations when majority of loci approach to homozygosity.

A simple additive-dominance model was sufficient to explain most of genetic variations for the expression of plant height in both the crosses, of pods per plant and pod length in Roba x G-6 cross as none of the scales were significant. This implied that selection could be practiced effectively at F_2 population for improvement of plant height in both the crosses, and for number of pods per plant and pod length in Roba x G-6 cross.

At least one of the scales was significant for grain yield per plant in both crosses, but only for number of pods per plant and pod length in DOR 794 x Red Wolayta cross. This indicated that epistatic effects contributed to the inheritance of the traits. The interaction components accounted for larger proportion of the variation, in addition to high dominance main effect for the traits in these crosses suggested that larger variation would be expected in later generation. Hence success of selection for grain yield in both the crosses, number of pods and pod length in DOR -794 x Red Wolayta cross would be realized in selection practiced in advanced generations when majority of loci approach to homozygosity. Moreover, the type of epistasis operating in the populations of the crosses Roba x G-6 and DOR-794 x Red Wolayta for grain yield, DOR 794 x Red Wolayta for number of pods per plant and pod length is duplicate type. Hence recurrent or gamete selection can be employed for the genetic improvement of these traits.

Table 1. Scaling test and gene effects for some metric traits in small-seeded bean line crosses.

Cross	scale				gene effect						Type of epstasis
	A	B	C	D	[m]	[d]	[h]	[i]	[j]	[l]	
Grain yield (gm/plot)											
Roba x G-6	-	-	**	-	27.7**	-1.1	18.8	15.4	-4.3	-7.9	D#
DOR 794 x Red Wolayta	-	-	**	**	24	-10.2**	46.8**	49.0**	-13.7*	-47.5**	D
Plant height (cm)											
Roba x G-6	-	-	-	-	65.1**	4.01**	-4.92	-	-	-	-
DOR 794 x Red Wolayta	-	-	-	-	50.9**	-5.1**	17.0	-	-	-	-
Number of pods/plant											
Roba x G-6	-	-	-	-	18.0	2.1**	24.0	-	-	-	-
DOR 794 x Red Wolayta	-	-	*	**	24.3**	-1.3	28.1**	27.8**	5.9	-22.9	D
Pod length (cm)											
Roba x G-6	-	-	-	-	10.2**	0.15	-1.25	-	-	-	-
DOR 794 x Red Wolayta	-	-	*	**	9.5**	-1.1**	2.1**	1.8*	-1.0	-1.8	D

#D= duplicate

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Yield Components Measured in a Climbing x Bush Bean RIL Population.

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Introduction:

Climbing beans are an important traditional component of agriculture in Central America and the Andean Region. Climbing beans are thought to have a higher yield potential than bush beans due to longer growth cycles and higher overall rates of biomass accumulation. The yield advantage of climbing beans is also evident when analyzing yield components. Compared to bush beans, climbing beans can have more pods per plant, more pods per raceme, longer pods and more seed per pods. Few studies have looked at the interaction of climbing bean yield and yield components with soil fertility levels. One of our long-term objectives with the climbing bean breeding program at CIAT (International Center for Tropical Agriculture) is to increase tolerance of advanced lines to low phosphorus conditions. Fertilization levels often affect the capacity of climbing beans to produce the biomass necessary to realize their higher yield potential. Therefore our specific research goals for this study were to understand how soil fertility levels affect climbing ability and how adaptation to low phosphorus soils affect components of the yielding ability of climbing beans. To do this we analyzed a population of recombinant inbred lines derived from the cross of a climbing bean, G2333, by a bush bean, G19839, grown under high and low phosphorus treatments, for yield, harvest index and yield components.

Materials and Methods:

A total of 84 $F_{5:8}$ recombinant inbred lines (RILs) from the cross G2333 x G19839 were grown under high and low phosphorus conditions in Darien (Valle) during the rainy season in semester 2002A. G2333 ('Colorado de Teopisca') is a climbing bean from Mexico that has a type IVa growth habit, while G19839 is a landrace from Peru with a type IIIa growth habit. The RILs were grown under two fertilization treatments, one at high phosphorus (fertilization of 300 kg/ha TSP (45 kg P2O5)) and one at low phosphorus (fertilization of 50 kg/ha TSP (7.5 kg P2O5)). The experiment had a randomized complete block design with two repetitions per fertilization treatment. Plots consisted of a single row that was 3 m in length, planted with a total of 30 seeds. The distance between seeds was 10 cm and between rows was 1.2 m. The parental genotypes, G2333 and G19839, were planted every 10 rows throughout the experiment to use as visual checks. The trials were protected with two preventative fungicide treatments at planting and again at flowering, and one insecticide treatment to control thrips and whitefly. The cropping systems consisted in a 2 m high bamboo and wire trellis, with strings tied to individual plants within the plot. The following variables were evaluated: pod length (PL), number of pods per raceme (P/B), pods per plant (P/P), seeds per pod (S/P), 100 seed weight (100s), harvest index (HI) and yield per plant (Y/P). Data was analyzed with a combined ANOVA in which sources of variation were: environment (high or low P), genotype (RIL) and genotype x environment. Pearsons correlation coefficients were estimated for all combinations of yield components.

Results and Discussion:

ANOVA results (Table 1) showed significant differences between the recombinant inbred lines for all variables. Significant differences were also observed between high and low phosphorus treatments for all variables except 100 seed weight. Genotype x environment interactions were significant for seeds per pod, pods per plant and especially significant for yield, indicating that phosphorus levels had a differential effect on the genotypes, whereby some genotypes responded better to high phosphorus while others were more severely affected by low phosphorus, and suggesting that these traits were of lower heritability. Meanwhile, the variables for pod length, pods per raceme, 100 seed weight and harvest index showed no significance in genotype x environment interaction even though they had strong genotype and location effects, indicating that these traits were less sensitive to a differential effect of phosphorus levels. Therefore, the heritability of these traits is likely to be higher than for the ones mentioned above. Yield and yield components, except for 100 seed weight and pod length, were highly correlated with climbing ability and the correlation coefficients were similar in both environments (data not shown). Beans with good climbing ability also had a slight advantage in terms of pod length and seed weight but this was not highly correlated with climbing ability especially in low phosphorus. Frequency histograms (Figure 1) showed lower yield and pods per plant in low phosphorus and a wider range among the RILs for these traits under high phosphorus, suggesting that better fertilization was more favorable for differentiating the yield potential of the genotypes.

Table 1. Analysis of variance for location, genotype and genotype x location effects for RILs from the G2333 x G19839 population grown under low and high phosphorus.

SOURCE	DF	PL	P/R	S/P	100S	P/P	HI	Y/P
Rep (Loc)	2	31.82 ***	0.05 ns	3.11*	0.43ns	6.58**	2.03ns	10.05***
Loc	1	150.25***	68.33***	42.71***	2.49ns	218.95***	7.34**	323.98***
RIL	84	1.97***	2.31***	1.89**	2.22***	2.94***	3.05***	3.01***
Loc x RIL	79	0.95 ns	1.25ns	1.54*	0.11ns	1.52*	1.14ns	2.47***

(a) Significance at p = 0.001 (***),0.01(**),0.05(*),or not significant (ns); (b) trait abbreviations given in text.

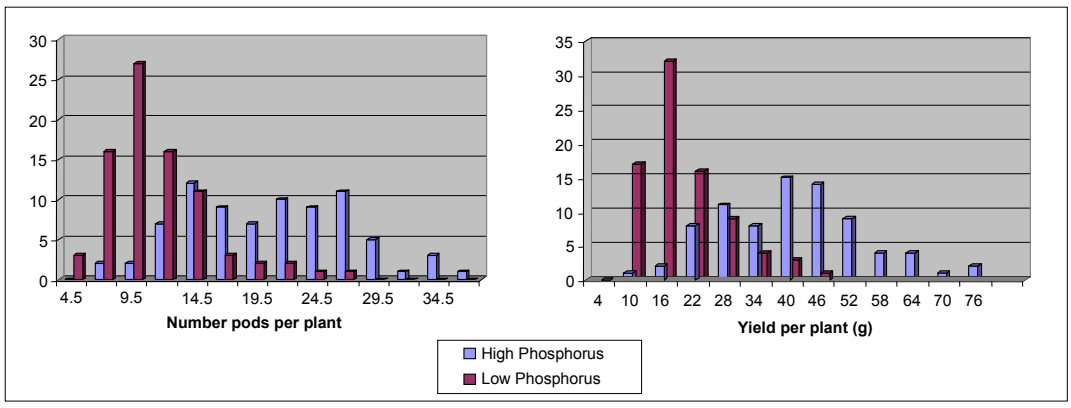

Figure 1. Frequency distribution of two yield traits in the G2333 x G19839 recombinant inbred line population under low and high phosphorus treatments.

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Performance of Common Bean Families from Crossing of Andean and Mesoamerican Lines

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Introduction

In some regions of MG state, Brazil, some races of *Phaeoisariopsis griseola* (Sacc.) Ferr. infect mainly cultivars of Mesoamerican origin, and the Andean cultivars like Jalo are resistant. Nevertheless, using Andean cultivars as source of resistance to this pathogen has been difficulted due to the impossibility of hybridization between beans of those two origins, because they are incompatible (Singh and Gutiérrez, 1984; Vieira et al., 1989). The genetic control of this incompatibility has been explained by the presence of two genes (Dl_1 and Dl_2) with double recessive epistasis (Shii et al., 1980). The Andean cultivars have a $dl_1dl_1Dl_2Dl_2$ genotype and the Mesoamerican $Dl_1Dl_1dl_2dl_2$. Among cultivars from the Mesoamerican type, the Carioca-MG shows high grain yield and upright plant type, however, it has small grains and it is susceptible to *P. griseola*. This cultivar probably has the genotype dl_1dl_1 dl_2dl_2 and therefore can cross with Andean cultivars. This work has the purpose to select families that associate the good seed size of carioca, high grain yield and *P. griseola* resistance from crossing Carioca-MG x ESAL 550 (Andean line group).

Material and Methods

Carioca-MG cultivar has cream color grains with dark brown stripes, the weight of one hundred seeds is about 20 to 22 g, upright plant type and it is susceptible to angular leaf spot. ESAL 550 line has large yellow grains, one hundred seed weight is around 50 g and it is resistant to *P. griseola*. In 2001, there were obtained F_1 and F_2 generation and lately the $F_{2:3}$ families. On February 2002, 194 $F_{2:3}$ families and two parents were evaluated in the field. The experimental design used was a simple lattice 14 x 14 and 1 meter line plot. From those 194 families 98 were selected in the $F_{2:4}$ generation that showed the severity of *P. griseola* under score 4 (Table 1). On fall-winter crop of 2002, sowed in July, those families and parents were evaluated using a triple lattice design 10 x 10 and two lines of two meters plot. 62 $F_{2:5}$ families were selected, and showed grains type like carioca and high grains yield. The 62 families and the parents were also evaluated in the dry season of 2003 that were sowed in February. The experimental design was a triple lattice 8 x 8 and two lines of two meters plot. Grain yield data of three generations and *P. griseola* severity score for $F_{2:3}$ and $F_{2:5}$ generations were used. $F_{2:4}$ generation was not evaluated due to absence of disease.

Results and Discussion

High difference was observed among families for severity scores of *P. griseola* ($P \leq 0,01$) showing that there is genetic variability. The estimates of genetic variance (σ^2_{Gi}) among families were relatively high (Table 1). Carioca-MG cultivar confirmed its susceptibility showing the highest score for pathogen severity (Table 1). ESAL 550 line practically did not show any

symptoms (Table 1). Although had occurred variation among the families in all cases the pathogen average severity was lower than 'Carioca-MG'.

High genetic differences of the families was observed for grain yield in three generations. The heritability (h^2_i) estimates were high except for $F_{2:4}$ generation (Table 1), although the average grain yield of the families was lower than their parents in $F_{2:4}$ and $F_{2:5}$. According to Jonhson and Gepts (2002) the Andean and Mesoamerican common bean had been domesticated in different regions, and developed specific genic complex and epistatic interactions. When crossed, those combinations are dissolved and hardly obtained promising lines. Nevertheless, there were identified some lines with high grain yield associated with high resistance to *P. griseola* and grain type closest to the market demanding standard.

Table 1. Genetic and phenotypic parameters estimates of grains yield ($g/2m^2$) and severity scores of *P. griseola* obtained in the $F_{2:3}$, $F_{2:4}$ and $F_{2:5}$ families from crossing Carioca-MG x ESAL 550;

		$F_{2:3}$		$F_{2:4}$	$F_{2:5}$	
		Grain Yield	Angular leaf spot[2]	Grain Yield	Grain Yield	Angular leaf spot[2]
Number of Families		194	194	98	62	62
Genetic Variance	σ^2_G	1041,77	0,208	1163,74	4937,81	0,222
	LI[1]	853,5	0,172	897,1	3582,6	0,161
	LS[1]	1285,4	0,256	1570,5	7242,4	0,325
Heritability	h^2 %	66,0	47,0	26,0	74,0	45,0
	LI[1]	54,0	28,9	-3,0	59,0	8,0
	LS[1]	74,0	60,1	48,0	83,0	65,2
Families Mean		441,4	3,03	473,7	393,9	3,75
	LI[1]	165,6	1,0	344,5	264.8	2,56
	LS[1]	1003,7	5,0	622,5	578,7	5,0
CV %		29,8	33,6	20,5	18,1	24,06
Parents Mean						
Carioca-MG		415,0	6,0	539,3	548,3	6,84
ESAL 550		262,8	1,0	482,70	438,4	2,4

[1]Inferior and superior limit of the estimate at 5% probability level, respectively.
[2] Scale of score from 1 to 9, been 1 (resistant) and 9 (susceptible).

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Genotypic Variation in Climbing Ability Traits in a Common Bean RIL Population

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Introduction:

Climbing beans are vines that can be grown in either monoculture using wooden or bamboo trellises or in intercropping with other support crops such as maize, but in either case an important characteristic of climbing beans is their vegetative vigor and climbing ability. A range of climbing bean architecture exists; some are extremely vigorous producing more biomass at the top of the plant (type IVb), while others distribute biomass more uniformly across their the length of their vines (type IVa). Different types are selected by farmers in given situations, depending on climate, cropping system, harvesting method and growing period. Few studies have analyzed the inheritance of climbing ability in common bean or analyzed the interaction of this trait with soil fertility levels. Information about climbing ability and its component traits could be used by plant breeders to develop climbing bean ideotypes for different production systems. Therefore one of our research objectives has been to develop methods to analyze climbing bean growth and apply these to genetic mapping populations. In this research we analyzed a population of recombinant inbred lines derived from the cross of a climbing bean, G2333, by a bush bean, G19839, grown under high and low phosphorus treatments, for traits involved with climbing ability.

Materials and Methods:

In this experiment we used a set of 84 $F_{5:8}$ recombinant inbred lines (RILs) from the cross G2333 x G19839, where G2333 is a climbing bean from Mexico with type IVa growth habit, while G19839 is a landrace from Peru with type IIIa growth habit. The RILs were grown in four experiments: 1) high phosphorus (HP) – Darien 2002A (2 reps); 2) low phosphorus (LP) – Darien 2002A (2 reps); 3) high temperature - Palmira 2002 B (2 reps); and 4) high phosphorus, cool temperatures - Popayán 2003A (3 reps). Plots consisted of single 3m rows planted with a total of 30 seeds. The distance between seeds was 10 cm and between rows was 1.2 m. The parental genotypes, G2333 and G19839, were planted every 10 rows throughout the experiment to use as visual checks. The cropping systems consisted in a bamboo and wire trellis, with strings tied to individual plants within the plot. The trellis had a height of 2 m. The following variables were evaluated for two plants each within a plot and averaged to produce plot values: plant height (PH), internode length (IL) and number of vines (NV). The latter two traits were evaluated at mid plant height. Climbing ability (CA) was evaluated on a 1 to 9 scale (where 1=highly aggressive climber and 9 = no climbing ability). The scale for climbing ability is an expanded scale compared to the scale for growth habit, which goes from I to IV. Climbing ability and plant height were measured both at flowering and again in pod filling stages. Data were analyzed by non-orthogonal contrasts to compare parents and by analysis of variance (ANOVA) in which sources of variation were: environment, genotype and genotype x environment. Randomized complete block designs were used for all four experiments.

Results and Discussion:

In the comparison of the parents, G2333 had significantly taller plant height and longer internode length than G19839 in all four environments (Table 1). The number of vines was also larger in G2333 than in G19839 in all environments, but difference were only significant in Palmira and in Popayán. Similarly, climbing ability (as measured on an inverse scale) was greater in G2333 than in G19839. Meanwhile, in the comparison of the recombinant inbred lines (RILs), the majority of traits showed significant differences, except for 1) climbing ability in the low phosphorus treatment in Darien and 2) number of vines in Palmira and in the high phosphorus treatment in Darien. In general, for both parents and RIL comparisons, the most favorable environment was Popayán, followed by the high phosphorus treatment in Darien, the low phosphorus treatment in Darien and Palmira. Broad sense heritabilities ranged from 0.12 to 0.86 and were higher in the more favorable environments than in the less favorable environments. It was also notable, that the phenotypic trait values of the RILs were wider than those for the parents indicating that transgressive segregation may be acting on climbing ability and its component traits.

Table 1. Phenotypic difference between parents and among RILs, and broad sense heritability (BSH^2) for climbing ability (CA), internode length (IL), number of vines (NV) and plant height (PH) in a recombinant inbred line (RIL) population derived from the cross G2333 x G19839 evaluated at flowering (1) and pod filling (2) growth stages in four environments.

Environment	**Trait**	Parents			RILs			BSH^2
		G2333	**G19839**	**F_c**	**MIN**	**MAX**	**F_c**	
HP- Dairen	PH1	1.97	0.85	8.70^{**}	0.28	2.39	2.46^{***}	0.71
	PH2	2.54	1.28	9.25^{**}	0.73	3.15	2.85^{***}	0.66
	IL2	18.53	7.00	19.41^{***}	3.50	26.50	5.07^{***}	0.81
	CA1	2.95	6.75	19.39^{***}	1.00	7.00	4.47^{***}	0.74
	CA2	2.95	5.60	10.11^{**}	1.00	6.00	3.78^{***}	0.79
	NV2	1.79	1.57	0.16^{ns}	0.50	4.00	1.14^{ns}	0.12
LP- Dairen	PH1	1.37	0.50	8.01^{**}	0.28	1.97	1.77^{**}	0.46
	PH2	1.78	0.96	2.91^{**}	0.50	3.00	1.68^{**}	0.42
	IL2	13.80	5.90	9.16^{**}	4.50	21.50	2.91^{***}	0.67
	CA1	4.00	6.45	4.22^{*}	1.00	7.00	1.39^{ns}	0.31
	CA2	3.90	6.25	4.00^{**}	1.00	7.00	1.36^{ns}	0.29
	NV2	1.23	1.01	0.32^{ns}	0.42	3.25	1.70^{**}	0.41
Palmira	PH2	1.65	0.71	10.52^{*}	0.28	2.20	2.40^{***}	0.61
	IL2	12.80	7.50	4.5^{ns}	4.50	19.95	1.45^{*}	0.34
	CA1	4.65	7.50	12.39^{***}	3.00	9.00	1.90^{**}	0.52
	CA2	4.50	6.95	3.93^{ns}	2.00	9.00	1.61^{*}	0.41
	NV2	2.67	1.42	14.04^{***}	0.38	2.25	1.24^{ns}	0.47
Popayán	PH1	2.29	0.86	37.88^{***}	0.18	2.75	4.54^{***}	0.80
	PH2	2.77	1.70	14.83^{***}	0.17	3.50	4.11^{***}	0.77
	IL2	19.49	12.04	9.89^{**}	5.00	26.50	2.93^{***}	0.67
	CA1	3.35	7.25	58.47^{***}	3.00	8.00	6.68^{***}	0.86
	CA2	2.25	4.95	21.77^{***}	1.00	9.00	5.22^{***}	0.82
	NV2	4.22	3.08	4.48^{**}	0.33	5.75	2.60^{***}	0.66

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RESPONSE OF DRY BEAN CULTIVARS AND LANDRACES TO SEVEN CROPPING SYSTEMS IN SOUTHERN IDAHO

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Introduction

Knowing the performance of dry bean cultivars and landraces under different cropping systems is essential for measuring gains from selection and development of high yielding cultivars for low to high-input sustainable organic and conventional farming systems. Our objective was to evaluate 16 medium-seeded cultivars and landraces of great northern, pinto, and red market classes for yield, 100-seed weight, and days to maturity in seven cropping systems in southern Idaho. Native Americans grew the two landraces, namely Common Pinto and Common Red Mexican, in low-input and dry-land farming systems without supplemental water, chemical fertilizer, and herbicide until the advent of irrigation systems in the Western U.S. in the early 1900's. Fourteen cultivars were developed and released between 1932 and 1999, of which UI 59 was a selection from the local great northern landrace, and all others were developed through hybridization. Since landraces were grown under low-input farming systems it is likely that they possess favorable alleles and quantitative traits loci (QTL) that support adaptation in such environments. In contrast, for the last 75 years breeding has been mostly carried out for irrigated farming systems under relatively high-input weed-free environments for resistance to diseases such as beet curly top virus, bean common mosaic virus, and root rots, as well as upright plant type and early maturity.

Materials and Methods

Four great northern, seven pinto and five red cultivars were evaluated in three on-experiment station cropping systems, namely conventional (EFC), continuous bean cropping (ECB), and drought (EDT). They were also evaluated in four on-farm cropping systems, namely conventional (OFC), low fertility soil (OLF), organic low-input (OGL), and organic high-input (OGH) in the Magic Valley in southern Idaho in 2003. A randomized complete block design with four replicates was used, and each plot consisted of four or eight rows, 25 or 50 ft long. The spacing between rows was 22 inches, and the EFC, ECB, EDT, and OLF trials were grown on the residual soil fertility. The OGL plots received only three irrigations until July 15 (i.e., middle of the growing season), and both OGL and OGH had exceptionally high populations of more than six weed species from approximately four weeks after emergence. Also, the OFC plots had excess salt content and suffered moderate water stress. Data were recorded for growth habit, days to maturity, 100-seed weight, and yield, and were analyzed separately for each cropping system. Subsequently, combined analysis was performed after testing for the homogeneity of variances. Genotypes and cropping systems were considered fixed effects and replicates a random effect.

Results and Discussion

The effects of cropping systems were highly significant (P <0.01) for seed yield, 100-seed weight, and days to maturity. Highly significant differences were observed among cultivars in all cropping systems. Moreover, cropping system and cultivar interactions were also highly significant, indicating that the rank-order of cultivars and landraces for seed yield, 100-seed

weight, and days to maturity changed from one cropping system to another. Therefore, evaluation and identification of high yielding cultivars specific to each cropping system would be needed. Furthermore, selection in contrasting cropping systems may be required for identification of broadly adapted high yielding cultivars across cropping systems. The mean seed yield of 16 dry bean landraces and cultivars was the lowest (271 kg ha^{-1}) in the OGL and the highest (3097 kg ha^{-1}) in OGH (Table 1). The mean seed yields were reduced by 62% in EDT and by 43% in ECB compared to the EFC. To our great surprise seed yields in the OFC were lower than in the ECB. Among the great northern, Matterhorn was the highest yielding across seven cropping systems, and UI 59 followed by US 1140 and UI 465 yielded significantly lower than Matterhorn. Pinto Mesa had the highest yields, and Topaz, UI 320, and Common Pinto had significantly lower yields than Buster, Othello, and Mesa. Among reds, UI 239 yielded higher, but not significantly, than NW 63 and Common Red Mexican, and all three yielded significantly higher than LeBaron and UI 259. Matterhorn in the ECB and OFC, Mesa in EDT, Buster in OGH, Othello in OGL, NW 63 in OLF, and Common Red Mexican in EFC were the highest yielding. Among all cultivars and landraces across all cropping systems the mean 100-seed weight ranged from 26 (Common Pinto) to 35 (Buster, Mesa, and UI 320) grams, and days to maturity varied from 83 (Common Pinto) to 95 (UI 259). The EFC yields were positively correlated with yields in EDT, OFC, and OGH. The ECB yields were positively associated with OFC and OLF yields. Similarly, The OFC yields were positively correlated with EDT and OLF yields. However, the OGL yields were not correlated with yields in any other cropping systems.

Table 1. Mean seed yield (kg ha^{-1}), 100-seed weight (SW, g) and days to maturity (DM) for 16 dry bean cultivars and landraces evaluated in seven cropping systems in southern Idaho in 2003.

Identification	MC	EFC	ECB	OFC	OLF	OGH	OGL	EDT	Mean	SW	DM
Matterhorn	GN	1905	1909	1276	3068	2808	149	979	1728	32	90
UI 59	GN	1622	661	478	2155	2815	213	333	1182	28	91
UI 465	GN	1797	816	906	2111	3083	327	687	1390	31	92
US 1140	GN	1517	751	616	2603	3178	332	529	1361	28	84
Bill Z	PT	1998	827	823	2194	3323	458	639	1466	30	90
Buster	PT	1927	1602	584	2474	3540	184	488	1543	35	94
Common Pinto	PT	1520	991	679	2477	2672	356	359	1293	26	83
Mesa	PT	2039	1006	1006	2808	3470	267	1312	1701	35	91
Othello	PT	1805	963	923	2374	3192	480	1199	1562	33	84
Topaz	PT	1559	375	271	1853	2830	293	155	1048	32	90
UI 320	PT	1407	891	693	1962	2969	207	502	1233	35	90
Common Red Mexican	RD	2162	938	779	2379	2977	211	1164	1484	27	88
Le Baron	RD	1732	833	625	1587	3111	288	640	1247	30	88
NW 63	RD	2008	1059	726	3104	3218	143	824	1583	28	89
UI 239	RD	1955	1498	983	2841	3497	242	724	1677	28	87
UI 259	RD	1850	1309	590	1752	2874	193	471	1291	30	95
Mean		1800	1026	747	2371	3097	271	688	1425	30	89
LSD(0.05)		204	741	269	836	596	198	630	262	1.7	3.3

PARTICIPATORY SELECTION OF YELLOW, BROWN, SUGAR AND TAN BEAN MARKET CLASSES IN EASTERN CONGO

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Introduction

Yellow, brown and tan bean cultivars are widely consumed and traded in the Great Lakes region of Eastern Africa. These cultivars are often grown and sold as mixtures. The region is known for some of the greatest diversity in bean germplasm. Yellow, brown and tan seed types are rated as highly important in DR Congo, Rwanda, Burundi, Angola, Zambia and parts of Ethiopia, Kenya, Tanzania, Uganda, Sudan, Madagascar and Mozambique. They account for 11% of Africa's bean production. Productivity of these grain types in constrained by diseases especially angular leaf spot, anthracnose, common bacterial blight, root rots and soil fertility stress factors especially acidity, low soil nitrogen and phosphorus. Development of yellow, brown and tan grain types is one of the priorities of the regional bean program. A regional program was started in 2001 to develop yellow, brown and tan bean cultivars with improved tolerance to two or more biotic and abiotic stresses for smallholder farmers in East and Central Africa. Selection for genotypes tolerant to priority production constraints from existing and new breeding populations was conducted collaboratively by INERA (Mulungu, DR Congo) and University of Nairobi (Kabete, Kenya). Eastern Congo is one of the leading producers of yellow, brown and tan grain types. These market classes account for 49.5 % of total bean production in North Kivu, 16.5 % in south Kivu, 10.5% in the Oriental Province and Ituri, and 8.20% in Oriental Kasaï. Eastern Congo is main producing bean area in DR Congo. The common bean is a staple food which provides more than 45% of protein in the diet of the poorer people of the countries comprising Burundi and Rwanda in the Great Lakes region. The breeding program has been focused on participatory development of new improved marketable bean varieties, in partnership with national and international research institutions, farmers and farmer's association, NGOs, private sector and other stakeholders. Participatory selection was adopted to improve identification of marketable lines and adoption.

Materials and Methods

Breeding populations were generated from crosses and backcrosses among parents with resistance to angular leaf spot, bean stem maggot, tolerance to low soil nitrogen, phosphorus and acidity, and locally important susceptible cultivars (Nakaja, Kirundo and Munyu). Additional segregating populations and advanced lines were received from the regional bean breeding program at Kabete, Kenya. Sources of resistance to angular leaf spot included G5686, Mexico 54, Jalo EEp 558, MAR 1 and A235. Resistance to bean stem maggot was contributed by Besh Besh, Acc 714, G 11727 and G 8074. COM 9315-1, G5889 and More 92018 provided tolerance to low soil nitrogen, VEF 88(40) to low soil P. AFR 708, RWR 1873 and LSA 144 were sources of tolerance low soil pH complex. The parents were crossed pairwise in a diallel scheme and the final combination used as male parent in the final cross. The hotspots for these constraints were created to screen the segregating populations derived from the crosses. Lines combining tolerance to two or three stresses and preferred grain type were selected following pedigree procedure in the early generations, followed by participatory evaluation by farmers on-station and on-farm.

Results and Discussion

Twenty-four 24 brown and tan bean market classes, 25 sugars, 28 red mottled and 17 yellow bean market class were finally selected in 2003. The characteristics of some of the lines are shown in Tables 1 and 2. Breeding capability has been strengthened by the local crossing activities which generated additional genetic diversity toward selection purpose. The new lines were coded as CODMLB or CODMLV which stand for Congo Democratic Mulungu Lines, Bush or Climbers as shown in the Tables 1 and 2.

Table 1. Grain type, 100-seed mass, duration to flowering and grain yield of bush bean lines after participatory evaluation over two seasons at Mulungu, DR Congo.

Line	Grain type	100-seed mass (g)	Days to 50% flowering	Days to 95% maturity	Grain yield (kg ha-^{1})		
					2002A	2002B	Mean
SEQ 1006	Red mottled	41.0	49	94	695	1874	1285
M'MAFUTALA (Check1)	Brown	22.8	42	94	850	1697	1274
KIRUNDO (Check 2)	Yellow	37.9	42	87	834	1689	1258
RWK 5	Sugars	17.1	49	90	838	1599	1218
M 1/98	Sugars	37.5	42	94	483	1838	1160
MLB 207/96B	Sugars	18.7	53	94	629	1669	1149
DOR 481	Sugars	24.0	42	94	873	1288	1058
ZKA 94-12M/95	Sugars	33.0	42	84	686	1346	1016
MLB 174/94B	Brown	24.0	42	94	875	1063	969
ZKA 98-6M/95	Brown	21.9	49	94	424	1222	950
MUNYU (Check 3)	Cream	48.0	40	84	470	1417	944
ITURI MATATA		34.5	40	84	379	351	367
GENOTYPES (G)					**	**	
SEASONS (S)					**	**	
G X S					**	**	
MEAN					612	1111	862
L.S.D$_{.05}$					250.7	501.6	392.1
C.V. %					25.0	27.6	28.1

**Significant at 1% probability level.

Table 2. Grain type, 100-seed mass, duration to flowering, maturity and grain yield of climbing bean lines selected after participatory evaluation over two seasons at Mulungu, DR Congo.

VARIETY NAME	Grain type	100-seed mass (g)	Days to 50% flowering	Days to 95% maturity	Grain yield (kg ha-^{1})		
					2002A	2002B	Mean
CODMLV 044	Purple	33.0	47	98	904	2125	1514
CODMLV 045	Brown	35.8	51	98	1002	1686	1344
G 59/1-2 (Check 1)	Brown	39.9	45	98	1014	1445	1229
CODMLV 046	Brown	30.2	51	110	596	1741	1169
CODMLV 047	Brown	35.2	47	98	468	1835	1151
CODMLV 048	Tan	36.0	45	98	468	1709	1083
MLV 135/97B	White	24.5	51	110	840	1321	1081
CODMLV 049	Tan	46.7	45	98	779	1364	1072
MLV 224/97A	Brown	33.7	51	110	542	1422	982
AND 10 (Check 2)	Sugars	33.8	51	110	921	998	959
GENOTYPES (G)					**	**	
SEASONS(S)					**	**	
G X S					NS	NS	
MEAN					628	1386	1007
L.S.D $_{.05}$					286.0	723.3	574.0
C.V. %					27.3	31.3	32.7

**Significant at 1% probability level.

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ECONOMIC EVALUATION OF BEAN-RESEARCH INVESTMENT IN MEXICO

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In Mexico, dry beans are the second most important crop after maize, both in terms of production and consumption. During the 1990s, the harvested bean area averaged 1.9 million hectares, with an average yield of 632 kg/ha, and an average production of 1.2 million Mt. Approximately 85% percent of the country's bean crop is grown under rainfed conditions. During the 1996-2000, the total harvested bean area decreased by 2.0%, average yield decreased by 2.5%, and production declined by 4.5%, compared to 1990-1995 (SAGAR 2000). As a result, there has been an increasing trend in requiring bean imports to meet domestic demand, especially after Mexico joined NAFTA in 1994. During the 1990s the Mexican government, through the Secretariat of Agriculture (SAGAR), started two programs—PROCAMPO and Alliance for the Countryside—to support farmers and promote the adoption of improved varieties for most important crops through the Kilo per Kilo subprogram.

In 1982, the Bean/Cowpea Collaborative Research Support Program (CRSP) signed an agreement with Mexico's National Research Institute for Forestry, Agriculture and Livestock (INIFAP) to collaborate in developing improved bean varieties for the semiarid highlands of Mexico's North-Central region. During 1990-2000, INIFAP released several improved bean varieties that were distributed via the Kilo per Kilo program and adopted by farmers in the semiarid region.

Numerous studied have demonstrated the critical role that increasing agricultural production plays in the process of economic development and the key contribution of research in promoting growth in agricultural production (Alston *et al.* 1998, 1999). However, now facing tighter budgets, research administrators are increasingly being asked to provide evidence than the costs of public-sector funded research are justified by the benefits.

Thus, the government of Mexico needs to justify its investments—as do other governments and donors—because the economic value of public investment may not be obvious. It is particularly difficult to observe the impact of bean research because the benefits are diffused over many years and millions of dispersed producers and consumers. Without an economic analysis, it is difficult to assess the social value of new technologies and to make informed judgments about the trade-offs in allocating scare scientific resources (Alston *et al.* 1998; Masters 1996). An economic impact assessment of bean research is essential to provide decision-makers with information needed to improve the allocation of research resources.

This study generates insights that meet the information needs of the main stakeholders of bean research investments: 1) government decision-makers, who desire information on the payoff of agricultural research, since it competes with alternative uses for public funds; 2) research administrators, who desire information on the expected payoffs from funds allocated to alternative research investments, and 3) the general public (consumers and producers included),

who has become increasingly concerned about the productivity of their tax payments and government investments (Norton and Davis 1981).

The objectives of the study were to describe Mexico's bean subsector, analyze the factors associated with adoption of the improved bean varieties released by INIFAP in the 1990s, identify factors that contributed to explaining the participation of farmers in the government's seed distribution program (Kilo per Kilo), and estimate the net social gains generated by public investment in agricultural research and extension to develop and distribute improved bean varieties in northern Mexico.

The study area includes the states of Chihuahua, Durango, and Zacatecas, which account for 62% (1.15 million hectares) of the Mexico's rainfed bean production area. The results reported in this study include a rapid appraisal assessment of the bean subsector, an evaluation of government support policies affecting the bean subsector, a statistical and econometric analysis of improved bean seed adoption and farmer participation in the Kilo per Kilo program (based on survey data), and an estimation of the economic returns to public investment in bean research and extension (using the economic surplus method). The farmers' survey focused on analyzing the adoption of the improved bean varieties released by INIFAP during 1990-1996: three pinto beans (Pinto Villa in 1990, Pinto Mestizo in 1996, and Pinto Bayacora in 1996); two black beans (Negro Altiplano in 1996 and Negro Sahuatoba in 1996); and one light-colored bean (Flor de Mayo M38 in 1994).

The adoption analysis indicates that the improved bean varieties Pinto Villa and Pinto Mestizo have been widely adopted in Chihuahua and Durango, that these varieties have yields that were 20.6% higher that traditional pinto bean varieties, and that the yield difference was statistically significant. The economic analysis indicates that if a closed economy model is assumed, the financial (US$ 1,853,360) and economic (US$ 3,083,879) NPVs are positive and the IRRs are 17.5 and 21.4%, respectively. If an open economy model is assumed, the financial (US$ 2,760,108) and economic (US$ 2,558,186) NPVs are positive and the IRRs are 21.3 and 20.7%, respectively. The results from both models are consistent and suggest that public investment in bean research and extension was profitable (opportunity cost of capital=10%). Thus, the government should continue investing in bean research in northern Mexico to promote agricultural development and to improve the level of welfare of farmers under rainfed conditions and low-income consumers.

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Selection of Climbing Bean Lines Tolerant to Common Bacterial Blight, Bean Common Mosaic and Web Blight

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Introduction

Climbing beans offer a new opportunity for increasing bean yields in East and Central Africa because of their high yield potential. Improved climbing beans were introduced by CIAT to the Great Lakes region (Rwanda, DR Congo and Burundi) in the late 1980's. They were rapidly adopted in this region because of traditional experience with climbing bean landraces. Improved climbing bean cultivars are attractive to farmers because of their high yield potential of 3:1 compared to bush cultivars in favourable environments. Climbing beans are particularly important in areas with high population pressure due to declining availability of arable land. They spread rapidly from Great Lakes region to Kenya and Uganda in the early 1990's. They are now being experimented with in nearly all nine countries comprising the East and Central Africa Bean Research Network (ECABREN). However, the expansion of climbing bean technology is threatened by diseases especially root rots, bean common mosaic, anthracnose, angular leaf spot. common bacterial blight and ascochyta, For example, Umubano which was released in Rwanda and Uganda, and is widely adopted in Western Kenya, is susceptible to fusarium wilt and bean common mosaic virus. As a result, many farmers in Rwanda stopped growing it. Cultivars resistant to these important diseases are required to stabilize production. The objective of this study was to screen a regional climbing nursery for disease resistance.

Materials and Methods

Seventy-five climbing bean lines were screened for disease resistance at the Kenya Agricultural Research Institute (KARI) station in Ol Jorok during 2001 and 2002. Ol Jorok (2350 masl) is a 'hotspot' for several bean diseases (bean common mosaic virus, anthracnose, angular leafspot, web blight, ascochyta and halo blight). The site has a uni-modal rainfall pattern starting in April/May with a peak in August. Soils are moderately fertile. The test lines originated from DR Congo (45), Rwanda (4) and CIAT (25). Each entry was sown on two, 5 m rows. Plants were staked to 2 m at two weeks after germination. The trial was replicated twice. The entries were rated at flowering (R6) and at mid-pod fill (R8). Disease assessment was based on natural epiphytotics. CIAT scale was used for disease rating.

Results and Discussion

Conditions were favourable for development of common bacterial blight, bean common mosaic (BCMV) and web blight. Some characteristics of the 26 lines selected for resistance to one or more diseases and vigour are shown in Table 1. Duration to flowering varied from 57 days for Nakaja to 94 days for G 50330. Thirteen of the selected lines were small seeded, 7 medium and 6 were large seeded. Twenty-five of 75 lines were rated resistant to common bacterial blight. Five of the lines susceptible to common bacterial blight failed to produce any seed. Thirty-three lines

were susceptible to BCMV. Both Umubano and Vunikingi, regionally popular cultivars, were susceptible to BCMV. Thirty-four lines were resistant to BCMV. Eight showed intermediate reactions. Six test lines were susceptible to web blight; 4 showed intermediate reactions and 65 were resistant. Disease pressure for anthracnose, ascochyta and halo blight was low and for angular leaf spot was moderate. MLV76/97A and A235 were susceptible to angular leaf spot. All other materials showed intermediate to resistant reactions to angular leaf spot. In 2002, most of the lines were destroyed by frost. These results indicate potential new sources for resistance to BCMV, common bacterial blight and web blight.

Table 1. Duration to 50% flowering, seed size, grain type and reaction to bean common mosaic virus, web blight and common bacterial blight of 26 climbing bean lines selected at Ol Jorok in Kenya (2001-2002).

Line	Days to flowering	#Seed size	Grain type	*CBB	*BCMV	Web blight	Other observations
MLV 59/97A	72	M	sugar	5	2	4	
MLV 76/97A	63	S	brown	1	1	2	
Kirundo	76	L	yellow	1	7	1	ALS susceptible
VCB 87012	70	M	brown	1	1	1	
Nakaja	57	S	brown	1	5	1	vigorous
AND 10	85	L	sugar	1	1	1	vigorous
VCB 81012	61	S	brown	1	7	1	
M'Sole	76	S	brown	1	2	2	
AFR 441	71	M	zebra	1	1	3	
MLV 198/97A	71	S	zebra	2	1	3	
MLV 6/90B	71	S	brown	1	1	9	
MLV 222/97A	69	S	white	1	1	1	vigorous
MLV 56/96B	62	S	brown	1	5	1	
MLV 227/97A	63	S	brown	1	1	1	
MLV 216/97A	85	S	black	1	1	1	
Cuarentino 0817	63	S	white	1	1	1	
SEQ 1006	70	L	zebra	1	3	4	
G59/1-2	71	L	brown	2	7	1	
Naindekyondo	76	S	white	1	2	1	
G50330	94	M	brown	1	2	1	
G24517	76	M	yellow	3	1	1	
G20875	86	L	brown	1	7	1	
Gisenyi	91	L	sugar	1	2	1	
G20833	85	S	black	3	9	1	
G31479	76	M	black	2	7	1	
G20751	84	M	yellow	2	7	1	

Seed size: S= small (< 25g/100 seeds), M= medium (25-39 g/100 seeds) and L=large (> 40 g/100 seeds)
* CBB= common bacterial blight, ALS= angular leaf spot and BCMV= bean common mosaic virus).

Screening for Drought tolerance in Eastern Africa

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Introduction

Drought is one of the most important constraints to bean production in East, Central and Southern Africa. Over 396,000 t of grain are lost annually in Africa due to drought (Wortmann et al, 1998). Drought may occur early in the season, mid-season or late in the cropping season. Drought is ranked as major constraint to bean production in Kenya, Ethiopia, parts of south western Uganda, northern and central Tanzania, South Africa, southern Rwanda, Sudan, Angola, central plateau of Madagascar and southeast DR Congo. Although the adverse effects of drought can be alleviated through irrigation, few smallholder bean growers in East and Central Africa (except in Sudan) have access to irrigation water. Bean production in this region is predominantly rain fed. Crop failures are frequent. Growing drought tolerant bean cultivars is probably the most cost-effective strategy for smallholder, resource-poor farmers in drought prone environments. However, few drought tolerant cultivars are available in sub-Saharan Africa. CIAT has been screening bean cultivars for drought since 1983 (Laing et al, 1983) as part of integrated genetic improvement of the common bean (Teran and Singh, 2002). Recently, an international drought nursery was constituted which included the most promising drought tolerant lines. This report highlights the performance of this nursery in trials conducted in Eastern Africa.

Materials and Methods

Thirty-six drought tolerant bean lines including two susceptible checks were evaluated at Thika, Kenya in 2001, 2002 and 2003. Each year, the trial was laid out in 6 x 6 lattice design with three replicates. The 36 genotypes were evaluated in drought stressed and non-stressed environments for the three cropping seasons. Each entry was sown on four, 5 m rows. Data was recorded from the two inner rows. Entries in non-stressed plots were provided with 1 to 2 supplemental irrigations. In stressed plots, the entries were grown under natural rain fed conditions. For data analysis, the cropping seasons (environments) and replications were considered as random effects, whereas irrigation treatments (stress levels) and genotypes were fixed effects. All data was analyzed using Genstat (6ed, 2002) statistical package. Two local cultivars, GLP x 92 and GLP 585 were included as checks.

Results and Discussion

There were significant grain yield differences due to environments, stress levels and genotypes (Table 1). Significant environment x stress levels, genotype x stress level, genotype x environment interactions were detected. This indicated that performance of the genotypes varied with stress level and with environments. Yield reduction due to drought was highest in 2001 (58%) but remained at 40% in 2002 and 2003. The ten most promising lines under both stress and no stress conditions are shown in Table 1. SEA 16 and SEA 20 consistently ranked among the top five best yielding lines under stress conditions for the three seasons. RAB 608, RAB 636 and INIB 35 ranked among the top five for two seasons under stress conditions. However, SEA 23, RAB 608, SEA 16 and RAB 618 were the best yielding lines under stress and no stress

environments. These four lines out yielded all the checks. These results indicate new possibilities of stabilizing bean yields in drought prone environments in Eastern Africa. These lines are potential sources for breeding drought tolerant marketable bean cultivars.

Table 1. Grain yield (kg ha-[1]) of drought tolerant lines grown under stress and no stress conditions over three seasons at Thika, Kenya, 2001-2003.

Genotype	2001		2002		2003		
	No stress	Stress	No stress	Stress	No stress	Stress	Mean
RAB 608	1435	986	935	513	3031	739	1273
SEA 23	2780	562	1048	585	2405	1032	1402
RAB 636	1196	621	1075	443	2551	983	1145
SEA 16	1885	792	1236	972	1467	1105	1243
RAB 618	1551	564	861	896	1956	861	1115
Pinto Villa	1437	279	1188	407	2119	836	1044
SEA 20	1298	806	1704	681	1139	862	1082
INB 38	1370	528	1269	1091	1149	841	1041
INB 35	1432	881	1207	992	1287	697	1083
INB 39	1468	388	1162	828	1180	823	948
Checks							
Tio Canela	1253	300	1264	664	1591	1059	1022
SEA 5	1934	570	1316	546	1040	546	992
GLP x 92	698	676	1124	265	1288	757	801
GLP 585	1154	685	1267	512	1113	523	876
Trial mean	1561	654	1072	652	1340	801	1037

Reps/Environments	
Environments (E)	**
Stress levels (S)	**
Genotypes (G)	*
E x S	NS
G x E	**
G x S	**
G x E x S	**
Residual	

*, **: Significant at 5 and 1% probability levels, respectively; NS= not significant

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'BRS CAMPEIRO': NEW BLACK BEAN CULTIVAR RECOMMENDED FOR THE SOUTH REGION OF BRAZIL

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Common beans is an important source of protein for the Brazilian people, especially those of low income, with a "per capita" consumption of 13.6 kg and a total production of 2.37 million tons in the 2001/2002 year. These numbers rank Brazil as the largest producer and consumer of common beans in the world. There is regional preference as to the seed color, with the carioca type predominating over the country. Overall consumption of black beans reach 17%, mainly for the State of Rio de Janeiro and those of the South Region.

The cultivar BRS Campeiro is the result of a mutational program to alter the color of the tegument of cultivar Corrente, developed by Embrapa Rice and Beans.

Seeds of the cultivar Corrente, cream color, were submitted to gamma radiation at the Nuclear Energy Center for Agriculture (CENA) at the University of Sao Paulo, Piracicaba, SP. Progenies from M_1 to M_6 were then conducted at Embrapa Rice and Beans to select for seed type and plant architecture by the pedigree method of breeding associated to bulk selection. Some breeding lines were selected at the initial steps and evaluated in replicated trials. From these emerged the line MT 95202057, with black seed, upright growth habit and superior yield potential.

From a total of 34 field trials for cultivar conducted in the South Region of Brazil MT 95202057 showed 32% greater seed yield than the average of the control cultivars (Table 1). With such a result, BRS Campeiro was released in 2003 for cultivation in the South Region of Brazil for the wet and dry cropping seasons.

Table 1. Yield of BRS Campeiro compared to the mean of two controls in the field trials from 1999 to 2002.

State	BRS Campeiro (kg/ha)	Mean for controls[1] (kg/ha)	Relative yield (%)	Number of trials
Rio Grande do Sul	1939	1550	125	5
Santa Catarina	2695	2060	131	17
Parana	2519	1857	137	12
Mean	2519	1907	132	-

[1]Controls: Diamante Negro and FT Nobre.

The cultivar BRS Campeiro has excellent seed color uniformity and a mass of 25.4 g per 100 seeds, besides high cooking quality (Table 2).

Table 2. Technological and industrial quality of seeds from the cultivar BRS Campeiro compared to other black bean cultivars.

Cultivar	Cooking time (minutes)	Soluble Solids(%)	Broth Color	Protein (%)	Fiber (%)	Tegument (%)
BRS Campeiro	25.00	8.86	Dark	22.80	14.00	8.84
BRS Valente	28.10	10.91	Light[1]	19.25	9.70	11.75
FT Nobre	28.48	11.05	Light[1]	21.60	----	13.48
Rio Tibagi	36.00	12.40	Dark	20.00	12.50	13.10
Diamante Negro	34.02	11.20	Light[1]	20.00	10.00	11.40

[1]Chocolate brown.

Under artificial inoculation BRS Campeiro shows resistant reaction to the bean common mosaic virus (I gene) and intermediate reaction to the *C. lindemutianum* pathotypes 89, 89-AS, 95 e 453. Under field conditions, it showed intermediate reaction to rust and angular leaf spot, and susceptibility to common bacterial blight.

The cultivar BRS Campeiro has upright plant habit under all growing conditions evaluated. It has also good resistance to plant lodging during its cycle of 85 days from emergency to physiological maturity.

Due to its high yielding potential and excellent cooking quality, upright plant architecture and resistance to lodging, the cultivar BRS Campeiro is a new option for black bean growers in the States of Rio Grande do Sul, Santa Catarina and Parana, for the wet and dry cropping seasons.

Genetic seed stocks are maintained by Embrapa Rice and Beans and basic seed is available at Embrapa Technology Transfer.

Institutions of participating scientists:

Embrapa Arroz and Feijão; Embrapa Trigo; Epagri - Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina; Coopercampos; Cefet0-Pato Branco; Iapar - Instituto Agronômico do Paraná; Embrapa Negócios para Transferência de Tecnologia/Escritório de Negócios de Ponta Grossa.

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'BRS REQUINTE': NEW COMMON BEAN CARIOCA CULTIVAR WITH DELAYED GRAIN DARKNESS

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Common bean constitutes the basic vegetal protein food in the Brazilian's daily diet, with a consumption, "in natura", of 16 kg/inhabitant/year. This leguminous crop is cultivated all year around, in several ecosystem diversities, in about 2,69 million ha, producing 2,34 million tons. These data classifies Brazil as the biggest common bean producer and consumer around the world. Usually, the Brazilian production has been enough in supplying the internal market, except for the black and white beans, which represent an average import of 80 and 20 thousand tons/year, respectively.

The Brazilian regions are well defined regarding to the preference for the type of grain, including traits such as size, color, form, shining, darkening and cooking quality. In Brazil, carioca is the most demanded grain type, representing around 70% of the total bean consumption. One of the greatest problems faced by the carioca grain type producers is the fast darkening of the grain tegument, economically depreciating the product and impeding its storage for long periods, what is a great disadvantages for the farmer.

The cultivar BRS Requinte was derived from the cross Carioca MG//POT 947/AN 910523 accomplished by Embrapa Rice and Beans. The F_2 to F_4 population was advanced in bulk. The F_5 population was planted at Embrapa Rice and Beans, inoculated with the pathotype 89 of *Colletotrichum lindemuthianum* and individual plant selections were made based on earliness, plant vigor and disease reaction. From the F_6 families it was selected the line LM 95102682 on the basis of its productivity, architecture and disease resistance.

In the year of 1997, LM 95102682 and 42 other lines were evaluated, in the National Bean Trial carried out in 11 environment, in the Brazilian States of Goias (2), Mato Grosso (1), Mato Grosso do Sul (3), Minas Gerais (1), Bahia (1), Pernambuco (2) and Espirito Santo (1).

The joint analysis of yield and other agronomic traits allowed the line LM 95102682 be promoted to the Regional Bean Trial of 1999/2000. In this trial it was evaluated with 12 other lines and five checks, in a completely randomized block design with four replications using the recommended technologies for the different cultivation systems, in a total of 29 environments in the States of Goias (10), Federal District (1), Minas Gerais (13), Mato Grosso (2) and Mato Grosso do Sul (3).

In the 29 regional trials, the line LM 95102682 outyielded the checks by 8,4% (Table 1). These results allowed its release in 2003 with the trade name BRS Requinte, for cultivation in the States of Goias/Federal District, Mato Grosso, Mato Grosso do Sul and Minas Gerais, during the dry and winter seasons. This new cultivar has a very uniform grain color, excellent cooking quality (Table 2) and the seeds averages 24.0 grams 100 $seed^{-1}$. BRS Requinte presents the

advantage of keeping grain tegument color with no major alterations for a longer period of time when compared with the checks.

This cultivar presents a semi-prostrate growth habit, low resistance to plant lodging in the majority of the bean production systems tested and requires 87 days from seedling stage to physiological maturity.

Table 1. Yield of the cultivar BRS Requinte compared to the mean of control cultivars in the years 1999 and 2000.

Region	State	'BRS Requinte' (kg/ha)	Mean for controls[1] (kg/ha)	Relative yield (%)	Number of sites
Southeast	Minas Gerais	3069	2820	110.3	13
	Goias/Federal District	2797	2818	100.5	11
Center West	Mato Grosso	1381	1259	114.7	2
	Mato Grosso do Sul	1997	1735	120.7	3
Mean		2709	2574	108.4	

[1]Controls: Perola and Iapar 81.

Under artificial inoculation, the cultivar BRS Requinte showed to be resistant to the bean common mosaic virus and resistant, intermediate and susceptible to 9, 7 and 8 *C. lindemuthianum* pathotypes, respectively. In the field trials, it was susceptible to angular leaf spot, rust and common bacterial blight.

Table 2. Technological and industrial grain quality of the common bean cultivar BRS Requinte compared to other cultivars of carioca grain type.

Cultivar	Cooking time (minutes)	Soluble solids (%)	Protein
BRS Requinte	22.0	10.0	20.1
Perola	29.0	9.6	21.3
Iapar 81	29.0	9.4	21.0

Genetic seed stocks are maintained by Embrapa Rice and Beans and basic seed is available at Embrapa Technology and Transfer.

Institutions of participating scientists:

Embrapa Arroz e Feijão; Embrapa Milho e Sorgo; Embrapa Cerrados; Empaer-MT; Agenciarural-GO; Universidade Federal de Viçosa; Universidade Federal de Lavras; Fesurv/Esucarv; Idaterra-MS and TecAgro - Tecnologia em Agricultura Ltda.

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'BRS VEREDA': NEW COMMON BEAN CULTIVAR FROM "ROSINHA" COMERCIAL GRAIN TYPE

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Brazilian bean production suffered significant impact as affected by strong social and economic changes during the last years. This situation suggests that individuals involved in the bean production chain should look for alternatives to better fit consumer demands, signaling plant breeders with the possibility to seek differentiated bean cultivars. On this purpose, Embrapa Rice and Beans releases BRS Vereda, of the "rosinha" (pink) grain type, differing from the traditional black and carioca commercial classes with the objective to supply regional market demands as well as broadening the consumer alternative choices.

BRS Vereda was originated from a multiple cross (HI 822510/CB 733743//LM 30013/Rosinha G2RMC), performed at Embrapa Rice and Beans The bulk method was used in F_2 and F_3 generations. In F_4, after inoculation with the pathotype 89 of *Colletotrichum lindemurhianum*, modified mass selection was performed and susceptible plants were eliminated. One pod per plant was collected from the remaining resistant plants to reconstitute the population. In the F_5 generation the same selection procedure was used, but the plants were harvested individually originating the F_6 families from where the LM 93203304 line was selected based on grain yield and erect plant type. In 1995 this line was assessed together with additional 24 lines and three controls in the National Trial, conducted under nine environments, in the States of Goias (4), Mato Grosso (2), Minas Gerais (2) and Espirito Santo (1). The joint analysis of the grain yield data and other agronomic characteristics provided the elements to promote LM 93203304 to the Regional Trial during the 1997/98 crop season. This time, LM 93203304 was assessed with eight additional lines and four controls in a randomized complete block design with four replications in 28 environments in the States of Goias (11), Federal District (2), Minas Gerais (7) and Mato Grosso do Sul (8) with average grain yield 11.2% superior than the controls (Table 1).

Table 1. Yield of BRS Vereda compared to the mean of control cultivars in the years 1997/1998.

Region	State	BRS Vereda (kg/ha)	Mean for controls (kg/ha)	Relative yield (%)	Number of sites
Southeast	Minas Gerais	2545	2259	112.7	7
Center West	Goias/Federal District	2746	2408	114.0	13
	Mato Grosso do Sul	1648	1662	99.2	8
Mean	-	2397	2156	111.2	

[1]Controls: Rosinha G2 and Roxo 90.

Based on these data it was released in 2002 with the trade name of BRS Vereda, for the States of Goias/Federal District, Mato Grosso do Sul and Minas Gerais. Even though grain yield in Mato Grosso de Sul had been 0.8% less than the controls, disease resistance and superior grain quality provided basis for cultivar indication for this State.

BRS Vereda has uniform grain size and color, average 100 grain mass of 26.3 g, excellent cooking quality and good grain appearance after cooked (Table 2).

Table 2. Technological and industrial quality of seeds from the cultivar BRS Vereda.

Cultivar	Cooking time (minutes)	Soluble solids (%)	Protein (%)	Whole grain (%)
BRS Vereda	27.0	10.8	22.8	95

Under artificial inoculation, BRS Vereda showed resistant reaction to common mosaic virus and to the following *C. lindemuthianum* pathotypes: 89, 585 and 95. In the field trials, it showed resistant reaction to rust, intermediate resistance to angular leaf spot and susceptibility to common bacterial blight.

BRS Vereda presents semi-erect plant type in any crop system and under a variety of soil and climate conditions where it was evaluated. It also presented good lodging resistance throughout its cycle of 93 days, in average, from emergence to physiological maturity.

Due to its superior yield potential and differentiated grain type, associated to excellent cooking performance, semi-erect plant type, resistance to lodging and to major diseases, is an interesting option for producers involved with specialty grain type production, providing a value added commodity for commercialization in the States of Goias/Federal District, Mato Grosso do Sul and Minas Gerais.

Genetic seed stocks are maintained by Embrapa Rice and Beans and basic seed is available at Embrapa Technology Transfer.

Institutions of participating scientists:

Embrapa Arroz e Feijão; Embrapa Milho e Sorgo; Embrapa Cerrados; Embrapa Transferência de Tecnologia/Escritório de Negócios de Sete Lagoas-MG; Embrapa Transferência de Tecnologia/Escritório de Negócios de Goiânia-GO; Empaer-MS; Agenciarural-GO; Universidade Federal de Viçosa; Universidade Federal de Lavras; Coopertinga; Fesurv/Esucarv.

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'BRSMG TALISMA': COMMON BEAN CULTIVAR WITH CARIOCA GRAIN TYPE

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Common bean in Brazil is cultivated all year in several ecosystems, in about 2,69 million ha with a grain production of 2,34 million tons. It constitutes the basic vegetal protein food in the Brazilian diet, totaling a consumption, "*in natura*", of 16 kg/inhabitant/year. States of Paraná and Minas Gerais are the biggest producers, representing 37,7% of the national production. In Brazil, the traditional preference of bean consumption lies on grain of the carioca commercial type.

BRSMG Talisma cultivar is the result of the network program among Lavras Federal University, Viçosa Federal University, Embrapa Rice and Beans and Agricultural State Enterprise of Minas Gerais (Epamig). It was released in 2002 for Minas Gerais and recommended for Paraná in 2003.

BRSMG Talisma is originated from a recurrent selection program based on a population performed in 1990 including BAT 477, IAPAR 14, FT 84-29, Jalo EEP, A 252; A 77, Ojo de Liebre; ESAL 645, Pintado and Carioca,. crossed in complete dialel scheme. In F_2, 2,000 seeds were evaluated in $S_{0:1}$ and $S_{0:2}$ generations being selected 13 families. They were recombined by intercross and, following the same previous scheme, it were selected 18 families of the first cycle. These families were intercrossed and the families were available in $S_{0:1}$, $S_{0:2}$, $S_{0:3}$ and $S_{0:4}$ in three locations in the State of Minas Gerais. This work allowed selection of CII-102 line.

From 1998 to 2001, this line was assessed with two controls (Carioca and Perola) in three different cropping seasons (rainy, dry and winter/irrigated) including 25 environments in Minas Gerais, showing 10.6% higher grain yield than the control cultivars. In 2000 and 2001, line CII-102 was also assessed with the same control cultivars in the rainy and dry cropping seasons in 10 environments in Parana State, out yielding the controls in 20.9% (Table 1).

Table 1. Yield of cultivar BRS MG Talisma compared to average grain yields of control cultivars in 1998 to 2002 in Minas Gerais and Parana.

State	Cropping seasons	Number of sites	BRSMG Talisma	Control Carioca	Control Pérola	Relative yield (%)
Minas Gerais	Rainy season	8	2192	1845	1875	117.8
	Dry season	12	2198	2043	2034	107.8
	Winter/irrigated	5	3311	2982	3146	108.1
	Mean	25	2418	2167	2206	110.6
Paraná	Rainy season	7	2480	1874	2022	127.3
	Dry season	3	1731	1618	1736	103.2
	Mean	10	2256	1797	1936	120.9
General mean			2372	2062	2129	113.2

BRSMG Talisma has uniform grain size and color of carioca type (beige with light brown stripes), average 100 grain mass of 26.5 g, excellent cooking quality and good grain appearance after cooked (Table 2).

Table 2. Grain technological and industrial quality.

Cultivar	Cooking time (minutes)	Soluble solids (%)	Protein (%)
BRSMG Talisma	28.5	9.8	23.8

Under artificial inoculation, cultivar BRSMG Talisma showed resistant reaction to the bean common mosaic virus and to 65 and 89 *C. lindemuthianum* pathotypes. In field trials, it showed intermediate reaction to angular leaf spot.

BRSMG Talisma presents a growth duration cycle varying from 75 to 85 days, in average, from emergence to physiological maturity, being earlier than Perola and Carioca cultivars.

Due to its superior yield potential, associated to excellent cooking performance, resistance to major diseases and earliness, BRSMG Talisma is an interesting option for producers involved with carioca grain type production, in the States of Minas Gerais and Parana.

Genetic seed stocks are maintained by Lavras Federal University and basic seed is available at Embrapa Technology and Transfer.

Institutions of participating scientists:

Universidade Federal de Lavras; Embrapa Arroz e Feijão; Embrapa Milho e Sorgo; Universidade Federal de Viçosa; Iapar - Instituto Agronômico do Paraná; Embrapa Soja: Embrapa Negócios para Transferência de Tecnologia /Escritório de Negócios de Ponta Grossa.

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'BRS MARFIM': NEW COMMON BEAN CULTIVAR FROM "MULATINHO" COMERCIAL GRAIN TYPE

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Dry beans is an important source of protein on daily diet of Brazilian urban and rural people. Due to good adaptability to a variety of weather and soil conditions the dry bean culture takes place in different production systems in small farms. Part of this production is used by the farmer subsistence and the exceeded is sold in the local market. In the Northeast of Brazil, there is a strong demand for mulatinho (small beige) grain type by the small farmers where this crop has a great socioeconomic importance mainly for the low income people. To fill this demand the Embrapa Rice and Beans is releasing the variety BRS Marfim.

BRS Marfim is originated from a multiple cross (BAT 85///A 375/G17702//A445/ XAN112), performed at CIAT. Embrapa Rice and Beans received the developed A774 line and promoted it to the Preliminary Trial in 1991. This line was assessed together with additional 19 lines and two controls in the National Trial, conducted under six environments, in the States of Goias (1), Pernambuco (2), Bahia (2) and Sergipe (2). The joint analysis of the grain yield data and other agronomic characteristics provided the elements to promote A774 to the Regional Trial during the 1995/96 crop season. This time, A774 was assessed with ten additional lines and five controls in a randomized complete block design with four replications in 14 environments in the States of Goias (4), Bahia (6), Pernambuco (1), Rio Grande do Norte (1), Ceara (1) and Paraiba (1) with average grain yield 11.0% superior than the controls (Table 1).

Table 1. Yield of cultivar BRS Marfim compared to the mean of two control cultivars in 1995 and 1996.

Region	**State**	BRS Vereda (kg/ha)	Mean for controls (kg/ha)	Relative Yield (%)	Number of sites
	Bahia	1525	1488	102.3	6
	Pernambuco	2667	2120	125.8	1
Northeast	Rio Grande do Norte	1817	1613	112.6	1
	Paraiba	1054	744	141.7	1
	Ceara	627	715	87.7	1
Center West	Goias	2626	2319	113.2	4
Mean	-	1844	1687	111.0	

[1]Controls: IPA 6 and Bambui.

Based on these results the line A774 was released in 2002 with the trade name of BRS Marfim, for the states of Goias, Bahia, Pernambuco, Rio Grande do Norte, Paraiba e Ceara. Even though grain yield in Ceara had been 12,3% less than the controls, disease resistance and superior grain quality provided basis for cultivar indication for this state.

BRS Marfim has uniform grain size and color, average 100 grain mass of 26.6 g, excellent cooking quality and good grain appearance after cooked (Table 2).

Table 2. Technological and industrial quality of seeds from the cultivar BRS Marfim.

Cultivar	Cooking time (minutes)	Soluble solids (%)	Protein (%)	Whole grain (%)
BRS Marfim	30.0	9.3	22.1	85

Under artificial inoculation BRS Marfim showed resistant reaction to common mosaic virus and. to the following *C. lindemuthianum* pathotypes: 89, 453 and 95. In the field trials, it presented resistant reaction to rust, intermediate resistance to angular leaf spot and susceptibility to common bacterial blight.

BRS Marfim presents semi-erect plant type in any crop system and under a variety of soil and climate conditions where it was evaluated. It also presented good lodging resistance throughout its cycle of 89 days, in average, from emergence to physiological maturity.

BRS Marfim, due to its superior yield potential and differentiated grain type, associated to excellent cooking performance, semi-erect plant type, resistance to lodging and to major diseases, is an interesting option for producers involved with specialty grain type production, providing a value added commodity for commercialization in the States of Goias, Bahia, Pernambuco,Rio Grande do Norte, Paraiba and Ceara.

Genetic seed stocks are maintained by Embrapa Rice and Beans and basic seed is available at Embrapa Technology Transfer.

Institutions of participating scientists:

Embrapa Arroz and Feijão; Embrapa Transferência de Tecnologia/Escritório de Negócios de Goiânia-GO; EBDA - Empresa Baiana de Desenvolvimento Agrícola; IPA - Empresa Pernambucana de Pesquisa Agropecuária; Emparn - Empresa de Pesquisa Agropecuária do Rio Grande do Norte; Emepa - Empresa Estadual de Pesquisa Agropecuária da Paraíba; Epace - Empresa de Pesquisa Agropecuária do Ceará.

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'BRS PONTAL': NEW COMMON BEAN CULTIVAR WITH CARIOCA GRAIN TYPE

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The common bean breeding program strategy at Embrapa Rice and Beans is based on the demands of the participants of its agri-chain. Besides productivity increase, yield stability and the grain quality, the program also aims at the reduction of yield losses due to biotic and non biotic stresses. In Brazil, the traditional preference of bean consumption lies on grain of the carioca commercial type, what justifies the efforts in developing superior lines with this type of grain associated with the most common desirable commercial traits. Common bean needs to become more productive and competitive in the agricultural system to guarantee its sustainability in the Brazilian agribusiness. The development of new cultivars more productive and more resistant to non biotic and biotic stress, will turn it possible to farmers to get a more profitable crop with less environmental impact and, probably, will contribute for the consolidation of the common bean as consistent option for agricultural exploration.

The cultivar BRS Pontal was derived from the cross BZ3836 // FEB 166 / AN 910523 performed by Embrapa Rice and Beans. The F_2 and F_3 population was advanced in bulk. The F_4 population was planted at the Embrapa Rice and Beans, inoculated with the pathotype 89 of *Colletotrichum lindemuthianum* and only one pod/resistant plant was harvested to rebuild the plant population. In the F_5 generation it was used the same selection methodology; however, plants were harvested individually. From the F_6 families it was selected the line LM 95102774 on the basis of its productivity and disease resistance. In the year of 1997, LM 95102774 and 42 other lines were evaluated, in the National Bean Trial carried out in 11 environment, in 7 different Brazilian States [Goias (2), Mato Grosso (1), Mato Grosso do Sul (3) Minas Gerais (1), Bahia (1), Pernambuco (2) and Espirito Santo (1)].

The joint analysis of yield and other agronomic traits allowed the line LM 95102774 be promoted to the Regional Bean Trial of 1999/2000. In this trial it was evaluated with 12 more lines and five checks, in a completely randomized block design with four replications using the recommended technologies for the different cultivation systems, in a total of 36 environments in the States of Goias (13), Federal District (1), Minas Gerais (17), Mato Grosso (2) and Mato Grosso do Sul (3).

In the 36 regional trials, the line LM 95102774 outyielded the checks by 15,34% (Table 1). These data allowed its release in 2003 with the trade name BRS Pontal, for cultivation in the States of Goias/ Federal District, Mato Grosso, Mato Grosso do Sul and Minas Gerais,. This new cultivar has a very uniform grain color, excellent cooking quality (Table 2) and the seeds averages 26.1 grams 100 $seed^{-1}$. 'BRS Pontal' presents a semi-prostrate growth habit, low resistance to plant lodging in the majority of the bean production systems tested and requires 87 days from seedling stage to physiological maturity.

Table 1. Yield of the cultivar 'BRS Pontal' compared to the mean of control cultivars in the years 1999/2000.

Region	State	'BRS Pontal' (kg/ha)	Mean for controls[1] (kg/ha)	Relative yield (%)	Number of sites
Southeast	Minas Gerais	3014	2671	115.6	17
Center West	Goiás/Federal Ditrict	2747	2701	108.9	14
	Mato Grosso	1286	998	135.0	2
	Mato Grosso do Sul	2209	1735	131.0	3
Mean		2747	2455	115.3	

[1]Controls: Perola and Iapar 81.

Under artificial inoculation, the cultivar BRS Pontal showed to be resistant to the bean common mosaic virus and resistant, intermediate and susceptible to 11, 6 and 7 *C. lindemuthianum* pathotypes, respectively. In the field trials, it presented intermediate reaction to rust and common bacterial blight and was susceptible to angular leaf spot.

Table 2. Technological and industrial grain quality of the common bean cultivar 'BRS Pontal' compared to other cultivars of carioca grain type.

Cultivar	Cooking time (minutes)	Soluble solids (%)	Protein (%)
'BRS Pontal'	26.0	8.3	21.4
Perola	29.0	9.6	21.3
Iapar 81	29.0	9.4	21.0

BRS Pontal, is a new option for carioca bean growers in the States of Minas Gerais, Goias/Federal District, Mato Grosso and Mato Grosso do Sul.

Genetic seed stocks are maintained by Embrapa Rice and Beans and basic seed is available at Embrapa Technology and Transfer.

Institutions of participating scientists:

Embrapa Arroz e Feijão; Embrapa Milho e Sorgo; Embrapa Cerrados; Empaer-MT; Agenciarural-GO; Universidade Federal de Viçosa; Universidade Federal de Lavras; Fesurv/Esucarv; Idaterra-MS; and TecAgro - Tecnologia em Agricultura Ltda.

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'BRS TIMBO': NEW COMMON BEAN CULTIVAR FROM "ROXINHO" COMERCIAL GRAIN TYPE

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Dry bean is one of the most important crop in Brazil because it is daily component of the food of the Brazilian population and most of the production came from small farms. The Brazilian production in the past ten years was between 2.2 and 3.4 million tons. It was observed a decreasing in planted area and an increasing in yield. The market for different grain types from carioca (beige with brown strips) and black is in expansion in Brazil. There is a demand by industry for a product with different type and quality to attend people with high income. The dry bean breeding program of Embrapa Rice and Beans, aims at the development of genotypes with adaptation, resistance to main diseases, yield, reduced high of plant. As result of this program, Embrapa Rice and Beans is releasing BRS Timbo, a cultivar of the grain type (purple)"roxinho".

The cultivar BRS Timbo is originated from a multiple cross performed at CIAT (A252/XAN105//A373/A213///A445/XAN112//BAT447/A213). Embrapa Rice and Beans received the developed line FEB 163 and promoted it to the Preliminary Trial in 1991. This line was assessed together with additional 22 lines and three controls in the National Trial, conducted in 1993 under eigth environments, in the States of Goias (2), Mato Grosso (1), Mato Grosso do Sul (1), Minas Gerais (3) and Espirito Santo (1). The joint analysis of the grain yield data and other agronomic characteristics provided the elements to promote FEB 163to the Regional Trial during the 1995/96 crop season. This time, FEB 163 was assessed with seven additional lines and four controls in a randomized complete block design with four replications. in 26 environments in the States of Goias (8), Federal District (2), Minas Gerais (5), Mato Grosso (7) and Mato Grosso do Sul (4), with average grain yield 3.5% superior than the controls (Table 1).

Table 1. Yield of cultivar BRS Timbo compared to the mean of two control cultivars in 1995 and 1996.

Region	State	BRS Timbo (kg/ha)	Mean for controls (kg/ha)	Relative Yield (%)	Number of sites
Southeast	Minas Gerais	2787	2649	105.2	5
Center West	Goias/Federal District	2449	2372	103.2	10
	Mato Grosso do Sul	1544	1447	106.7	4
	Mato Grosso	1665	1653	100.7	7
Mean	-	2163	2089	103.5	

[1]Controls: Vermelho 2157 and Roxo 90.

Based on these results it was released in 2002 with the trade name of BRS Timbo, for the States of Goias, Federal District, Minas Gerais, Mato Grosso and Mato Grosso do Sul.

BRS Timbo has uniform grain size and color, average 100 grain mass of 19.3 g, excellent cooking quality and good grain appearance after cooked (Table 2).

Table 2. Technological and industrial quality of seeds from the cultivar BRS Timbo.

Cultivar	Cooking time (minutes)	Soluble solids (%)	Protein (%)	Whole grain (%)
BRS Timbo	30.0	9.5	23.43	92

BRS Timbo is resistant to common mosaic under artificial inoculation. It also presented resistance reaction to the following *C. lindemuthianum* pathotypes: 55, 89, 453, 585. In the field trials, it presented resistant reaction to rust, intermediate resistance to angular leaf spot and susceptibility reaction to common bacterial blight.

BRS Timbo presents semi-erect plant type in any crop system and under a variety of soil and climate conditions where it was evaluated. It also presented good lodging resistance throughout its cycle of 87 days, in average, from emergence to physiological maturity.

BRS Timbo, due to its superior yield potential and differentiated grain type, associated to excellent cooking performance, semi-erect plant type, resistance to lodging and to major diseases, is an interesting option for producers involved with specialty grain type production, providing a value added commodity for commercialization in the States of Goias, Federal District, Mato Grosso, Mato Grosso do Sul and Minas Gerais.

Genetic seed stocks are maintained by Embrapa Rice and Beans and basic seed is available at Embrapa Technology Transfer.

Institutions of participating scientists:

Embrapa Arroz e Feijão; Embrapa Milho e Sorgo; Embrapa Cerrados; Embrapa Transferência de Tecnologia/Escritório de Negócios de Sete Lagoas-MG; Embrapa Transferência de Tecnologia/Escritório de Negócios de Goiânia-GO; Empaer-MT; Empaer-MS; Agenciarural-GO; Universidade Federal de Viçosa; Universidade Federal de Lavras; Coagril; Fesurv/Esucarv.

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'BRS GRAFITE': BLACK BEAN CULTIVAR RECOMMENDED FOR THE WEST CENTRAL AND SOUTHEAST BRAZIL

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Common beans is an important source of protein for the Brazilian people, especially those of low income, with a "per capita" consumption of 13.6 kg and a total production of 2.37 million tons in the 2001/2002 year. These numbers rank Brazil as the largest producer and consumer of common beans in the world. There is regional preference as to the seed color, with the carioca type predominating over the country. Overall consumption of black beans reach 17%, mainly for the State of Rio de Janeiro and those of the South Region.

Black bean production has been below national needs, leading to 50-80 thousand tons of importation per year. The breeding program at the Embrapa Rice and Beans aims to develop evaluate and release improved cultivars with broad adaptation to the growing regions. The objective is to reach self sufficiency and finally reach amounts enough to export.

BRS Grafite is derived from the cross made at Embrapa Rice and Beans in 1986 between lines AN 5125867/Mexico 168. Bulk selection was utilized from F_2 to F_4 generations. Selection for resistance to the pathotype 89 of *Colletotrichum lindemuthianum*" was done in F_5. Remaining resistant plants were harvested individually to give rise to F_6 families. These families were evaluated for yield and upright plant architecture, resulting in the selection of line LM 95103904.

The above line, with 26 other breeding lines and three controls, was evaluated in a National trial in nine sites in the States of Goias (2), Mato Grosso do Sul (2), Minas Gerais (1), Rio de Janeiro (1), Bahia (1), Espirito Santo (1), and Mato Grosso (1). LM 95103904 overcame all of the other lines and controls in the joint statistical analysis. The line was then evaluated in the field trial for cultivar release with eleven lines and two controls in eleven sites in the States of Goiás, Minas Gerais, Rio de Janeiro and Federal District (Table 1). It was released in 2003 with the trade name BRS Grafite, for cultivation in the Southeast and West Central regions of Brazil for the Autumn/Winter growing seasons, with irrigation.

Table 1. Yield of BRS Grafite, in Autumn /Winter growing seasons, compared to the mean of two control cultivars in 1999 and 2000.

Region	State	BRS Grafite (kg/ha)	Mean for controls (kg/ha)	Relative Yield (%)	Number of sites
Southeast	Rio de Janeiro	2251	2063	109	8
	Minas Gerais	3599	3323	108	4
Center West	Goias/Federal District	2789	2831	99	7
Mean	-	2733	2586	106	-

[1]Controls: Diamante Negro and FT Nobre.

The BRS Grafite has uniform color and a mass of 25.2 g for 100 seeds. It has excellent cooking quality with 20 min cooking time and a chocolate brown color broth (Table 2).

Table 2. Technological and industrial quality of seeds from the cultivar BRS Grafite compared to other black bean cultivars.

Cultivar	Cooking time (minutes)	Soluble solids (%)	Broth Color[1]	Protein (%)	Fiber (%)	Tegument (%)
BRS Grafite	20,00	8,46	Light[1]	20,06	14,00	8,85
BRS Valente	28,10	10,91	Light[1]	19,25	9,70	11,75
FT Nobre	28,48	11,05	Light[1]	21,60	----	13,48
Rio Tibagi	36,00	12,40	Dark	20,00	12,50	13,10
Diamante Negro	34,02	11,20	Light[1]	20,00	10,00	11,40

[1]Chocolate brown.

The cultivar BRS Grafite showed resistant reaction to bean common mosaic virus strains (I gene) and to *C. lindemutianum* pathotypes 55, 89, 95 and 453. Under field conditions it showed resistance to rust, intermediate reaction to angular leaf spot and susceptibility to golden mosaic and common bacterial blight.

It has semi-upright plant architecture under the evaluated conditions of soil and climate. It has also good resistance to lodging, with a growing cycle of 90 days from emergency to physiological maturation.

BRS Grafite has been released for its high yield potential, excellent grain quality, upright growth habit, and resistance to some important diseases. The recommendation is for the States of Rio de Janeiro, Minas Gerais, Goias and Federal District.

Genetic seed stocks are maintained by Embrapa Rice and Beans and basic seed is available at Embrapa Technology Transfer.

Institutions of participating scientists:

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Names of Common Bean Varieties Released in Central America and the Caribbean

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Collaborative research in Central America and the Caribbean during the last 20 years has permitted the development and release of several improved common bean (*Phaseolus vulgaris* L.) cultivars which have had a significant level of adoption and impact in the region (Mather *et al*., 2003; Johnson and Klass, 1999). Due to their superior performance across the region, several improved cultivars were released in various countries during a short period of time. Unfortunately, National Bean Programs involved in the release process often gave these cultivars different names in different countries. Collaborative bean research activities have been conducted in Central America, Mexico and the Caribbean since the 1980´s, by the PROFRIJOL Bean Research Network and under the scientific leadership of CIAT (Centro Internacional de Agricultura Tropical). In the 1990´s, scientists of the bean research programs from the University of Puerto Rico and the Escuela Agricola Panamericana (EAP), Zamorano, Honduras, supported by the Bean/Cowpea CRSP Program, joined the PROFRIJOL network and became involved in the development of bean cultivars for the region. Organizations or persons that were not previously involved in the collaborative research that resulted in the releases of small red and black bean cultivars for Central American may not be familiar with the names currently being used for a specific cultivar in different countries. This situation has already caused some confusion concerning the correct identity of bean cultivars. The purpose of this publication is to serve as a reference to guide researchers, producers, seed dealers, public officials and brokers, in their decisions with regards to the use of improved cultivars for commercial production of grain or seed in the region or elsewhere. Its content only refers to those bean cultivars that are known with at least two different names in the region. In addition, the following provides descriptions of the most important traits of these cultivars.

The small red breeding line DOR 364, derived from the cross BAT 1215//RAB 166/DOR 125, was released in 1990 as the cultivar “Dorado” in Honduras and “CENTA Cuscatleco” in El Salvador. Other releases of the line DOR 364 were “DORICTA” in Guatemala in 1992, “DOR 364” in Nicaragua in 1993 and “Delicias 364” in Cuba in 1999. Dorado has a quantitative resistance to bean golden yellow mosaic (BGMY), expressed as reduced yellow mosaic symptoms (Beebe, 1994; Miklas *et al*., 1996). Dorado carries the dominant *I* gene for resistant to bean common mosaic (BCM). Dorado has a type II plant, an intermediate maturity of 72-74 days after planting (DAP) and good and stable yields in diverse environments. DOR 364 has a dark, shinny red kidney seed shape and an individual seed weight of 0.21 g.

The small red breeding line DOR 482, derived from the cross DOR 367//DOR 364/IN 101, was released in Honduras as the cultivar “Don Silvio” in 1992 and in El Salvador as “Rojo Salvadoreño” in 1997. Don Silvio has a higher BGYM resistant than Dorado due to the addition of the recessive gen *bgm-1* that confers resistance to chlorosis, transferred to its parental line DOR 367 from the source line A429 (inherited from the original source “Garrapato”). It also carries the dominant *Bgp-1* gene that confers resistance to pod deformation in the presence of BGYM (Molina and Beaver, 1998), and the dominant *I* gen for resistance to BCM. Don Silvio has a type II plant and an intermediate maturity of 70-72 DAP. Don Silvio has a shinny dark red, kidney shaped seed and an individual seed weight of 0.24 g.

The small red breeding line MD 30-75, derived from the cross DOR 483//DOR 391/ Pompadour J, was released in Honduras in 1996 as the cultivar "Tio Canela 75" (Rosas *et al*., 1997). In 2000, this line was released in El Salvador as "CENTA 2000", in Panama as "Rojo Chiricano" and in Nicaragua as "INTA Canela". Tio Canela 75 is a BGYM resistant cultivar that carries the QTL, *bgm-1* and *Bg-p1* resistant genes. Tio Canela 75 also carries the dominant *I* gene for resistance to BCM. It is well adapted to several environments, has a type II plant and an intermediate maturity of 70-72 DAP. Tio Canela 75 has a shinny red, ovoid shaped seed and an individual seed weight of 0.22 g.

During the 2002 and 2003, the small red breeding line EAP 9510-77, derived from the cross Tio Canela 75/DICTA 105, was released as the cultivar "Amadeus 77" in Honduras, "INTA Rojo" in Nicaragua, "CENTA San Andres" in El Salvador, "Cabecar" in Costa Rica and "IDIAP R3" in Panama. Amadeus 77 is a BGYM resistant cultivar carrying the QTL, *bgm-1* and Bg*p-1* genes for resistance to BGYM. It also carries the dominant *I* gene for resistance to BCM. Amadeus 77 was developed as a heat tolerant line for the coastal regions of Central America. It has a type II plant and an early maturity of 68-70 DAP. Amadeus 77 has light shinny red, ovoid elongated seed shape and an individual seed weight of 0.25 g.

The small red breeding line EAP 9510-1 (a sister line of EAP 9510-77), was released as the cultivar "Carrizalito" in Honduras in 2003 and " Telire" in Costa Rica in 2004. Carrizalito is a BGYM resistant cultivar carrying the QTL, *bgm-1* and *Bgp-1* genes for resistance to BGYM. Carrizalito also carries the dominant *I* gene for resistance to BCM. Carrizalito has been identified as a high yielding cultivar. It has an upright type III plant and an early maturity of 68-70 DAP. Carrizalito has a shinny red, ovoid seed shape and an individual weight of 0.22 g.

The black seeded line DOR 390, derived from the double cross DOR 364/G18521//DOR 365/LM 30630, was released as "ICTA Costeña" in Guatemala in 1992, "Negro Tacana" in Mexico in 1994 and "Tomeguin 93" in Cuba in 1996. DOR 390 is a BGYM resistant line (*QTL* from DOR 364) and carries the dominant *I* gene for resistance to BCM. DOR 390 has a type II plant and intermediate maturity of 74 DAP. DOR 390 has a black opaque, kidney shaped seed with an individual seed weight of 0.21 g.

The black seeded line DOR 500, derived from the double cross DOR 364/G18521//DOR 365/IN 100, was released as "Negro Tropical" in Mexico and "INTA Cardenas" in Nicaragua in 2002. DOR 500 is a BGYM resistant line and carries the *I* gene for resistance to BCM. DOR 500 has a type II plant and intermediate maturity of 70-72 DAP. DOR 500 has a black opaque, kidney shaped seed with an individual weight of 0.22 g.

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NOTICE OF NAMING AND RELEASE OF CLARET, A NEW, UPRIGHT, DISEASE RESISTANT SMALL-RED DRY BEAN (*PHASEOLUS VULGARIS*, L.) CULTIVAR

G.L. Hosfield, A.N. Hang*, and M.A. Uebersax

The Agricultural Research Service, United States Department of Agriculture and Washington State University announce the joint release of Claret, a new small-red dry bean (Phaseolus vulgaris L.) cultivar. The new release was developed to bring earliness, disease resistance, and improved seed characteristics and canning quality into the small-red market class. Earliness in small-red beans affords one the opportunity to use an early maturing cultivar as a second crop in a double-crop system

Claret, breeding line No. ARS-R93365, was derived from a cross made in 1990 between the accessions, X90116 and X90124. Both parents of Claret are USDA-ARS recurrent selections with complex pedigrees. The two parents of Claret were S_2 selections from the C_1 cycle of recurrent selection. Selection criteria were Type IIA growth habit, early maturity, and seed characteristics commensurate with the requirements of commercial small-red bean market class. The F_1 seed were grown and plants self pollinated in the spring of 1991 in the greenhouse. Six, single plant F_2 selections from the cross were made on the population coded 91L-5223 in the summer of 1991 in a nursery grown near Saginaw, Michigan. The selections were based on upright Type IIA growth habit, early maturity and seed characteristics commensurate with the small-red market class. In 1992 and 1993, seed was advanced in nurseries in Michigan and Puerto Rico. In 1993, the $F_{2:6}$ breeding line was given the permanent code No. ARS-R93365 and seed were planted in replicated yields trials at the Bean and Sugar Beet Research Farm near Saginaw, MI. ARS-R93365 was tested from 1993 to 2002 in Mid-Michigan and 1997 to 2001 at Othello, Washington. Claret was also tested over 49 locations in the Cooperative Dry Bean Nursery during 2000, 2001, and 2002. Based on yield in the respective trials, general appearance in the field at maturity, overall agronomic performance, and canning characteristics, ARS-R93365 was cited for release in 2003 and given the name Claret.

In 12 tests from 1993 to 2002 in Mid-Michigan and Othello, Washington, Claret yielded 2,564 kilograms per hectare (2,289 pounds per acre) and ranged from a low yield of 1,608 kilograms per hectare (1,436 pounds per acre) in 1998 to a high of 4,029 kilograms per hectare (3,597 pounds per acre) at Othello, Washington in 2001. Claret had an average yield of 2,677 kilograms per hectare (2,390 pounds per acre) over 49 locations in the Cooperative Dry Bean Nursery. Claret yielded 87% of Rufus, a check variety, (Type III growth habit) in 9 tests, but 108% of Garnet, a check variety, (Type III growth habit) in 6 tests.

Claret combines the upright and short-vine growth habit (Type IIa) characteristic of its parents with the seed size, shape, and pigmentation preferred for beans of the small-red market class. The strong main stem and upright growth habit gives Claret a superior lodging resistance compared to the viny and prostrate growth habit of small-red commercial cultivars, (for example, Rufus and Garnet). Plants of Claret average 43 cm in height and have the narrow profile appearance characteristic of the dry bean architecture. Claret has white flowers and blooms 45 days after planting, which is similar to other small-red cultivars. Claret matures, on average, 87 days after planting and ranges in maturity from 73 to 120 days depending on the location and season. Claret is considered an early maturing cultivar. Claret matures uniformly and its "dry down" at maturity is very uniform.

Claret has an attractive garnet seed color, and a noticeable black hilum ring which is characteristic of small-red commercial cultivars. Individual seeds are oval, . 1.2 x 0.8 centimeters in length and width, plump at the surface tangential to the hilum, and gently rounded at the apices, giving them a more attractive appearance than Rufus and Garnet, which have rhomboid-shaped seeds. Seed mass of Claret averaged 35.3 grams per 100 seed compared to 34.2 and 29.2 grams per 100 seed for Rufus and Garnet, respectively. Claret exhibited a consistent and highly appealing canning quality, which enabled this new variety to readily stand out among the numerous small-red breeding lines in the canning evaluations conducted in the Michigan State University Pilot Processing Laboratory. In the canning trials, Claret's visual appeal was significantly superior to Garnet.

Claret is susceptible to bean common mosaic virus but carries the Ur-3 gene for resistance to bean rust [*Uromyces appendiculatus* (Pers.:Pers.) Unger] disease making it the second commercial small-red cultivar developed and released by USDA, ARS to have rust resistance. Claret is susceptible to bean common blight [*Xanthomonas campestris* pv. *phaseoli* (Smith) Dye] disease. Claret has been reported to be tolerant to high temperatures and low soil water potential in the Cooperative Dry Bean Nursery location at Parma, Idaho.

Claret was developed by the dry bean breeding team at East Lansing, Michigan and Prosser, Washington consisting of Dr. G.L. Hosfield of the U.S. Department of Agriculture, Agriculture Research Service, Sugarbeet and Bean Research Unit, East Lansing, Michigan; Dr. Mark A. Uebersax of Michigan State University, Department of Food Science and Human Nutrition; and An N. Hang of Washington State University, Irrigated Agricultural Research and Extension Center, Prosser. Seed of Claret for experimental purposes may be obtained from A.N. Hang. Michigan State University has no seed for distribution. Claret small-red dry bean is being released as an exclusive variety.

Genetic material of Claret will be deposited in the National Plant Germplasm System where it will be available for research purposes.

REGISTRATION OF FIVE COMMON BEAN GERMPLASM LINES RESISTANT TO COMMON BACTERIAL BLIGHT: W-BB-11, W-BB-20-1, W-BB-35, W-BB-52, AND W-BB-11-56

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Common bacterial blight is one of the major diseases of dry beans worldwide. The disease is caused by the bacterium *Xanthomonas campestris* pv. *phaseoli (Xcp)=X. axonopodis pv. phaseoli.* Few commercial common bean (*Phaseolus vulgaris* L.) cultivars are resistant to common bacterial blight caused by Xcp. Nebraska Great Northern No. 1, Sel. 27 has been used as a standard and is recognized as having a useful level of resistance to the bacterium. However, the resistance in this cultivar is not as great as that found on some tepary beans (*P. acutifolius* Gray), (McElroy, 1985, Freytag, 1989, Zapata, 1989).

The University of Puerto Rico, the Agricultural Research Service, U.S. Department of Agriculture, and Cornell University cooperatively announced five common bean germplasm lines as: Wilkinson (W) – Bacterial Blight (BB) -11, -20-1, -35, -52, and -11-56 in 1990. These germplasm lines represent the culmination of more than 20 years of crossing and testing by Dr. R.E. Wilkinson at Cornell to pyramid mostly minor gene effects for common bacterial blight resistance caused by Xcp in the common bean (*P. vulgaris*) and nearly 10 years collaboration involving field testing, inoculation and selection of these lines in Puerto Rico (PR). This cooperative work was supported in part by grants from the U.S. Agency for International Development (AID/CM/TA-C-73-26), AID/TA-C- 1296, AID/DSAN/XII-G-0261 and CBA-UPR-18 (83-CRSP-2-2160), and the NY State Dry Bean Growers and Shippers fund.

Bacterial blight resistance is available in three lines with determinate growth habit which have been more susceptible than indeterminate beans to common blight and two indeterminate bushy vine types. Superior level of bacterial blight resistance were developed in lines W-BB-20-1 and W-BB-11-56 a bush and bushy vine type, respectively. The germplasm lines W-BB-20-1 and W-BB-11-56 (Zapata et al., 1991) have been used successfully as parental lines for breeding for resistance to common bacterial blight on recently released lines USNA-CBB-1 and USNA-CBB-2, respectively (Miklas et al., 2001a and 2001b). The other three lines are two bush or determinate types and one bushy vine or indeterminate type with a superior level of resistance to bacterial blight better than Great Northern No.1, Sel. 27. Another achievement was the development of a germplasm line with bush type and snap bean characteristics such as W-BB-52 with resistance to most of the bacteria tested.

The high level of Xcp resistance in the W-BB lines was developed by pyramiding minor genes for resistance from several sources such as GN-1, Sel.27, PI 207262, PI 180745, PI 180746, and 65859 (Table 1) primarily through a reciprocal backcross program. The reciprocal backcross process of pyramiding genes for resistance began with crossing two plants that derive resistance from two different sources. After two generations of screening the F_3 and F_4 for bacterial blight, the resistant selections were backcrossed to each parent and the resulting progenies were ultimately intercrossed. Typically one or more generations of selfing and screening for resistance followed this last cross. The progeny from the two backcrosses was screened for resistance for two generations. Additional minor genes from other sources of Xcp resistance were combined through a parallel procedure. Then these two small pyramids were combined through a reciprocal backcross procedure. It was assumed that the progeny from the

reciprocal backcross carried most of the genes for resistance from each original source. This assumption was taken because a high degree of recessiveness in the genes for resistance from many sources was observed. The reciprocal backcross procedure has the advantage of producing a higher percentage of homozygosity in which the recessive genes for resistance can be expressed. Also, it is suspected that some minor genes may be best expressed in the presence of certain other minor genes. If this is the case, it adds importance to the need for recovering "all" the resistance genes from both parents. Regardless of the real reason for why it works, experience has shown that the reciprocal backcross procedure is essential to accomplishing a satisfactory pyramiding of recessive genes for resistance.

Resistance to common blight was determined by screening promising families for their latent period (length of time between inoculation and symptom expression). Indicator plants used were 3M-152 (highly susceptible Puerto Rico line), Redkote (susceptible), and Sel 27.(resistant). The indicator plants developed symptoms at 3, 4, and 7 days after inoculation, respectively. The experiments were conducted in growth chambers at 85F. In the beginning it was possible to conduct a test every week but as soon as the incubation time increased the observation period also increased. Generally the material that was used to plant one experiment in the growth chamber consisted of one or more related groups or families together with various support material. Also, when F2 populations were screened, one or both parents were included, especially when no obvious phenotypic markers were involved, to help confirm that a cross had been obtained, as to get a measure of the effect of pyramiding genes for resistance. Plants that were saved from a screening test were transplanted to larger pots in the greenhouse and held for seed production and possible crossing. All crosses were made at Cornell with resistance screening conducted by multineedle wound inoculation (Zapata et al., 1985) of primary leaves on 8-day old seedlings with 10^8/CFU in a controlled growth chamber at about 85 F (29 C) (Zapata et al., 1991). Symptom development was observed daily. Plants showing hypersensitive reaction were discarded. Susceptible plants showing chlorosis, progressive necrosis were discarded as soon as detected. Only plants showing no symptoms or having a longer incubation period than Sel. 27 were maintained.

From 1979-1985 Dr. M. Zapata of the UPR in collaboration with Dr. R.Wilkinson of University of Cornell evaluated breeding lines under tropical conditions using inoculation with local strains of the pathogen at the UPR Fortuna Substation (Zapata et al., 1985). There was also some selection for resistance to ashy stem blight, as the fields had a high inoculum level of *Macrophomina phaseolina.* Seeds from plants selected for Xcp resistance were sent to Cornell for incorporation into the crossing program. The progenies of the crosses in the F_1 generation were returned to PR for evaluation.

In field plantings during five summer seasons (1986-1990) at the UPR Fortuna Substation, Dr. G. Freytag, USDA-ARS and Dr. M. Zapata inoculated with local Xcp strains and individual plant selections in the F_3 generation were made from heterogeneous lines selected for Xcp resistance. Plant rows from resistant plants were grown in nurseries during the winter season at the USDA-ARS Isabela Research farm and selected for plant habit and yield potential. Resistance on foliage of individual plants at flowering was confirmed three times by using multi-needle inoculations under controlled greenhouse environments at Mayagüez using 4 pure strains from the American Type Culture Collection (ATCC) and from two local sources. The results are presented in Table 2.

Table 1. Sources of resistance to *Xanthomonas campestris* pv. *phaseoli* used to develop the W-BB lines.

	Sources of resistance					
Lines	GN-1[1]	Sel. 27 [2]	PI 207262[3]	PI 180745[4]	PI 180746[5]	65859[6]
W-BB-11	+[7]	+	+	+		
W-BB-20-1	+	+	+	+	+	+
W-BB-35	+	+	+	+	+	+
W-BB-52	+	+	+			+
W-BB-II-56	+	+	+		+	

[1/]GN-1 = Univ. of Idaho Great Northern #1.
[2/]Sel. 27 = Great Northern Nebraska #1 Selection 27.
[3/]207262 = Plant Introduction 207262 from Colombia, SA.
[4/]180745 = [5/]180746 = Plant Introduction (*P. coccineus* x *P. vulgaris*) from Germany.
[6/]65859 = (*P. vulgaris* x *P. coccineus*) from P.A. Lorz, Univ. of Florida.
[7/]+ = Indicates source of resistance present in the line.

Table 2. Reaction of individual bean lines to inoculation with *X. campestris pv. phaseoli* under greenhouse and field conditions.

	Greenhouse Xcp pathovar/origin					Field
Identitiy	*phaseoli* ATCC 9563	*phaseoli* PR 820	*fuscans* ATCC 11766	*vignicola* ATCC 11648	*glycines* ATCC 17915	*phaseoli* field strains PR
W-BB-11	I	I	T	I	I	T
W-BB-20-1	I	I	I	I	I	R
W-BB-35	I	I	S	I	I	T
W-BB-52	I	I	R	I	I	S
W-BB-II-56	I	I	I	I	I	R

I- Immune, no lesions; R = Resistance, very small (1-3mm), chlorotic but non-progressive lesions; T = Tolerant slow disease development, takes 8-10 days under controlled growth conditions and 44 days after inoculation under field conditions to develop 25% chlorotic lesions and less than 25% of necrotic lesions; S = Susceptible under controlled growth conditions takes 6 days to show symptoms and 44 days to develop necrosis on 50% of the inoculated tissue under field conditions.

Botanical Description

Line W-BB-11 (from Cornell line 84-4216-1) has a bushy vine (Type III) plant habit, a height of 70 cm, straight pods 5-7 cm long and small, gray to black seed weighing 0. 27g/ seed.

Line W-BB-20-1 (from Cornell line 84-4446-1) has a determinate bush (Type I) plant habit, a height of 40 cm, slightly curved pods 5-8 cm long and white seed weighing 0. 24g/seed. This line has the I gene resistance to Bean Common Mosaic Virus (BCMV).

Line W-BB-35 (from Cornell line 84-4454-1) has a determinate bush (Type I) plant habit, a height of 30 cm, late season, broad, straight pods 5-8 cm long and a large, rounded, yellow ship brown seed weighing 0.43g/seed. This line has a protected (probably by bc2-2) I gene resistance to BCMV.

Line W-BB-52 (from Cornell line 84-4610-3) has a determinate bush (Type I) plant habit, a height of 20 cm, early season, flat, curved pods 7-9 cm long with some snap characteristics and long, cream colored seeds weighing 0.30g/seed. This line does not have the I gene for BCMV resistance.

Line W-BB-11-56 (from Cornell line 85-8250-1) has a bushy vine (Type III) plant habit, a height of 70 to 120 cm, late season, curved pods 7-10 cm long with some snap characteristics and a good seed set in Puerto Rico of a pinto seed type weighing 0.25g/seed. This line has protected by bc2-^{2} I gene resistance to BCMV.

Identification of resistance using genetic markers

A portion of the resistance to common bacterial blight was derived from GN#1 Sel. 27 as indicated by the presence of the SCAR (sequence characterized amplified region) marker linked with a quantitative trait locus for resistance to common bacterial blight on linkage group B10 SAP-6-820 Miklas, 2000 et al., (Table 3). Suitable markers to identify the other sources of resistance have not been developed yet.

Table 3. Identification of bacterial blight resistance using four genetic markers (SCARs).

	SCARS Markers			
Lines	XAN 159 B-8 [1/] Su 91	OAC 88 B-8 R-7313	GN#1 Sel 27 B-10 SAP-6	XAN 159 B-6 BC 420
W-BB-11	-[2/]	-	+	-
W-BB-20-1	-	-	+	-
W-BB-35	-	-	+	-
W-BB-52	-	-	+	-
W-BB-11-56	-	-	+	-

[1/] Indicates the linkage group.

[2/] - Indicates absence and + presence of the marker for resistance

Seed availability

Seed of the germplasm lines from the F_8 generation is available upon request to the Bean Program, Tropical Agricultural Research Station, Agricultural Research Service, U.S. Department of Agriculture, P.O. Box 70, Mayagüez, Puerto Rico, 00681 or to Dr. Mildred Zapata, Crop Protection Department. University of Puerto Rico, P. O. Box 9030, Mayagüez, Puerto Rico, 00681-9030. We ask that appropriate recognition of source be given when this germplasm contributes to a new cultivar.

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ASTRO – INIA

FIRST CHILEAN "COSCORRON" CULTIVAR WITH AN UPRIGHT DETERMINATE BUSH GROWTH HABIT

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Origin. Astro-INIA was obtained through a single selection from a commercial bean crop of Coscorrón Granado INIA cultivar, in 1995. Until now, all the "coscorron" cultivars were type III plant, with the consequently difficulties for cropping and harvesting.

Plant characteristics. Astro-INIA is a determinate bush type I plant, with an erect robust main stem that reach about 48 cm height, lateral branches that also are erects. The foliage is dark green. Flowers are bicolor with light purple standard petal and lilac wing petal. Flowering is around 51 days after planting. The pod has a dark red background with a few light green striates. At the green shelled bean stage, it is 11 to 14 cm long, 8.5 g weight and harbor 3 to 6 grains.

Grain. Astro-INIA produce a "coscorron" grain type, with a cream background and light yellow striates. The shape is ovoid and the weight of 100 seed is about 54 g.

Vegetative growth. The "coscorron" cultivars in Chile are used for fresh grain consumption, named " poroto granado", or green shelled bean, and earlier productions are demanded for farmers. Astro-INIA reach the first harvest, as green shelled bean, after 85 days of seeding; 5 to 7 days earlier than the "coscorron" type III cultivars. For dry grain Astro-INIA require 105 days between planting and harvest.

Disease resistance. Astro-INIA is immune to *Bean common mosaic*, Type and NY-15 strains, but susceptible to NL-3 strain. Besides, this cultivar is susceptible to severe strain of *Bean yellow mosaic*.

Yield. Astro–INIA reach 3 ton/ha as dry grain and 8.5 ton/ha as green shelled bean (included the pod). To produce such yields is necessary to seed 120 kg/ha and get a stand of 18 to 20 plants/m^2.

Quality. Astro-INIA is used as green shelled bean or dry grain. It has an excellent flavor, thin skin, soft grain and produces a light color soup.

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e-mail:jwyatt6@utk.edu

Yellowstone Bean Company
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Phone:406-662-3622
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Mildred Zapata
Dept. of Crop Protection
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Mayaguez, PR 00680
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BEAN IMPROVEMENT COOPERATIVE - Financial Statement

BALANCE ON HAND JANUARY 1, 2003		$8,997.28
INCOME CATEGORIES		
Back Issues	437.00	
Dues	4,228.00	
Dues CD	190.00	
Interest	70.76	
Page Charge	25.00	
Total Income Categories	**4,950.76**	
EXPENSE CATEGORIES		
Bank Fees	114.00	
BIC Meeting	225.90	
Postage & Copying Charges	1,989.25	
Printing	2,303.00	
Total Expense Categories	**4,657.42**	
GRAND TOTAL	$293.34	
BALANCE ON HAND DECEMBER 31, 2003		$9,290.62