The XLVI

Report of The

# BEAN IMPROVEMENT COOPERATIVE

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# THE 46th ANNUAL REPORT OF THE BEAN IMPROVEMENT COOPERATIVE

**The Bean Improvement Cooperative (BIC)** invites all members and other interested parties to join us at the Seventeenth Biennial Meeting in Sacramento, California from October 24-30, 2003. In addition, there are associated meetings with our colleagues in the North American Pulse Improvement Association (NAPIA), Crop Germplasm Committees and the Regional W-150 Committee before and after the BIC/National Dry Bean Council (NDBC) sessions. Our local organizers are Paul Gepts, Fred Bliss, Bob Gilbertson, Chet Kurowski and Steve Temple. Please refer to the information provided by the local organizing committee in this report of the BIC, and look for other information on the BIC web site and the call for abstracts that will be mailed directly by the local organizing committee to all BIC members later this year. Please share this information with interested colleagues who would like to attend these meetings and/or join the BIC.

On behalf of the BIC, I would like to recognize Steve Antonius and George Kotch for their years of dedicated service on the BIC Coordinating Committee. I wish to welcome Ken Kmiecik and Chet Kurowski as new industry representatives who joined the coordinating committee in 2003. Our organization has always had a strong commitment from its members who have devoted their time and energy to creating a positive atmosphere of cooperation and enthusiasm for those just beginning their exciting careers and to those who have come to the end of their productive and rewarding careers with beans. Please review the call for nominations for the Frazier-Zaumeyer Distinguished Lectureship, the BIC Meritorious Service Award and the BIC Achievement Award, and forward your nominations to the Awards Committee Chairperson, Howard Schwartz by July 1, 2003. A current list of BIC Committee Membership, and those who have received BIC Awards throughout the history of the Bean Improvement Cooperative is provided in the 2003 issue of the BIC to assist you in nominating colleagues for these awards.

In 2001, Dr. Dermot Coyne was the first recipient of the **Frazier-Zaumeyer Distinguished Lectureship**. The purpose of the Lectureship is to honor a distinguished colleague and invite the award recipient to deliver the keynote opening address at the biennial BIC meeting. The selected individual should have made a significant contribution to bean research over the previous 5-10 year (or longer) period. In addition the recipient would provide a short review (maximum 6 pages) for publication in the BIC report and be featured on the BIC web site. The Lectureship would be distinct from the other BIC Achievement and Meritorious Service Awards and holders of these awards are not excluded from being awarded the Frazier-Zaumeyer Distinguished Lectureship. The Lectureship recognizes the original BIC founder members, Dr. 'Tex' Frazier, distinguished bean breeder and Dr. Bill Zaumeyer an equally distinguished bean pathologist. The Awards Committee in agreement with the BIC President and the Local Meeting Committee Chair will choose the successful recipient of the Lectureship in 2003. The Lectureship will be awarded at the meeting in Sacramento and nominations are requested from the membership. Since the first session of the BIC meeting in Sacramento will focus on the topic of germplasm in recognition of the achievements of Dr. George Freytag and the publication of the monograph: Taxonomy, Distribution, and Ecology of the Genus *Phaseolus* (*Leguminosae–Papilionoideae*) in North America, Mexico and Central America, the awards committee would prefer to receive nominations of individuals for the Frazier-Zaumeyer Distinguished Lectureship with expertise in the general areas of germplasm collection, utilization and enhancement.

Please bookmark and access the BIC web page www.css.msu.edu/bic for current BIC information. In this issue, the BIC continues to publish annually, short review articles on a topic of current interest to members. The mini-reviews will be limited to six (6) pages and are designed to be more expansive, and address a topic of current interest in bean improvement. Members are asked to submit review topics for consideration. In the 2003 edition Dr. M.A. Pastor Corrales summarizes information on 41 disease resistant dry bean germplasm releases from USDA-ARS and State Experimental Stations in Michigan, Nebraska and North Dakota. **Dr. James D. Kelly, BIC President**

## BIC COMMITTEE MEMBERSHIP - 1957 to 2003

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, Monis
1985 Emery, Hagedorn, Sandsted, Schwartz
1987 Emery, Hagedorn, Sandsted
1989 Coyne, Silbernagel, Wallace
1995 Coyne, Dickson, Stavely
1997 Coyne, Stavely, Schwartz
1998 Coyne, Stavely, Schwartz
1999 Coyne, Stavely, Schwartz
2000 Coyne, Stavely, Schwartz
2001 Hosfield, Magnuson, Schwartz
2003 Hosfield, Magnuson, Schwartz

## BIC MERITORIOUS SERVICE & ACHIEVEMENT AWARD RECIPIENTS

| Year | Recipients |
|---|---|
| 1970 | Melvin E. Anderson- Rogers Bros. Seed Co., Plant Pathologist<br>William A. Frazier- Oregon State Univ., Horticulturist (**BIC Founder** & **Coordinator**, 1957-67)<br>Walter H. Pierce- Asgrow Seed Co., Plant Pathologist<br>William J. Zaumeyer- USDA, Plant Pathologist |

1971 Walter H. Burkholder- Cornell Univ., Plant Pathologist
James R. Douglass- USDA, Entomologist
Howard S. Gentry- USDA, Plant Explorer
Charles W. Hungerford- Univ. of Idaho, Plant Pathologist
Herbert A. K. Lamprecht- Pl. Breeding Inst. of Sweden, Geneticist
John J. Natti- Cornell Univ., Plant Pathologist
Melbourne C. Parker- Gallatin Valley Seed Co., Plant Breeder
Francis L. Smith- Univ. of California, Agronomist
Robert E. Wester- USDA, Plant Breeder

1973 Leslie L. Dean- Univ. of Idaho, Plant Pathologist
Nicolaas Hubbeling- Inst. of Phyto. Res.- Netherlands, Pl. Pathologist

1975 M. Wayne Adams- Michigan State Univ., Plant Breeder
Dermot P. Coyne- Univ. of Nebraska, Plant Breeder (BIC **Coordinator**, 1968-76)
Shigemi Honma- Michigan State Univ., Plant Breeder
Max. L. Schuster- Univ. of Nebraska, Plant Pathologist

1977 Douglas W. Burke- USDA, Plant Pathologist
Roelof Prakken- Utrecht Univ. of the Netherlands, Geneticist
Clibas Vieira- Univ. Federal de Vicosa of Brazil, Agronomist

1979 Barbara J. Ballantyne- New South Wales, Plant Pathologist
Donald J. Hagedorn- Univ. of Wisconsin, Plant Pathologist
Marshall LeBaron- Univ. of Idaho, Agronomist

1982 Eelco Drijfhout- Agr. Inst. of the Netherlands, Plant Breeder
Donald H. Wallace- Cornell Univ., Plant Breeder
Donald R. Wood- Colorado State Univ., Plant Breeder

1983 Leland W. Hudson- USDA, Horticulturist
Roger F. Sandsted- Cornell Univ., Horticulturist

1987 Michael H. Dickson- Cornell Univ., Plant Breeder (BIC **Coordinator**, 1976-87)
Aart van Schoonhoven- CIAT, Entomologist
Frederick A. Bliss- Univ. of Wisconsin, Plant Breeder
Matt J. Silbernagel- USDA, Plant Pathologist

1989 Axel L. Andersen- Michigan State Univ., Plant Breeder/Pathology
John D. Aktin- Asgrow Seed Co., Plant Breeder
Colin L.A. Leakey- England, Geneticist
Alfred W. Saettler- USDA/ARS, Plant Pathologist
Arthur P. Sprague- Del Monte, Plant Breeder
James R. Steadman- Univ. of Nebraska, Plant Pathologist
J. C. "Mike" Tu- Agriculture Canada, Plant Pathologist
James D. Kelly- Michigan State University, Plant Breeder [Achievement Award]

1991 Iver L. Jorgensen- Northrup King & Co., Plant Breeder
John L. Morris- Rogers/NK Seed Co., Plant Breeder
Rosario Provvidenti- Cornell University, Plant Pathologist
Shree P. Singh- CIAT, Plant Breeder
J. Rennie Stavely- ARS/USDA-Beltsville, Plant Pathologist
Daniel Debouck- IBPGR, Rome, Plant Geneticist [Achievement Award]
Paul L. Gepts- Univ. of Calif.-Davis, Plant Geneticist [Achievement Award]
Pat Barnes-McConnell- Bean/Cowpea CRSP, Director [Achievement Award]

1993 Hubert L. Bannerot- INRA, Versailles, Plant Breeder
Cesar Cardona- CIAT, Entomologist
Robert B. Colville- Del Monte Foods, Variety Development
George L. Hosfield- ARS/USDA, East Lansing, Genetics/Nutrition
Oswaldo V. Voysest- CIAT, Agronomy/Germplasm Evaluation
James S. Beaver- Univ. of Puerto Rico, Plant Breeder [Achievement Award]

1995 Howard F. Schwartz- Colorado State University, Plant Pathologist (BIC **President**, 1988-97)
Kenneth F. Grafton- North Dakota State University, Plant Breeder [Achievement Award]

1997 George Emery- Ferry Morse, Plant Breeder
James D. Kelly- Michigan State University, Plant Breeder (BIC **President**, 1998-2003)
Steve Magnuson- Harris Moran, Plant Breeder
David Nuland- University of Nebraska, Bean Extensionist
Phillip Miklas-USDA-ARS, Prosser, WA, Plant Geneticist [Achievement Award]

1999 James R. Baggett - Oregon State University, Plant Breeder
James S. Beaver - University of Puerto Rico, Plant Breeder
Phillip McClean - North Dakota State University, Geneticist [Achievement Award]
James Myers - Oregon State University, Plant Breeder [Achievement Award]

2001 Dermot P. Coyne – University of Nebraska, Plant Breeder [Frazier - Zaumeyer Distinguished Lectureship]
Mark J. Bassett – University of Florida, Plant Geneticist
Soon J. Park – Agriculture and Agri-Food Canada, Plant Breeder
Mark A. Brick – Colorado State University, Plant Breeder [Achievement Award]
Ron Riley – Syngenta, Plant Breeder [Achievement Award]
Juan Carlos Rosas – Escuela Agricola Panamericana, Honduras, Plant Breeder [Achievement Award]

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

### MARK JULIAN BASSETT

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

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

Dr. Bassett has made several important contributions in the area cytogenetics of the common bean. He discovered that chromosome bridges formed in F 1 plants of P. *coccineus x P. vulgaris* crosses. He induced chromosome translocations by pollen irradiation that produced semisterility in heterozygotes. He also developed homozygous translocation lines, for which the intercrosses were analyzed cytologically. Dr. Bassett developed the five primary trisomics of common

bean with distinctive plant phenotypes and developed the karyotype at diplotene for common bean chromosomes. He discovered a system of cytoplasmic male sterility with maintainer and restorer (partial) genes. He induced a male sterile (ms) mutant with full female fertility, and the *sbms* mutant for male sterility
pleiotropic for a marker trait. He also developed an induced mutant do (dwarf outcrossing) for high outcrossing rates with full male and female fertility with *Fin.* Dr. Bassett supervised inheritance studies for the bean golden yellow mosaic (BGYM) resistance genes *bgm* and *bgm-2.* He has developed snap bean germplasm from an interspecific cross with superior BGYM resistance.

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

## SOON J. PARK

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

Dr. Park developed non-nod, super-nod, and ineffective nodulation. mutants for biological nitrogen fixation genetics and breeding studies in common bean and compared their agronomic performance. This research lead collaboration with several other researchers in U. S. and Germany as well as in Canada. He has been actively breeding several market classes of dry bean cultivars for resistance to anthracnose, bean common mosaic (BCMV), common bacterial blight (CBB), root rots, and white mold, among other characteristics. Dr. Park has extensively used exotic germplasm. to broaden the genetic base of common bean cultivars for Canadian bean growing environments and this includes interspecific crosses with P. *coccineus* and *P. acutifolius* to introduce resistance to common bacterial blight, root rot and white mold. In addition to releasing several gerinplasm. lines, Dr. Park has released 20 dry bean cultivars (in five market classes), two soybean cultivars, and one cultivar each of adzuki bean *(Vigna angularis)* and mung beans *(Vigna radiata).* He has written a book chapter and published 65 refereed and 50 non-refereed research articles.

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

## B I C AWARDS - NOMINATION REQUEST

The Bean Improvement Cooperative has proudly acknowledged outstanding contributions made by many of its members to bean research and education. The **Meritorious Service Award** has been presented to over 50 of our colleagues during the 44-year history of the BIC. These recipients have devoted many years of their illustrious careers to bean research and education, and have consistently provided outstanding service to our organization.

The BIC Coordinating Committee and Awards Committee offers a special award for BIC members who have devoted less time to their "bean careers" than our Meritorious Service Award recipients. The **BIC Achievement Award** acknowledges a scientist with fewer than 15 years of post-graduate service who has demonstrated outstanding contributions to bean research and/or education.

The BIC Coordinating Committee and Awards Committee proudly announce the second **Frazier-Zaumeyer Distinguished Lectureship**. Nomination for this award should be sent to the Awards Committee.These awards will be presented at the next BIC Biennial Meeting to deserving candidates nominated by their peers and selected by the BIC Awards Committee. Award recipients will be acknowledged at the BIC Biennial Meeting in Sacaramento CA from October 24-30, 2003. Please help us select worthy recipients.

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## BIC AWARD NOMINATION

Return by July 1, 2003 to:

Dr. Howard F. Schwartz
BIC Awards Committee Chairman
Dept. of Bioagricultural Sciences and Pest Management
Colorado State University
Fort Collins, CO 80523-6987
USA

The other Awards Committee members are:

Dr. George Hosfield
Dr. Steve Magnuson

Nominee: Name: _____

Address: _____

_____

_____

_____

Discipline: _____

Nominated for: _____ Meritorious Service Award _____Achievement Award

_____ Frazier-Zaumeyer Distinguished Lectureship

Nomination Submitted by: _____

Date of Submission: _____

[Please include a 1-page typewritten summary statement giving place of birth, date and name of institution granting each degree, career history and accomplishments of the nominee]

# 2003 BIC/NAPIA MEETINGS

## SACRAMENTO, CALIFORNIA

The BIC/NAPIA biennial meeting and associated meetings will be held Oct. 24-30, 2003 at the Embassy Suites Hotel in downtown Sacramento. This hotel is a brand new hotel opened in 2002 and is well located near the major attractions of downtown Sacramento, including Old Sacramento with its eateries and bars, the State Railroad Museum, the Well's Fargo Museum, the Downtown Plaza Mall, etc. It is within walking distance from the State Capitol and Sutter's Fort. For more information about Sacramento, go to http://www.sacramentocvb.org/ .

The Embassy Suites Hotel (http://www.embassysuites.com/en/es/hotels/index.jhtml;jsessionid=4FRRS30FK5TDDJ31AOR2K3Q?ctyhocn=SACESES ) has many amenities including complimentary airport transportation, daily newspaper, indoor pool, whirlpool, sauna and fitness center. The Hotel is holding a block of rooms for this conference. The rate of $ 124.00 includes a free breakfast cooked to order and a nightly two-hour beverage reception (not included are a 12% room tax and an assessment fee of $ 1.50 per night). **Please make reservations directly with the hotel at 1-800-EMBASSY. Be sure to mention that you are attending the "UC Davis BIC-NAPIA" meeting to get the special rate and credit the meeting so that we can get lower meeting room rates.** Reservations have to be made before October 1, 2003. After this date, the rooms will return to the hotel and rates will be substantially higher.

Registration information, fees, and final meeting agenda will be made available to members and other interested individuals in later mailings. If individuals or groups are interested in helping sponsor coffee breaks, publication costs associated with printing the Abstracts and Proceedings, and/or awards for outstanding student presentations, please contact the BIC president or Dr. R. Gilbertson of the local organizing committee (phone: +1-530-752-3163; email: rlgilbertson@ucdavis.edu). Other members of the local organizing committee are: Paul Gepts (phone: +1-530-752-7743; fax: +1-530-752-4361; email: plgepts@ucdavis.edu); Fred Bliss (phone: +1-530-666-0931; email: Fred.Bliss@seminis.com; Chet Kurowski (email: C.Kurowski@harrismoran.com); and Steve Temple (phone: +1-530-752-8216; email: srtemple@ucdavis.edu).

### First Call for Papers for the BIC

This is the first call to alert authors who desire to present oral or poster papers at the 2003 Biennial Meeting of the BIC and associated meetings. The deadline for receiving abstracts is **Friday August 15, 2003.** Abstract received after the August 15 deadline may be placed in the poster sessions if the oral sessions have filled up. (Authors will be notified if this placement is necessary). Details about the format of **Abstracts**, **Oral presentations** (1 only per registrant) and **Poster presentations** will be provided in forthcoming mailings, as will information on audiovisual equipment available during the meetings.

# TENTATIVE AGENDA

| | |
|---|---|
| Friday, Oct. 24 | 7:00 am - 9:00 am NAPIA Registration<br>9:00 am - 12:00 pm NAPIA meeting<br>1:00 pm - 5:00 pm NAPIA meeting |
| Saturday, Oct. 25 | 8:00 am - 12:00 pm NAPIA meeting<br>If necessary: 1:00 pm - 5:00 pm NAPIA meeting |
| Sunday, Oct. 26 | Wine tasting tour, Napa Valley for BIC and NAPIA participants (separate registration)<br>6:00 pm - 8:00 pm BIC Registration |
| Monday, Oct. 27 | 7:30 am - 9:00 am BIC Coordinating Committee Breakfast<br>7:00 am - 9:00 am BIC Registration<br>9:00 am - 12:00 pm BIC/National Dry Bean Council Opening Session<br>1:00 pm - 3:30 pm BIC Oral presentations<br>3:30 pm - 5:30 pm BIC Poster presentations<br>7:00 pm - 9:00 pm BIC Genetics Committee |
| Tuesday, Oct. 28 | 9:00 am - 12:00 pm BIC Oral presentations<br>1:00 pm - 3:30 pm BIC Oral presentations<br>3:30 pm - 5:30 pm BIC Poster presentations<br>7:00 pm - 9:00 pm *Phaseolus* Crop Germplasm Committee |
| Wednesday, Oct. 29 | 9:00 am - 12:00 pm BIC Oral presentations<br>1:00 pm - 6:00 pm Tour of UC Davis and local seed company (included in registration but requires advance sign-up)<br>7:00 pm BIC Mixer and Awards Banquet |
| Thursday, Oct. 30 | 9:00 am - 5:00 pm W150 Multistate Project |

A final agenda, registration materials, and additional meeting information will be sent to all BIC members in June/July 2003.

# TAXONOMY, DISTRIBUTION, AND ECOLOGY OF THE GENUS *PHASEOLUS* (LEGUMINOSAE–PAPILIONOIDEAE) IN NORTH AMERICA, MEXICO AND CENTRAL AMERICA

*George F. Freytag* and *Daniel G. Debouck*

*Phaseolus* beans are a fascinating group! So much variability exists that five distinct species have been domesticated—a size, shape, color pattern and flavor to satisfy most everyone, and nutritious, too! This lavishly illustrated monograph is the most comprehensive botanical treatment of beans to date. It starts with a brief history about the former taxonomical treatments of the genus, and goes on with the taxonomical criteria and a presentation about discriminant characteristics. It presents a full description of each section and species, its distribution and habitat, relationships with other species, uses and potentially useful traits, and historical notes. Color pictures, line drawings and distribution maps lead easily to the right identification of each species. The compilation of over 5,000 bean natural populations through collections and herbarium specimens sets the ground for present and future conservation efforts of these unique plant and food resources. This monograph will be most welcomed by bean breeders, pathologists, botanists and legume taxonomists, conservationists and natural history enthusiasts. It will be the companion book for naturalists on their field trips in the southern United States and most of Central America. It will serve as a reference for future works in documentation of neotropical biodiversity.

*Sida, Botanical Miscellany* No. 23. ISSN 0833-147, ISBN 1-88878-11-1, xviii + 300 pp, 97 b/w figures and distribution maps, 5 color plates (60 figs.), 7" × 10". 31 Dec 2002. $40.00 + p&h ($4.00 Domestic; $8.00 outside USA). Texas residents add $3.30 sales tax. Orders: Yonie Hudson, Publications Assistant, Botanical Research Institute of Texas, 509 Pecan Street, Fort Worth, Texas 76102-4060, USA, 817-332-4441 x 32. 817-332-4112 fax, yhudson@brit.org. Credit Card (Visa or MasterCard only) Number, Expiration date, and Signature. http://www.brit.org/sida/sbm/sbm23toc.htm

# Monitoring gene flow between wild relatives and landraces of common bean in Costa Rica

R.I. González-Torres[1], E. Gaitán[2], M.C. Duque[2], O. Toro[3], C. Ocampo[3], J. Tohme[2] & D.G. Debouck[3]
[1]Facultad de Ciencias, Universidad Nacional de Colombia; [2] CIAT Biotechnology Research Unit; [3] CIAT Genetic Resources Unit, A.A. 6317 Cali, Colombia.

## *Introduction*

Gene flow is the spread of genetic material among populations and may constitute an important evolutionary force to create genetic variability. We study possible cases of gene flow in accessions of *Phaseolus vulgaris* L. collected in the Central Valley of Costa Rica (Araya et al. 2001). The present study aims to establish the movement of genes in the complex wild/weed/cultivated of this crop model as a result of cross pollinations. We focus on intermediate, weedy forms in order to (1) find evidence of gene flow, and (2) quantify its importance and direction.

## *Materials and Methods*

Seeds were collected in 1987 and 1998 from six populations (Zarcero, Quircot, Tarbaca, Aserrí, Jérico and Chaguites). A morpho-agronomic evaluation, i.e. pod dehiscence, "agouti" testa (the wild phenotype being dominant in F1 and segregating materials), and seed size (quantitatively inherited), was used to select 95 weedy seeds possibly resulting from gene flow. We used phaseolin analyzed by SDS-PAGE (Gepts et al. 1986), and simple sequence repeats (SSR) of genomic DNA (Reichow & Smith 2001). To determinate the direction of gene transfer we used chloroplast DNA polymorphisms determined by PCR-RFLPs (Chacón Sánchez 2001) and single nucleotide polymorphism (SNPs) (Tost et al. 2002) of specific regions mainly of intergenic spacers and introns of cpDNA. One hundred eighty-seven original seeds of the complex plus wild controls and cultivated varieties have been analyzed.

## *Results and Discussion*

The analysis of phaseolin (controlled by nuclear genes) provides quick and reliable indication for origin and possible introgression. In the study area the predominant phaseolin was "S" and "Simple 4 (S4)" in the cultivated and wild forms, respectively. We found different phaseolins in the wild populations, i.e. Tarbaca: "S4", "M1" and "M6"; Jérico: "S" and "S4"; Quircot: "S4", "Het. (S+I)" (for heterozygote pattern between the S and I phaseolins; Ocampo et al. 2000), "S" and once "CH"; Zarcero: "S"; Aserrí: "S4"; and Chaguites: "S4". FI 5941 would indicate gene flow from cultivated into local wild forms (Table). In cultivated materials we found introductions of Andean materials (called 'Chileno') into the Central Valley. However, FI 6346 may indicate introgression and chloroplast capture. We observed forty alleles for the three microsatellites (Gaitán-Solís et.al. 2002) studied in the six populations, besides the characteristic alleles for each population. The weedy types i.e. FI 5765 and FI 5941 share microsatellites with cultivated FI 6323 and FI 6310, respectively, evidenced by a multivariate focus approached through an analysis of multiple correspondence (SAS version 8.12). The determination of haplotypes of cpDNA is important to quantify the phenomenon of 'cytoplasm capture', where the cytoplasm is taken out of the maternal parent after a series of backcrosses with the pollen donor parent. Therefore, we desire to establish the currently known haplotypes A-M (Chacón Sánchez, 2001) for the different materials realizing specific PCR-RFLPs testing for the gain or loss of a restriction site. According to Chacón Sánchez (2001), wild common beans in Costa Rica have a specific haplotype 'H', while haplotype 'G' is present in wild forms of Guatemala, Honduras, El Salvador, and Colombia. We needed to quickly distinguish between 'H' and 'G' through sequencing the fragment *rps14-psaB* spacer. We found two SNPs, one that is distinctive of 'H', and another one, *ndhA intron*, that separates 'H' and 'G'. Inferring from all markers of gene flow used in this study, we have found twenty-three cases of introgression between wild and cultivated beans, so far only in the Quircot population (eight of them discussed in the Table)

| FI No. | 100-seed weight | Testa color | Phaseolin | Haplotypes and their current distribution | Comments/ Possible gene flow direction |
|---|---|---|---|---|---|
| FI 5765 | 13 g | Beige/ Brown / Black | Simple 4* | L Mexico-Colombia | Wild phaseolin, bigger seed, wild testa, foreign haplotype. Introduced cultivated several times crossed with pollen of wild material (capture). Shares microsatellites with FI 6323. |
| FI 5941 | 14.4 | Deep purple | S | F G H S Ecuador Guatemala Costa Rica, resp. | Cultivated phaseolin, bigger seed, cultivated testa, and possible wild haplotype H. Wild material crossed (several times ?) with pollen of cultivated materials. Shares microsatellites with FI 6310 . |
| FI 5942 | 12.6 | Deep purple | S | J Mexico | Phaseolin of cultivated material, smaller seed, cultivated testa, Mexican haplotype. Introduced cultivated crossed with pollen of wild beans. |
| FI 5963 | 13.5 | Beige/Black | Simple 4 | J Mexico | Phaseolin as in the wild, but bigger seed, wild testa, haplotype from Mexico. Introduced cultivated materials crossed with pollen of local wild beans. |
| FI 5965 | 15.7 | Beige/ Black | Simple 4 | K Mesoam. Cultiv. | Wild phaseolin, bigger seed size, wild testa, haplotype of cultivated Mesoamerican. Cultivated material crossed with pollen of wild beans. |
| FI 6310 | 19.3 | Red | S | J Mex | Cultivated phaseolin, smaller seed size, cultivated testa, haplotype J. Possibly crossed with intermediate (highly related microsatellites with FI 5941). |
| FI 6323 | 17.1 | Black | S | K Mesoam.cultiv | Cultivated phaseolin, smaller seed size, cultivated testa, haplotype K. Possibly crossed with intermediate (shared microsatellites with FI 5765). |
| FI 6346 | 25.1 | Red/ Beige | T | K Mesoam.cultiv | Andean phaseolin, smaller seed size, cultivated testa, haplotype K; possible introduction from the Andes and chloroplast capture with local cultivated materials. |

- A novel type of phaseolin with simple pattern (Ocampo et al., 2000).

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# EVALUATION AND IDENTIFICATION OF NEW COMMON BEAN BRIDGE CULTIVARS

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During the domestication of the common bean (*Phaseolus vulgaris* L.) two gene pools, Middle American and Andean, were established. Due to the co-evolution host-pathogen, disease resistance genes of Middle American origin are usually effective against pathogens of Andean origin and vice-versa. For this reason, bean breeders have a unique opportunity to pyramid resistance genes derived from the two gene pools in the same background and develop cultivars with complementary resistance to several races of different pathogens. However, upon the establishment of the two gene pools a reproductive barrier was also formed and this may lead to hybrid lethality in several crosses (Vieira et al., 1999).

Singh et al. (1984) detected two genes involved with the control of bean incompatibility, $DL_1$ and $DL_2$. Small seeds of Middle American origin usually present the genotype $DL_1DL_1dl_2dl_2$, which is incompatible with the genotype $dl_1dl_1DL_2DL_2$ present in medium and large seed cultivars of Andean origin. Incompatibility would be due to the presence of a dominant allele ($DL_1$ and $DL_2$) in the two loci governing this trait. In other words, plants with genotype $DL_1\_DL_2\_$ would be abnormal. However, cultivars with genotype $dl_1dl_1dl_2dl_2$ always produce normal hybrids.

The main goal of this work was to evaluate the efficiency of bean cultivars mentioned in the literature as good bridge cultivars. These cultivars were used to bridge the cross between the Andean cultivar Jalo EEP558 and Middle American cultivar Rudá. New cultivars were also tested for their ability to bridge that same cross.

## Material and methods

The cultivars evaluated were: G2858, Diacol Calima and Ica Pijal, cited by Singh et al. (1984); CNF10, Milionario, Rio Vermelho, CNF261 and Small White, cited by Vieira et al. (1989); and the new cultivars tested were KW765, KW780 , GGWax, Novo Jalo, AND277 and BAT 93.

The Andean cultivars were crossed with Rudá, and the Middle American cultivars were crossed with Jalo EEP558. The crosses were done in March 2002, in a greenhouse of the Biotechnology Research Institute (BIOAGRO) of the Federal University of Viçosa (UFV), Minas Gerais, Brazil. All hybrids derived from cultivar Jalo EEP558 were crossed with cultivar Rudá and vice-versa.

To confirm the Andean or Middle American origin of the cultivars we used a RAPD primer which is able to distinguish between individuals belonging to the two gene pools (Dr. Ana Lilia Alzate-Marin, personal communication).

## Results and Discussion

All bean cultivars involved in this study were initially tested by the RAPD technique with a primer which is able to distinguish between plants from the two gene pools, Middle American or Andean, and their classification was confirmed (Figure 1).

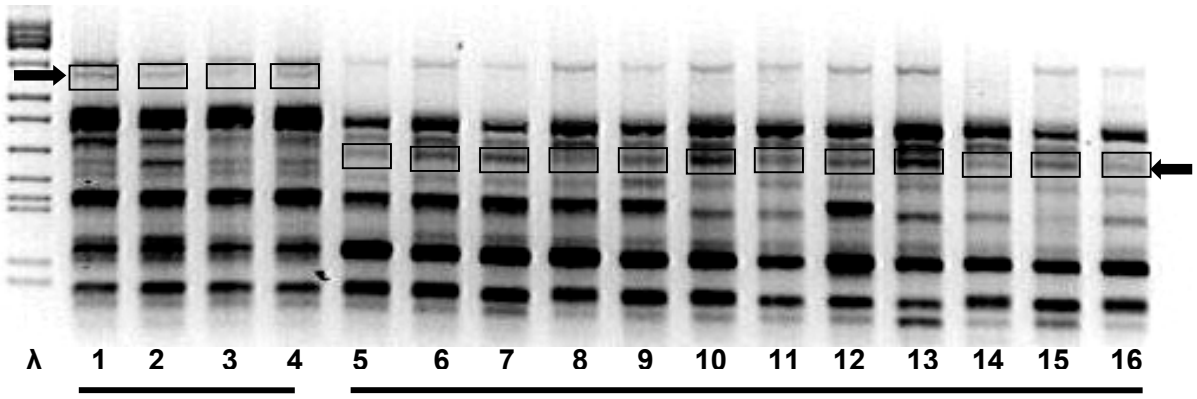


**Figure 1** – Electrophoretic analysis of DNA amplification products obtained by the RAPD technique. The lanes are as follows: λ, lambda phage DNA cut with enzymes *Eco*RI, *Hin*dIII and *Bam*HI (size markers); 1, Jalo EEP558; 2, Diacol Calima; 3, AND 277; 4, Novo Jalo; 5, Rudá; 6, Milionário; 7, Rio Vermelho; 8, BAT 93; 9, Ica Pijal; 10, KW780; 11, CNF261; 12, CNF10; 13, G2858; 14, GGWax; 15, Small White; 16, KW765. The upper arrow indicates a DNA band typical of Andean cultivars; the lower arrow indicates a DNA typical of Middle American cultivars. These bands were delimited by a rectangle for clarity reasons.

The crosses of the Andean cultivars with cv. Rudá showed that cv. Diacol Calima, AND 277 and Novo Jalo were compatible with Rudá. The hybrids were crossed with Jalo EEP558 and the populations obtained segregated 1:1 (viable:non-viable plants). These results are in accordance with the model, which proposes that two dominant and complementary genes govern incompability in common, beans (Singh et al., 1984). The crosses of the Middle American cultivars with Jalo EEP558 showed that cv. Rudá, Milionário, Rio Vermelho, BAT 93, Ica Pijal, GGWax and G2858 were incompatible with that cultivar and that CNF10, CNF261, Small White, KW765 and KW780 were compatible. The viable hybrids were crossed with Rudá and the populations obtained also segregated 1:1. Cultivars G2858, Ica Pijal, Milionário and Rio Vermelho which are cited in the literature as good bridge cultivars were not effective in the crosses we tested. Among the new bridge cultivars tested AND277, Novo Jalo, KW765 and KW780 were effective to bridge the cross between Rudá and Jalo EEP558.

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# GENE FLOW AMONG WILD *Phaseolus lunatus* L. POPULATIONS IN THE CENTRAL VALLEY OF COSTA RICA

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The Central Valley of Costa Rica is considered as a region of diversity for the wild *Phaseolus lunatus* form. Lima bean is a self-compatible annual or short-living perennial species with a mixed mating system. Despite small population sizes (66% of populations with fewer than 30 individuals), frequent bottlenecks, a low allogamy rate (t < 10%), major alleles at several loci, and restricted genetic neighborhood area (NA = 56 m²), isozyme studies revealed very few heterozygous individuals ($H_o$ = 0.013) and significant polymorphism within population ($G_{ST}$ = 0.575). Using microsatellites markers, few heterozygous individuals ($H_O$ =0.012) and high intrapopulation polymorphism ($G_{ST}$ = 0.303) were also found (Baudoin *et al.*, 2000). This significant intrapopulation diversity could be due in part to the existence of gene flow. According to Slatkin (1981, 1985a), gene flow encompasses several mechanisms of gene exchange among populations, including movement of gametes, zygotes, individuals or groups of individuals from one place to another, and extinction and recolonization of entire populations.

To assess gene flow, 9 populations from Heredia were scored with 10 pairs of microsatellites primers isolated from *Phaseolus vulgaris* L. by the *Centro International de Agricultura Tropical* (CIAT). Two models were applied : i) Wright's island model (1951). The mean rate of migration (Nm) was calculated by analysing ten microsatellites loci using the Crow and Aoki (1984) formula. ii) Slatkin's private alleles model (1985b) and the corrected estimate of Nm by Slatkin and Barton's method (1989). The estimation of gene flow was made using the *Genepop* software (Raymond, Rousset, 1995). An average of the genetic differentiation coefficients was calculated by the SpaGeDI software according to classes of distance between pairs of wild populations (Hardy, Vekemans, 2002).

*Estimation of gene flow*

The fixation index ($F_{ST}$) was 0.346, and average inbreeding coefficient within populations was high ($F_{IS}$ = 0.916). The number of migrants per population and per generation from Wright's method was 0.47. By the method of Slatkin using private alleles, Nm was estimated at 0.099 for n = 10, 0.075 for n = 25, and 0.060 for n = 50. The mean number of individuals per population was 33 and the mean frequency of the private alleles was 0.35. The corrected number of migrants per population and per generation was 0.06. Nm is underestimated with Slatkin's method when the seed number per population is heterogeneous (Slatkin, 1985b).

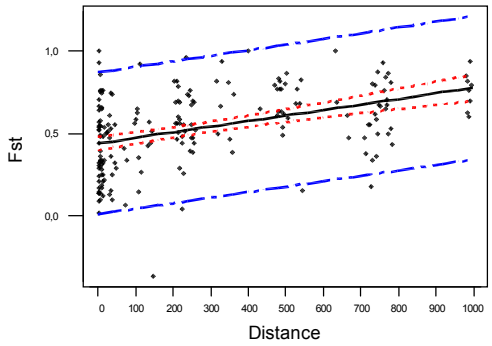


Figure 1. Linear relation between geographic distance (in m) and genetic coefficient differentiation ($F_{ST}$).

*Spatial structure of genes and isolation by distance*

For distances ranging between 0 and 997 m, a linear relation (P = 0.000) between genetic coefficient differentiation and geographic distance was obtained (Fig. 1). Geographic distance explained 18% of the divergence among populations. A linear relation between gene flow and geographic distance (P = 0.005) was also noticed for this range of distances. Beyond 1000 m, however, no relation between genetic coefficient differentiation of gene flow and geographic distance was observed.

Assuming no selection of populations and an equilibrium between genetic drift and gene flow, genetic differentiation coefficient between populations is inversely related to gene flow between populations ($Nm = (1-F_{ST})/(4F_{ST})$; Slatkin, Barton, 1989). The genetic differentiation coefficient decreased from 0.64 to 0.43 while Nm increased from 0.14 to 0.33 comparing distance classes "227-997m" and "0-226 m". Such genetic differentiation coefficients characterize populations with very significant divergence and weak to moderate gene flow (Wright, 1978).

## Conclusion and prospects

The island model and isolation by distance models were employed to measure indirectly the cumulative effects of gene flow. Wright's method is adequate for situation where equilibrium between genetic drift and gene flow has been reached in a large number of populations, which are constant in size and never go extinct (Whitlock, Mc Cauley, 1999). Slatkin and Barton (1989) compared indirect methods for estimating average level of gene flow and showed that $F_{ST}$ and rare alleles methods yield comparable estimates under a wide variety of conditions and found that $F_{ST}$ is likely to be more useful under realistic conditions. With enzymes (previoust work) and microsatellites (this study), low to moderate levels of gene flow (0.06 to 0.47) were noticed in wild Lima bean populations in the Central Valley of Costa Rica. Both enzymes and microsatellites markers showed that very great divergence (Wright, 1978) occurs among populations. This is probably due to restricted gene flow, with genetic drift therefore playing a major role in the genetic structure of Lima bean populations in the study area.

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# QTL ANALYSIS OF AN ANDEAN ADVANCED BACKCROSS POPULATION FOR YIELD TRAITS DERIVED FROM WILD *P. VULGARIS*

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## Introduction:

The genetic diversity of cultivated *P. vulgaris* is thought to be larger than that of wild common bean due to a genetic bottleneck that occurred during domestication. Yield increasing alleles may still reside untapped in the wild accessions that could be exploited to improve cultivated beans. The advanced backcross method has been shown to be a useful method for incorporating wild germplasm into cultivar breeding programs for tomato and rice. Although wild beans have been used before to transfer resistance to diseases and insects, as in the noted case of the Arcelin gene that provides resistance to bruchids, the studies presented here are among the first to attempt to obtain a higher yield potential from wild beans. The objective of this research was to conduct a molecular analysis of an Andean advanced backcross population to find quantitative trait loci from a wild bean that could be useful in the improvement of cultivated beans.

## Materials and Methods:

The population that was analyzed was derived from the Mexican wild accession G24404 and the Colombian, large red-seeded, "Radical" type variety, ICA Cerinza and represented the BC2F3 generation. The population consisted of 95 selected lines that were evaluated in three environments and an additional non-selected 62 lines that were evaluated in one of the locations for a total of 157 lines. The experiment was grown in two seasons in Popayán and one season in Darien. Three repetitions in a lattice design were used for each of the experiments. A total of 65 microsatellite markers were used to evaluate the introgression level in the full set of lines. Individuals with unexpected alleles were eliminated from the population for the sake of the quantitative analysis. Quantitative trait loci (QTL) were identified through a) single-point regression of the phenotypic data onto the marker genotypes using the software program qGENE, assuming the BC2S1 mode and b) interval and composite interval mapping analysis (IM, CIM) using the software program QTL Cartographer. Marker order was inferred for 50 markers with known locations on other genetic maps of beans. Linkage analysis was used to estimate the genetic distance between markers and to place 10 unmapped markers that were linked at 10cM or less from a mapped marker.

## Results and Discussion:

The results showed that the selected lines had a significantly lower introgression rate over all loci than the additional unselected lines indicating that selection had eliminated some amount of introgression. However together the selected and additional lines had rates of introgression over all loci that were not significantly different from those expected. For example the chi-square test for the average number of introgressed individuals across all loci was not significantly different than expected. However, segregation distortion was variable depending on the region of the genome that was assayed as indicated by chi-square tests showing significant deviation of the observed ratio from the expected ratio Segregation distortion was found to be most intense in the middle of chromosome B2 and on one arm each of B4 and B10. Mild segregation distortion occurred on chromosome B5 and on one arm of B3 and B9. Selection was always against the wild allele and always led to a predominance of the cultivated allele. Since selection was made for return to recurrent parent plant architecture and seed type this was presumed to be due to selection against genes in these regions that had a negative phenotypic effect on these traits. Other wild traits such as shattering that would be

undesirable in the advanced backcross lines were also eliminated by selection. Lack of any introgressions on chromosome B1 could have been a result of fewer microsatellites being screened for this chromosome or that introgressions for this chromosome were eliminated due to linkage drag with the *Fin* and *Ppd* genes. Wild alleles at these loci would condition indeterminate growth habit and photoperiod sensitivity. Both of these traits would be agronomically negative factors that were selected against in this cross, since the desired plant type was a early-flowering, bush bean like the recurrent parent, Cerinza.

Significant QTLs for yield were found on chromosomes 4 during the seasons in Darien 1999B and Popayan1998B, however in these cases the significant positive loci were associated with the recurrent parent Cerinza allele and the wild alleles were negative in their effect (Table 1). This indicates that there were still a large number of the alleles transmitted from the wild parent that had negative effects on yield and that remained to be eliminated from the progeny. In contrast, one QTL for yield in Popayan 1999A was associated with the wild allele and was found on chromosome 9. This QTL was not detected with yield data from any other season, indicating that there is a significant QTL x environment interaction for yield QTL as might be expected. However, several QTLs from the wild parent were found associated with unmapped markers in all three seasons. Both Darien and Popayan are good growing environments for the Cerinza variety and therefore were appropriate testing sites for advanced backcross progeny derived from Cerinza. The yield QTLs were not associated with later maturity and flowering or reduced seed size, since independent QTLs were located for these traits. Days to maturity and days to flowering as well as number of pods per plant and number of seed per plant were correlated. QTLs for these component traits were less variable across seasons. The results presented here, suggest that wild beans can be a source of genes for higher yield and yield component traits in cultivated beans and that advanced backcross strategies can be successful at transferring these genes into commercial seed-types.

**Table 1.** Yield QTLs identified by interval (IM) or composite interval (CIM) mapping analysis for the Cerinza x G24404 advanced backcross population .

| QTL | Location | Chr. | Nearest Marker | Method | LOD | Fuente | Additivity Kg/ha |
|---|---|---|---|---|---|---|---|
| *Yld*4.1 | Darien 99b | 4 | BMd015 | IM | 3 | Cerinza | 469.93 |
| | Darien 99b | 4 | BMd015 | CIM | 3.3 | Cerinza | 463.52 |
| *Yld*4.2 | Popayan 98b | 4 | BMd008 | IM | 2.7 | Cerinza | 260.35 |
| | Popayan 98b | 4 | Pv-ag004 | CIM | 4 | Cerinza | 249.18 |
| *Yld*9.1 | Darien 99b | 9 | Pv-at007 | CIM | 3.1 | G24404 | 266.23 |

# WILD BEANS AS SOURCE OF RESISTANCE TO *Colletotrichum lindemuthianum*

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Dry beans (*Phaseolus vulgaris* L.) are one of the most important leguminous crop in Brazil. It is the host of several fungus, bacterium and virus diseases. Among the air borne fungal diseases anthracnose, caused by the fungus *Colletotrichum lindemuthianum* (Sacc. & Magn.) Scrib., is one of the most important due to its constant appearance in the field and to the losses it causes. This disease affects susceptible cultivars established in local with moderate to low temperature and high relative humidity. Losses due to the disease can be as high as 100%.

Different strategies are used to control the disease such as cultural practices, chemical control and genetic resistance. The principal cultural practices include the use of disease free seeds and crop rotation. Although chemical control is not ecologically accepted, most of the time farmers do not have any other choice. Anthracnose control through fungicides includes chemical seed treatment and aerial foliage spray. Due to fungicide prices, these practices are used mainly by medium and large farmers. Consequently, the use of resistant cultivar, especially by small farmers, is the most practical and economical way to safely control the disease. However, the pathogenic variability presented by the causal agent makes the development of new resistant cultivars more complex in a breeding program.

Common beans are considered to present a narrow genetic basis mainly because only a small number of wild beans genotypes was domesticated through the evolution process. From the breeding point of view, to broaden the genetic basis of common beans, it is necessary to use the genetic diversity available in the cultivated as well as in the wild beans to enhance the possibility to find new useful genes.

The objective of this study was to test different wild beans of the Embrapa Rice & Beans germplasm bank in order to identify new resistant source to anthracnose.

The experiment was conducted in Embrapa Rice & Beans, Santo Antonio de Goiás, Goiás, Brazil. It was used 118 wild beans genotypes (*Phaseolus vulgaris* var. *aborigineus*) from the bean germplasm bank. Each entry were sown in lines of 0,7 m long, spaced by 0,2 m, in an isolated nursery. One line of the susceptible cultivar CNPF 10 was sown every 10 lines of the tested genotypes. Each entry was inoculated with the following *C. lindemuthianum* pathotypes: 89 (Alfa-Brazil), 95 (Kappa), 453 (Zeta) e 585 (Alfa-Brazil-TU Susceptible). Before each inoculation the isolated nursery was irrigated. For inoculation it was used a spore suspension of 1,2 x $10^6$ conidia $ml^{-1}$. After each inoculation the isolated nursery was covered with a black plastic, during the first night, to ensure high humidity for spore germination. Symptoms were evaluated 8-10 days after inoculation according to a 1 to 9 scale where 1 = no visible symptoms and 9 = death of much of the plant tissues. Plants rated 1 to 3 (incompatible reaction) were considered resistant and 4 to 9 (compatible reaction), susceptible.

From 118 genotypes evaluated, only 20 were considered resistant to all pathotypes tested (Table 1). Entry 8202 showed a mixture of susceptible/resistant reaction for the pathotype 585 and probably is a mixture of pure lines that needs to be purified. Entries 8155, 8306 and 8336, and 8052 and 8061 were rated 2 when inoculated with pathotypes 453, and 89 and 453, respectively. In conclusion, some of wild entries were resistant to all tested *C. lindemuthianum* pathotypes. These genotypes are very useful to enhance the resistance level of black and carioca beans that are widely grown in Brazil.

*Table 1*. Common bean wild genotypes resistant to pathotypes 89, 95, 453 and 585 of *Colletotrichum lindemuthianum*.

| Identification | Pathotypes | | | |
|---|---|---|---|---|
| | **89 (Alfa-Brazil)** | **95 (Kappa)** | **453 (Zeta)** | **585 (Alfa-Brazil TU S)** |
| 8050 | 1 | 1 | 1 | 1 |
| 8052 | 2 | 1 | 2 | 1 |
| 8061 | 2 | 1 | 2 | 1 |
| 8089 | 1 | 1 | 1 | 1 |
| 8090 | 1 | 1 | 1 | 1 |
| 8108 | 1 | 1 | 1 | 1 |
| 8109 | 1 | 1 | 1 | 1 |
| 8150 | 1 | 1 | 1 | 1 |
| 8155 | 1 | 1 | 2 | 1 |
| 8163 | 1 | 1 | 1 | 1 |
| 8169 | 1 | 1 | 1 | 1 |
| 8172 | 1 | 1 | 1 | 1 |
| 8174 | 1 | 1 | 1 | 1 |
| 8175 | 1 | 1 | 1 | 1 |
| 8195 | 1 | 1 | 1 | 1 |
| 8202 | 1 | 1 | 1 | 4/1 e 3/6 |
| 8208 | 1 | 1 | 1 | 1 |
| 8265 | 1 | 1 | 1 | 1 |
| 8306 | 1 | 1 | 2 | 1 |
| 8310 | 1 | 1 | 1 | 1 |
| 8336 | 1 | 1 | 2 | 1 |

# HEMAGGLUTINATING ACTIVITY OF LECTINS OF A WEEDY COMMON BEAN POPULATION

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## Introduction

Lectins are proteins of non-immune origin that have the potential of binding in both, specifically and reversible forms, to carbohydrates expressed on the surface of cellular membranes. One of the most important sources of lectins in nature is the dry seed of leguminous crop species (common beans, peas, lentils, etc.), with lectin content ranging from 2 to 10% of total protein (Van Damme, E.J.M., et. al, 1997). There has been a great interest in the last decades on the medicinal properties of natural plant extracts with biological activity, used as chemical protectors and cytotoxic agents on transformed cells. The objective of this study was to identify lectins in crude extracts of dry seeds collected from a weedy bean (*Phaseolus vulgaris* L) population. Partially purified extracts from such bean seeds were used to assess the biological effect of lectins on the development of transformed cells (HeLa).

## Materials and methods

Dry seed samples were collected from a local weedy common bean (*Phaseolus vulgaris* L.) population, which naturally grows near by the city of Durango, Mexico. Eleven different samples of about 400-500 seeds each were collected early December, 2001 from plants growing along a 200 m line. Immediately after harvesting, seeds were stored at $4^{o}$ C. Before cotyledons were milled (Micro-Mill), seed coat from each seed was removed, and then bean powder was stored at $4^{o}$ C until used. Protein (Bradford, 1976) and carbohydrate content (Dubois, 1956) were determined. Electrophoresis was undertaken for lectin identification (Laenmli, 1970). Specific hemagglutinating (Jaffé, 1980) and chemical activities (Neogrády, 1994) were determined. Protein partitioning was carried out on crude extracts (C.E.) using ammonium sulfate varying concentrations from 0 to 80%. Precipitated fractions were dialyzed using a 12,000 Da nitrocellulose membrane.

## Results and discussion

Electrophoretic profiles were obtained (SDS-PAGE) and six different protein groups were found regarding to their molecular weights from 97,000 Da (Phosphorylase) to 14,400 Da (data not shown). Results also indicated that those proteins present in the crude seed extracts did not show chemical specificity to any of the following sugars: D(-) ribose, (D (+) mannose, D(+) galactose, D(-) arabinose, fructose, lactose, dextrose, maltose). Genetic differences were found among seed samples for total hemagglutinating activities using crude extracts such that PS2, PS4, PS6, PS9, and PS10 had the higher values compared to a commercial dry bean cultivar, Flor de Mayo M38 (Table I).

Table 1. Hemagglutinating activities of crude extracts from weedy common bean seeds.

| Bean sample | 100 seed weight (g) | Specific activity (U/mg) | Total activity (U) |
|---|---|---|---|
| P S1 | 7.68 | 1280 | 429664 |
| P S2 | 10.93 | 1280 | 1011436* |
| P S3 | 8.52 | 1280 | 895024 |
| P S4 | 12.38 | 1280 | 1068128* |
| P S5 | 15.04 | 640 | 773920 |
| P S6 | 10.51 | 640 | 1317760* |
| P S7 | 14.62 | 640 | 375408 |
| P S8 | 10.00 | 640 | 917560 |
| P S9 | 13.17 | 1280 | 1833728* |
| P S10 | 22.59 | 640 | 1416360* |
| P S11 | 17.75 | 640 | 701120 |
| F.M. M38 | 34.66 | 640 | 837,120 |

Concanavalin A, used as a reference 640 (U/mg)
* High total activity

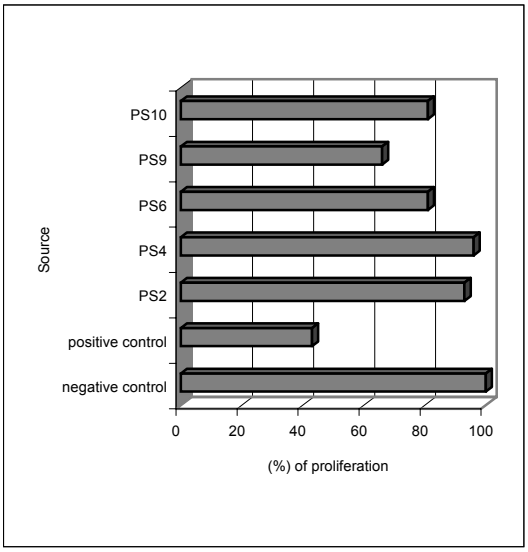


Fig.1. Biological effect of crude protein extracts on HeLa cells

Biological effect of crude protein extracts on the proliferation of HeLa cells was also evaluated using those seed samples with the highest hemagglutinating activities, PS2, PS4, PS6, PS9 and PS10 (Fig. 1). Crude extracts from the weedy bean sample PS9 (50 µg/ml) inhibited HeLa cell proliferation by 34% while the positive control (PHA-E) did it by 57% at the same concentration (50 µg/ml). This is a very important fact considering that crude seed extracts were used. Each one of the seed samples had an inhibition effect on HeLa cell proliferation in comparison to the negative control. PS2 and PS4 had the lower non-proliferation effect in comparison to the positive control with 5% and 7%, respectively. PS6 and PS10 had an intermediate effect (20%). Next step will be taken using PS9 for compound purification with biological activity since this sample had the highest effect on HeLa cells.

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# USE OF INBRED BACKCROSS METHOD TO INTRODUCE RESISTANCE TO WHITE MOLD FROM EXOTIC GERMPLASM INTO COMMON BEAN

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## Introduction

White mold, caused by *Sclerotinia sclerotiorum*, is a serious disease of common bean (*Phaseolus vulgaris*) that results in substantial yield loss and reduced seed quality. Sources of physiological resistance to white mold in cultivated dry bean are relatively rare. A strategy to increase the genetic variability for resistance in cultivated bean varieties is to introgress genes from primitive landrace varieties and related wild relatives of bean. The genetic basis of bean cultivars is extremely narrow and only a small portion (<5%) of the available genetic diversity in bean has been used, despite nearly a century of organized common bean improvement (Singh, 1999). Since only a small number of genes with large phenotypic effects control the inheritance of traits involved in domestication of *P. vulgaris* (Koinange et al., 1996) the recovery of the cultivated phenotype from wild and cultivated crosses should not be difficult. The inbred backcross method has recently received attention as an effective method to transfer more complex quantitative traits from unadapted or diverse germplasm into otherwise adapted, productive cultivars (Bliss, 1993; Tanskley and McCouch, 1997; Hartman and St. Clair, 1998). A similar strategy is being applied to improve the levels of resistance to white mold in cultivated dry bean through the introgression of germplasm from novel exotic and wild genetic sources.

## Material and Methods

Seven populations were developed through inbred backcross method by crossing four plant introduction (PI) accessions, including wild genotypes and landraces, with three adapted dry bean cultivars (Table 1). The plant introductions PI 318695, PI 313850, PI 325685, and PI 313609 were originally selected as potential sources for white mold resistance based on positive greenhouse tests conducted at MSU, NDSU and UNL (Kolkman, 2000). The greenhouse tests included the straw test (Petzoldt and Dickson, 1996), the leaf-agar plug assay (Steadman et al., 1997) and the oxalic acid assay (Kolkman and Kelly, 2000). The accessions PI 325685, and PI 318695 are wild type accessions and could provide new genetic diversity for resistance to white mold and other important traits in cultivated beans. Accession PI 313850 was also identified by Miklas et al. (1999) as having putative physiological resistance to white mold. Due to photoperiodism problems, the crosses between the accessions and adapted navy (Bunsi and Huron) and black (Tacana) bean cultivars were made under short days in a growth chamber during the summer of 2000. Field screening for white mold resistance of unadapted germplasm is not practical, since morphological and phenological traits, such as photoperiod sensitivity and climbing growth habit confound evaluation for physiological resistance. To overcome the problem of lack of adaptation two backcrosses were made to introgress traits from the wild genotypes into the adapted cultivated background and one backcross was made to introgress traits from cultivated landraces. The resulting lines were advanced to $BC_2F_3$ or $BC_1F_4$ generation in the greenhouse.

## Results and Discussion

[1] Supported by CAPES fellowship - Brazil

The inbred backcross progenies ($BC_2F_{3:4}$ and $BC_1F_{4:5}$) from the seven populations were grown in a preliminary field test in Saginaw, MI, in 2002. The populations and the number of lines evaluated in the field in Saginaw, in 2002 are shown in Table 1. The lines were visually rated for agronomic traits and both populations with Tacana were selected along with the cross of PI 318695 and Huron for further studies because they were agronomically adapted. Only a few of the best lines in the other populations were selected due to an overall lack of desirable agronomic traits particularly in the crosses with Bunsi. The populations produced using the inbred backcross approach should provide a unique opportunity for the evaluation of new novel sources of resistance to white mold in an adapted cultivated genetic background. Field evaluation was not previously possible due to problems of adaptation of wild bean germplasm lacking the domestication syndrome traits (Koinange et al., 1996). The next step in this study is to evaluate the inbred lines through greenhouse tests and field screening to determine if they carry unique traits for physiological resistance to white mold. A genetic analysis to detect the genomic regions that contribute to white mold resistance is proposed depending on the results of the greenhouse and field screenings.

Table 1. Number of inbred backcross lines developed in crosses between unadapted PI accessions with putative physiological resistance to white mold and three commercial dry bean cultivars.

| AccessionPI | Improvement status | Origin | 100 seed weight(g) | Generation | Tacana crosses | Bunsi crosses | Huron crosses |
|---|---|---|---|---|---|---|---|
| 318695 | Wild | Mexico | 3.5 | $BC_2F_{3:4}$ | 116 | 93 | 37 |
| 313850 | Cultivated | Peru | 57.2 | $BC_1F_{4:5}$ | 97 | 115 | - |
| 325685 | Wild | Mexico | 3.6 | $BC_2F_{3:4}$ | - | 100 | - |
| 313609 | Cultivated | Colombia | 60.6 | $BC_1F_{4:5}$ | - | - | 80 |

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# TRAIT CORRELATIONS IN CLIMBING BEANS

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## Introduction:

The most outstanding characteristic of climbing beans is their high yield potential compared to more commonly grown bush beans. Climbing beans have been an important component of traditional societies in Central America and the Andes for centuries. More recently, climbing beans have become important in certain areas of Africa. The principal limitation to the expansion of climbing bean technology into new areas has been the lack of new varieties. Most currently-available climbing beans come from high-altitude areas of Central and South America and do not grow well in lower elevations or hotter climates. An urgent need exists for climbing bean varieties that are adapted to lower elevations (800 to 1800m) and resistant to the diseases encountered there. The objective of this research was to test for mid-altitude adapted climbing beans.

## Plant Materials

Data was collected on two yield trials of I) 55 accessions from Rwanda, Mexico and the CIAT Core collection; and II) 55 advanced Andean breeding lines from CIAT. Both experiments were planted in Darien (1450 masl) and Palmira (1000 masl) during the rainy seasons in semesters 2001A and 2000B, using randomized complete block designs and two repetitions each. In all experiments, G685 and G2333 were used as visual checks. The genotypes were all planted at a low density of 10 plants per meter of linear row, where plots consisted of a single 2m row with 1.2 m between rows and vines were supported on bamboo and wire trellises at an approximate height of 2.0 m above the ground. Data collected included yield per plant (Y/P), pods per plant (P/P), grain per plant (G/P), 100 seed weight (100s), days to flowering (DF), days to maturity (DM) and harvest index (HI) based on stem and pod weight. Agronomic adaptation (AA) and climbing ability (CA) were evaluated on 1 to 9 scale (where 1=good and 9 = poor). The scale for climbing ability is an expanded scale compared to the accepted values for growth habit scale, which go from I to IV. Plant height (PH), raceme length (RL), number of pods per raceme (NP), pod length (PL), number of vines per guide (NV) and internode length at a height of one meter above the ground (IL) were evaluated for two plants per row and averaged to produce plot values.

## Results and Discussion

Yield, yield components and agronomic adaptation were higher on average in Darien then in Palmira for both groups of genotypes. The check varieties, G685 and G2333 and G2337 performed much better in Darien than in Palmira, indicating their lack of heat tolerance and adaptation to lower elevations. Palmira was a warmer and less hospitable location for climbing beans, and therefore a good site for selecting heat tolerance in climbing beans. Meanwhile, Darien was an ideal mid-elevation site where there was good performance by a wide range of germplasm. In both trials, there was significant correlation between traits (Table 1). As expected, climbing ability was correlated with both plant height and internode length. Climbing ability was evaluated visually on a whole-plot basis and was a rapid and accurate substitute for quantitative phenotypic measurements that are time consuming and must be taken on a per plant basis. Yield per plant, yield components, such as pods per plant and the visual evaluation of agronomic adaptation were correlated with climbing ability in the germplasm study in Darien but not always in Palmira. In the advanced breeding lines there was less variability for climbing ability than in the germplasm trial so differences in yield were not correlated with this factor but rather with agronomic adaptation. Days to maturity was correlated with climbing ability (and its components). The majority of the climbing beans in both environments matured in 100 to 120 days from planting. For both experiments, significant correlations across sites were seen between a genotype's 100 seed weight, climbing ability, plant height, days to maturity and internode length in Darien and Palmira, showing that these traits have medium to high

heritability. Yield, pods per plant and agronomic adaptation were not correlated between sites, indicating that these traits, as expected, have lower heritability.

In conclusion, we have seen significant correlations between traits associated with climbing ability. To address this, we developed several scales for agronomic adaptation and climbing ability that will be useful for the selection of breeding lines without time-consuming phenotypic measurements of plant height, internode length, etc. The sensitivity of climbing beans to genoytpe x environment interaction will have to factored into our breeding program for climbing beans. We are dealing with the issue of specific adaptation by using a parallel selection system over several sites.

**Table 1.** Correlation values (r) between traits in (I) 55 accessions from Rwanda, Mexico and the core collection and (II) 55 advanced Andean breeding lines grown in two sites Palmira (P) and Darien (D) in 2001A.

| Characteristic | Site | P/P | P/P | Y/P | Y/P | CA | CA | PH | PH | DM | DM | IL | IL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Trial** | | **I** | **II** | **I** | **II** | **I** | **II** | **I** | **II** | **I** | **II** | **I** | **II** |
| **Pods per plant** | P | 1.000 | 1.000 | 0.161 | 0.626 | 0.167 | -0.252 | -0.124 | 0.112 | -0.142 | -0.104 | 0.009 | 0.015 |
| (P/P) | D | 1.000 | 1.000 | 0.690 | 0.871 | -0.347 | 0.007 | 0.170 | 0.165 | 0.273 | -0.260 | 0.252 | 0.059 |
| **Yield per plant** | P | | | 1.000 | 1.000 | 0.088 | -0.157 | -0.076 | 0.252 | -0.110 | -0.190 | -0.074 | 0.157 |
| (Y/P) | D | | | 1.000 | 1.000 | -0.539 | 0.010 | 0.296 | 0.189 | 0.475 | -0.337 | 0.448 | 0.127 |
| **Climbing Ability** | P | | | | | 1.000 | 1.000 | -0.845 | -0.352 | -0.580 | -0.094 | -0.710 | -0.327 |
| (CA) | D | | | | | 1.000 | 1.000 | -0.596 | -0.076 | -0.626 | -0.136 | -0.772 | -0.220 |
| **Plant Height** | P | | | | | | | 1.000 | 1.000 | 0.441 | -0.194 | 0.649 | 0.700 |
| (PH) | D | | | | | | | 1.000 | 1.000 | 0.237 | 0.043 | 0.722 | 0.107 |
| **Days to Maturity** | P | | | | | | | | | 1.000 | 1.000 | 0.301 | -0.227 |
| (DM) | D | | | | | | | | | 1.000 | 1.000 | 0.398 | 0.006 |
| **Internode length** | P | | | | | | | | | | | 1.000 | 1.000 |
| (IL) | D | | | | | | | | | | | 1.000 | 1.000 |
| **Correlation between sites** | | 0.204 | 0.108 | -0.092 | 0.072 | 0.803 | 0.212 | 0.397 | 0.169 | 0.527 | 0.341 | 0.534 | 0.289 |

# RANDOM AMPLIFIED POLYMORPHIC DNA VARIATION WITHIN SOME BLACK BEAN LANDRACES (*Phaseolus vulgaris* L.) FROM MEXICAN HIGHLANDS

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**SUMMARY**. Thirty landraces of black common bean (*Phaseolus vulgaris* L.), 24 of them with shiny seed coat and six with opaque seed coat, from Mexican highlands were analyzed by RAPD (Random Amplified Polymorphic DNA). Amplification with six random primers generated 36 reproducible bands, of which 14 bands were polymorphic. In the cluster analysis shiny seed coat individuals were divided into five subclusters ca 87% genetic similarity. Opaque seed coat individuals were divided onto two main branches with ca 73% genetic similarity. The average intervarietal similarities estimated by Jaccard Similarity Coefficient ranged from 0.69 to 0.97 for the 24 shiny seed coat beans and from 0.71 to 0.94 for the opaque seed coat bean landraces. In the join cluster analysis between shiny and opaque seed coat bean separation of genotypes was not very clear, even though a notable variation in phenotypic characters among them was observed.

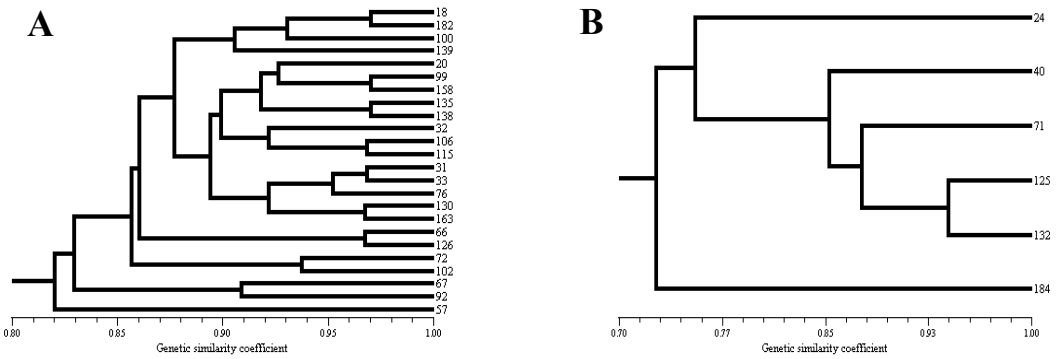
**INTRODUCTION**. The common bean is the most important grain legume for direct human consumption in Mexico and is second source of vegetable protein intake of the population. The annual per capita consumption is 15 kg ca. The biggest areas in which beans are grown in Mexico are in the north and middle part of the country, however in some areas of the middle and south of Mexico the predominant production system for the common bean is subsistence agriculture which is characterized by diversity, both within and between crops. There are regional preferences based on seed morphological characteristics, in the highlands there are regions in which light color varieties are preferred while in others black seeded beans are preferred especially of shiny seed coat even though in the south area of the country the opaque seed coat is preferred. Differences on shape and seed size, within black seeded beans are observed. It is necessary to know genetic diversity in beans to use it in breeding programs.
The objective of this study was to detect genetic diversity within shiny and opaque seed coat black beans (*Phaseolus vulgaris*) from Mexican highlands based on RAPD analysis.

**MATERIALS AND METHODS**. Thirty landraces of black seeded dry beans (six opaque and twenty four shiny seed coat) originating in highlands of six states of middle and south of Mexico were studied. Within every landrace, seeds phenotypically similar were selected based on seed color and size. DNA was extracted from leaves according to the protocol described by Llaca (1992). Polymerase chain reaction (PCR) was performed in a volume of 25 µL containing 50 mM KCl, 10 Mm Tris-HCl, 1.5 mM MgCl2, 0.2 mM of each dNTPs, 0.36, 50 ng genomic DNA and 1.0 unit of taq DNA polymerase (GIBCO-BRL). Thermal cycle used was: one cycle 94 °C for 3 min, then three cycles 94 °C for1 min, 36 °C for 1 m and 72 °C for 2 min; then 36 cycles 94 °C for 10 sec, 40 °C for 20 sec and 72 °C for 2 min. and finally 72 °C for 5 min. Each cultivar was scored for the presence or absence of every amplification product and the data entered into a binary data matrix. Coefficients of similarity of Jaccard's were calculated; cluster analysis, using the UPGMA method (unweighted pair-group method with arithmetical averages) was performed; and a dendrogram was produced using the Numerical Taxonomy Multivariate Analysis version 1.6 (2).

**RESULTS AND DISCUSSION**. A total of 15 primers were evaluated for their ability to prime PCR amplification; 10 of these showed evidence of polymorphism. Six primers were ultimately selected (OPF10, OPF13, OPK12, OPV06, OPI09, AC20) for further evaluation, which produced a total of 36 clear, easily detectable bands, 14 of which were polymorphic among the group of black seeded dry beans (*Phaseolus vulgaris* L.). The size of the DNA fragments ranged from 550 to 2100 bp. The number of scorable RAPD fragments generated per primer varied between 5 and 7 and the number of polymorphic bands per primer ranged from 1 to 5.

Associations among the 24 shiny seed coat landraces are presented in Figure 1A. The accessions were subdivided into five subclusters c.a 87% genetic similarity. The most closely associated shiny seed coat individuals were 99 and 158; 135 and 138 all of them are from different sites of the state of Puebla, and also 18 and 182 which are from different states. The average intervarietal similarities estimated by Jaccard Similarity Coefficient ranged from 0.69 to 0.97 for the 24 shiny seed coat bean landraces. Opaque seed coat individuals were divided onto two main branches with c.a 73% genetic similarity (Figure 1B). The result of pair-wise comparisons within the opaque group indicated that Jamapa (number 184) variety used as a reference was least similar to all other genotypes (less than 0.78 similarity coefficient when compared to the other five accessions). This could be due to fact that Jamapa is originated from lowlands even though the genotype used has been adapted to highlands through selection.

The results indicates that there exist a relative high level of similarity within most of the shiny and opaque seed coat beans, however it is necessary to increase the number of oligonucleotides to measure diversity.




s for shiny (A) and opaque (B) black bean landraces based on cluster analysis (UPGMA) of genetic-similarity estimates (Jaccard's coefficient) from RAPD data.

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# Promising common bean landraces (*Phaseolus vulgaris* L.) from Bulgaria

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**Introduction:** Dry bean is the most important legume crop in Bulgaria and it's grown by both commercial and small scale farmers. Except of Northeastern Bulgaria, in region "Dobrudja" where most beans are produced on large holdings in modern production system, in other regions are usually cultivated by small landholders mainly landraces. In the region "Dobrudja" are grown bred varieties during the last 50 years. Local forms and populations are characterized with great variability resulting from introductions, the spontaneous selection process and long-term farm selection (Ganeva, 1978). They have a considerable potential value to the country. In Bulgaria as well as in other European countries, large white seeded types are preferred obtaining better prices in the market. The main objective of this study was to make morphological and agronomic characterization related to the seed yield and its components. A preliminary study with molecular markers using RAPDs analysis was performed on a part of the samples.

**Material and Methods:** The evaluation trials were done during 1999-2001 on 21 accessions of P. vulgaris collected during 1995 from South and Southeastern part of the country and one check-breeding variety. Each accession was sown in two rows with 5m length and 0.7m inter row in two replications in IPGR (Sadovo, Bulgaria). From each accession and each replication were collected 10 plants for biometrical measurements. Comparatively study was made on morphological and agronomic characters (plant height; weight/pl; number of pods/pl.; weight pods/pl.; number of seeds/pl.; weight of seeds/pl.; weight of 100seeds; shape and color of seeds). Evaluation was undertaken following the IBPGR Descriptor (Rome, 1982). For molecular characterization, DNA was extracted from young leaves according to the protocol described by Cenis (1992). RAPD analysis was carried out using random decamer primers from Operon Technologies. This analysis was performed at the National Plant Breeding Station, Elvas, Portugal.

**Results and Discussion:** The Bulgarian bean landraces show a large diversity in most of the traits studied (Fig.1). Seed type (color and size) illustrates the variability of Phaseolus vulgaris. In addition, these characteristics are important descriptors in terms of marketing and agronomic production. The more common ones are white (14 accessions), the rest are black (2 accessions), purple (1 accession), mixed (5 accessions). The seed shape could be kidney, ovoid and truncate fastigiated. The most important characteristics related to the production are number of pods/plant and number of seeds/plant. Among all landraces ns: 4,6,11,17,18 and 21 show the highest value of these characters. According to the seed size almost the accessions belonged to the group of medium size (25-40g), except the accessions number 7,9,12,16 that have large seed with 100 seed weight more than 40g with white color and kidney shape. The preliminary study using RAPD markers revealed their ability to produce polymorphisms among the different accessions. These results means that it is possible distinguish the accessions by this technique and evaluate the genetic diversity of the material. Further studies are in progress with the aim to characterize all the accessions.

**Conclusions**: Some populations were chosen to be utilized in breeding programmes with the objective to improve the quality of seed and the architecture of the plant.

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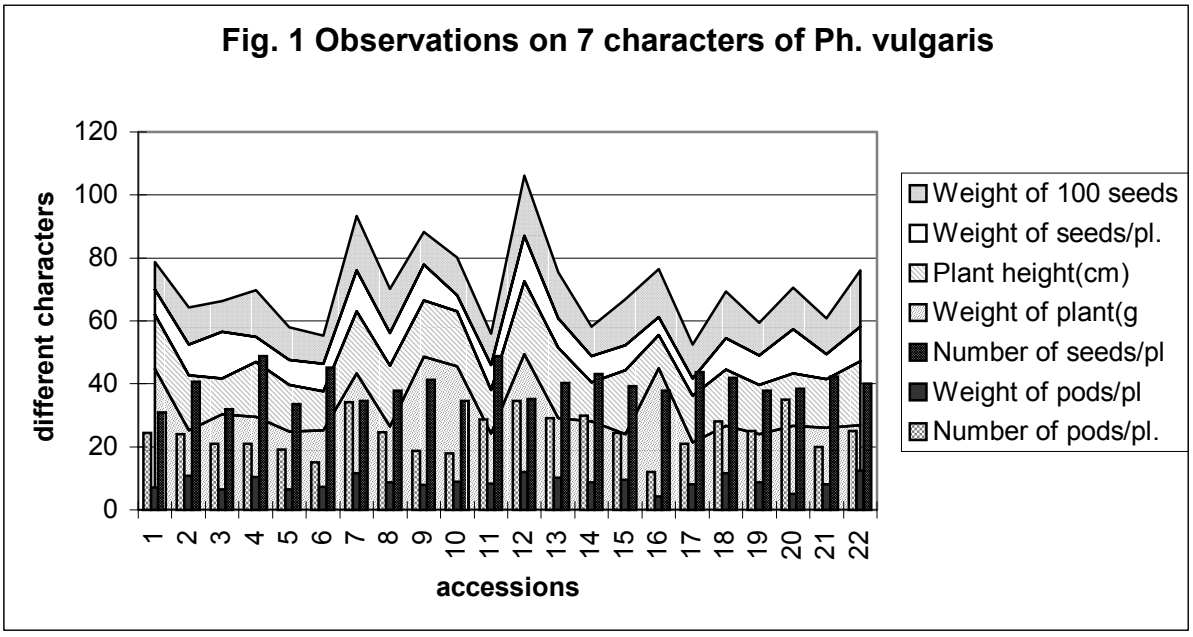


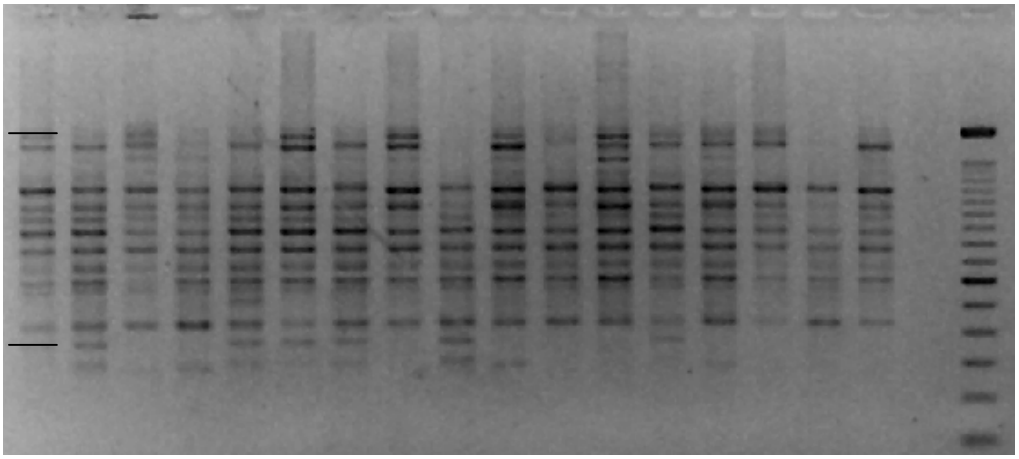

Fig. 2 – RAPD amplification pattern obtained with primer OPL05 in 17 accessions of *P. vulgaris*. Arrows indicated polymorphic bands.

# COLLECTING COMMON BEAN (*PHASEOLUS VULGARIS* L.) GERMPLASM IN BRAZIL - I

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## Introduction

Beans are an important source of protein in the diet of the Brazilian population and are farmed in an array of cropping systems, in areas ranging from less than one hectare to hundreds, under rainfed conditions or supplementary irrigation, providing three major harvests per year (Yokoyama et al., 2000.). To support these so diversified cropping systems, and to preserve genetic diversity, collecting, conservation and evaluation of germplasm have been practiced in the country for many years. Taking in consideration the long history of bean cultivation in the State of Santa Catarina, as well as the significance and representativeness of this region for dry beans, an expedition was set up to collect adapted common bean germplasm (Nadal, 1992).

## Objectives

This expedition was organized to explore regional common bean types and to obtain a representative collection of landraces farmed in Southern Brazil. Emphasis was also given to the collection of some ethnic, botanical and economic information, and to determine the extent of genetic erosion in the region.

## Methods

### Target area

At the Embrapa Rice and Beans Germplasm Bank a review of information and literature on regions with traditional bean farms was performed (Fonseca & Vieira, 1986). The available documentation on previous expeditions in the last 25 years was extensively examined and the target collecting area chosen was in the Northwest region of the State of Santa Catarina in Southern Brazil.

### Identification of sites

To increase the chances of success journeys, contacts were made with experimental stations in the area, which, in turn, contacted local extension offices in each county. The extension office informed the planting season and time of harvest to help the collecting team to organize the proper time schedule and farms to be visited. That procedure avoided waste of time and provided precise samplings in specific sites where farmers have been growing traditional cultivars for more than 20 years, with some properties actually growing them for more than 40 years.

### Sampling strategy

The sampling procedure targeted seeds from the farmer's own stocks kept in cellars, conditioned in bags, boxes or any other container. Cereal brokers and small roadside markets were also visited. Data collected for each sample was recorded in a logbook at the moment of sampling, considering several parameters: the county; the local variety name; the period of time the variety was being utilized; its origin, if known; and any other information provided by the farmers such as market opportunity or the traditional dishes they made with that type of beans (Fonseca & Freire, 1998). That data is important for the "passport" of each sample.

**Economic and social aspects**

The original landscape of most of the region had already been deforested; almost all native pine (*Araucaria angustifolia*) and hardwood has already been used for lumber or fuel. Most of the highlands are now being used for pasture and agriculture is practiced in the valleys. The majority of the population is of European ancestry, who migrated to the region in the 19th century. They are mostly Germans, Italians and Poles, very fond of traditional values. Their conservative life styles have largely contributed to the preservation of traditional varieties for so many years. Genetic diversity is well preserved in the region, because farmers market the commercial enhanced varieties, but keep on cultivating the old ones for self consumption.

**Results**

Eighty two samples of *Phaseolus vulgaris* were collected in July 2000, in 13 municipalities. This total was composed of the following types: 37.8% of the samples were small black tegument beans; 25.6% large seed types (white, colored and mottled); 12.1% small red; 3.6% yellow; 2.4% carioca (small, buff color seeds with brown stripes); 1.2% roxinho (small purple); 6% small brown; and 10.9% mixed (Table 1). All samples were introduced in the Rice and Beans Research Center Germplasm Bank for preservation and for characterization and evaluation by a multidisciplinary team. All germplasm may be shared with individuals and organizations from Brazil and abroad signing the Exchange Material Agreement (Acordo de Tansferência de Material - ATM).

**Table 1.** Common bean germplasm collected in the State of Santa Catarina

| Bean type | No. of samples |
|---|---|
| Small black | 31 |
| Small red | 10 |
| Large seed types (various colors) | 21 |
| Small brown | 05 |
| Carioca (buff color with brown stripes) | 02 |
| Small yellow | 03 |
| Roxinho (small, purple) | 01 |
| Other types | 09 |
| Total | 82 |

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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).

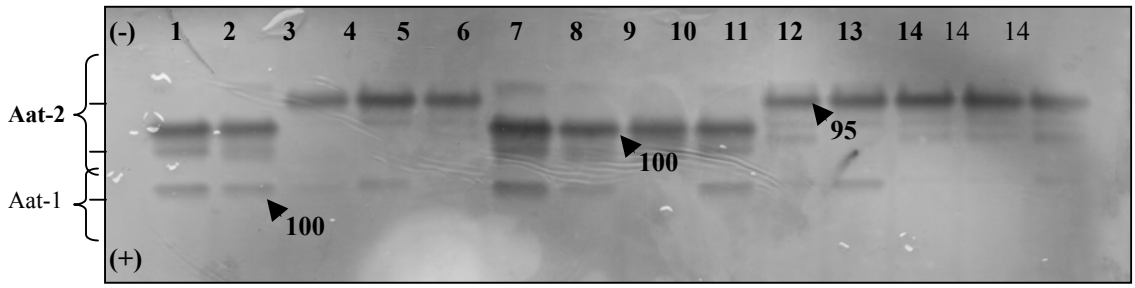


Fig. 1. Polyacrylamide gel phenotypes observed for aspartate aminotransferase (AAT). Individuals in lanes 1 and 2 are cultivated (var. *acutifolius*), individuals 6 and 7 are wild var. *acutifolius*, and individuals 8 and 9 are wild var. *tenuifolius*. The rest are classified as *P. parvifolius* (lane 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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# HISTOLOGICAL STUDY OF IMMATURE INTERSPECIFIC EMBRYO ABORTION BETWEEN *Phaseolus vulgaris* L. And *P. polyanthus* GREENM.

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## Introduction

More than 200 pathogens have been reported attacking common bean, some of them causing considerable economic losses (Graham *et al.* 1997, Singh 1999). Good sources of resistance have been found mainly in *Phaseolus coccineus* L. and *P. polyanthus* Greenm. (Baudoin *et al.*, 1992). Some interspecific crosses have been attempted by Lecomte (1997) and Geerts (2001) between *P. polyanthus* (♀) and *P. vulgaris*. Although fertilized ovules were obtained, up to 60 % of globular embryos failed within three to five days to develop due to undefined incompatibility barriers between embryo and mother plant. The aim of this study is to elucidate causes of embryo abortion in reciprocal crosses between *P. vulgaris* and *P. polyanthus*.

## Material and Methods

A wild (G 21245) and a cultivated (NI 637) genotype of *P. vulgaris* (PV) and two cultivated genotypes (NI 1015 and G 35348) of *P. polyanthus* (PP) were used. Accessions were selected on the basis of their good ability to flower in growth chamber conditions. Different combinations were made using either PV or PP as female partner. Since all pods obtained by crossing PP (♀) x PV aborted between 5 to 7 days after pollination (DAP), seeds were collected every day, from auto-pollinated flowers and flowers pollinated by either PV or PP. Histological observations were made from these seeds. Methacrylate resin sections, 2 µm thick, of 2 to 6 day-old hybrid seeds were used to examine the stage of embryo development and the state of seed tissues. For each cross, five pods of maternal genotypes (PP or PV), containing three or seven seeds respectively, were examined. These observations are aimed to determine the main causes of abortion and the developmental stages at which interspecific embryos should be rescued.

## Results and Discussion

In the comparative histological study, we only considered hybrid seeds, if pollen had germinated on the stigmatic surface and pollen tube residues were observed in sections of the micropylar canal. Number of hybrid seeds observed per cross is summarized in **Table 1**.

**Table 1.** Numbers of hybrid seeds obtained between *Phaseolus vulgaris* (PV) and *P. polyanthus* (PP) and reciprocal crosses.

| ***Female*** | ***Male*** | | | |
|---|---|---|---|---|
| | G 21245 (PV) | NI 637 (PV) | NI 1015 (PP) | Total |
| G 21245 (PV) | | | 123 | 123 |
| NI 1015 (PP) | 46 | 11 | | 57 |
| G35348 (PP) | 37 | 16 | | 53 |
| Total | 83 | 27 | 123 | 233 |

Embryos aborted at different developmental stages depending on the genotypes used. In more than 20 % of the seeds obtained by using PV (G 21245) as a female, a two-celled embryo could be obtained while less than 10 % of hybrid embryos reached this stage in the reciprocal crosses PP (♀) x PV (Geerts *et al.*, 2002). When using PV as a female, the first division was initiated 3 DAP and embryo developed to an early globular stage within 6 days in 50 % of the cases. Mature hybrid seeds were obtained. In contrast, when using PP as a female, first division

was initiated between 4 to 5 DAP and only 4 embryos out of 107 showed more than two cells 6 DAP. Most of them (74.1 %) did not divide and remained unicellular. All seeds aborted between 6 to 7 DAP.

Differences between early embryo abortion in reciprocal crosses are mainly related to the endosperm development. While a rapid division of primary endosperm nucleus (PEN) is observed in PV (♀) x PP seeds, allowing the further development of the embryo which is initiated 2 to 3 DAP, PEN stay uninucleated in PP (♀) x PV seeds during the first four DAP, limiting nutrient exchange between maternal tissue and zygote. Moreover, our results showed that zygotes of PP (♀) x PV seeds were still able to divide 5 DAP when PEN had divided at least once. This suggests that embryo abortion in PP (♀) x PV seeds could be related to a decrease in nutrient exchange at the beginning of its development, increasing the time at which first division can occur rather than incompatibilities between hybrid embryo and endosperm. This hypothesis is supported by the observations of Lecomte *et al.* (1998) describing wall thickening of the endothelial cells in PP seeds that are tangential while they are radial in PV seeds. Histological differences between maternal tissues in reciprocal crosses could thus be a key factor in the abortion processes.

Later in the hybrid embryo development, the proliferation of the endothelium was clearly described as the main factor of embryo abortion. Differences in the developmental rate of this endothelium proliferation between reciprocal crosses could be attributed to genetic factors or to the rate of endosperm development. In PP (♀) x PV seeds proliferation could be limited to one to five cells in thickness due to the poor endosperm development, while in PV (♀) x PP seeds the development of multinucleated endosperm could lead to a greater endothelial cell proliferation and subsequent later embryo abortion.

We also observed hypertrophy of vascular elements at the chalazal end. Hypertrophy was mainly located at hypostase level. This direct disruption of nutrient transfer was observed in all crosses without significant differences between them. Hypertrophy of vascular elements was observed 3 to 4 DAP and not later (Geerts *et al.*, 2002).

The importance of the abnormalities observed during embryo development depended to a great extent on the compatibility between the genotypes crossed. Results also suggest that the appropriate time for rescue of PP (♀) x PV embryos is at the early globular stage. A pod culture technique was described by Geerts (2001) and Toussaint *et al.* (2002) allowing the development of two-five day-old PV and PP embryos. This technique is now applied to hybrid pods.

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# GENETIC ANALYSIS OF CROSSES BETWEEN CULTIVATED TEPARY BEAN AND WILD *PHASEOLUS ACUTIFOLIUS* AND *P. PARVIFOLIUS*. -

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**Introduction:** Cultivated tepary bean (*Phaseolus acutifolius*) has several wild relatives. First are the wild accessions within the species itself, these include two variants (var. *acutifolius* and var. *tenuifolius*) and second there are the wild accessions belonging to the closely related species, *P. parvifolius*. The genetic diversity within cultivated tepary beans is small, therefore the objective of this research was to study the variability generated by crossing cultivated tepary beans by several of their wild relatives. These crosses were analyzed for polymorphism and segregation in the F2 generation using common bean microsatellites. The crosses can be expected to incorporate added genetic diversity into the cultivated tepary beans which may be useful for breeding this neglected crop and will also be used to generate recombinant inbred lines that can be analyzed for biotic and abiotic stress tolerance genes segregating in the populations derived from these crosses.

Methodology**: We used three contrasting tepary parents to develop two reciprocal F2 populations (Table 1). Parents included the genotypes G40022 (cultivated *P. acutifolius* from Arizona, USA), G40186 (wild *P. parvifolius* from Jalapa, Mexico) and G40240 (wild *P. acutifolius* var. *tenuifolius* from Durango, Mexico). Crosses were made with hand emasculation and both the F1 and F2 plants were grown in 9-inch pots in the greenhouse and single harvested. The F3 seed from each of 120 F2 plants was harvested from the greenhouse and was field-planted at CIAT headquarters in semester 2002A and 2002B. Seed scarification was used to increase the germination rate on both these sets fo reciprocal cross populations. In the F2 generation, data was collected on a series of phenotypic characteristics and furthermore, leaf tissue was collected for each individual plant grown in the greenhouse so that DNA could be extracted with standard methods. Ninety-four plants were analyzed for each of the AP populations and fourty-six plants were analyzed for each of the AT populations. A total of 68 common bean microsatellite markers were tested for polymorphism on the parents of each population. Polymorphic microsatellites were run on all individuals of the population along with the parents.**

**Results and Discussion:** Phenotypic analysis showed that both the AP and AT populations were segregating for several phenotypic traits and several of these appear to be simply inherited, notably stem color and flower color. Meanwhile, growth habit, plant height, flowering date, maturation date, leaf size, leaf color, pod size, yield and yield components were more quantitative traits in all the populations. Leaf shape was probably an oligogenically inherited trait because there were gradations between the narrow leaf of the wild tepary bean and the wider leaf of the cultivated tepary bean. For the molecular survey, the rate of parental polymorphism was roughly equivalent for both the AP (28 microsatellites or 40.6%) and AT (29 microsatellites or 43.3%) populations. Of these a total of 25 and 20 microsatellites were selected to run on the AP and AT populations, respectively. Significant segregation distortion was observed for 44%, 52%, 15% and 25% of these markers in the populations AP-1, AP-2, AT-1 and AT-2,

respectively (Table 1). The average segregation distortion was higher for the cross between *P. acutifolius* and *P. parvifolius* (48.0%) than between *P. a.* var. *acutifolius* and var. *tenuifolius* (20.0%). The segregation distortion results suggest that there is a greater distance between the parents of the AP population than the AT population. Supporting this hypothesis was the observation of genetic incompatibilities and hybrid lethals and dwarfs in the cross between *P. acutifolius* and *P. parvifolius* but none between *P. acutifolius* var. *acutifolius* and var. *tenuifolius*. In each pair of reciprocal crosses the use of the cultivated *P. acutifolius* as the female parent reduced the amount of segregation distortion, while the use of the wild parent, either *P. parvifolius* or *P. acutifolius var. tenuifolius* increased the amount of segregation distortion. This may reflect the possibility that a cytoplasmic factor for incompatibility is more significant when the wild tepary beans are used as females than when the cultivated tepary bean is used as the female parent. An additional observation was that the maternal allele was always favored over the parental allele in all of the crosses used in this study, however this was more notable in the *P. acutifolius* x *P. acutifolius* var *tenuifolius* crosses than in the *P. acutifolius* x *P. parvifolius* crosses. Garvin and Weeden (1994; Journal of Heredity 85: 273-278) made a series of inter-varietal crosses between *P. a.* var. *acutifolius* and var. *tenuifolius* and studied the resulting populations with isozymes and RFLPs, finding a similar levels of segregation distortion in this type of population, however our analysis is the first that we know of to reveal the high levels of segregation distortion in the inter-specific crosses between *P. acutifolius* and *P. parvifolius*. Segregation distortion and hybrid lethal incompatibilities are common with both intra-specific (eg. Andean x Mesoamerican *P. vulgaris*) and inter-specific crosses (*P. vulgaris* x *P. acutifolius*) within the genus *Phaseolus* and cytoplasmic effects have been observed in other crosses between species within the genus, including in crosses between *P. acutifolius* and ***P. vulgaris*.**

**Table 1.** Number of microsatellite markers showing segregation distortion (SD) and average frequency of cultivated (C), wild (W) and heterozygous (H) genotypes for four F2 populations developed for the genetic analysis of tepary bean.

| PopCode | Type of Cross | Female Parent | Male Parent | No. Indiv | No. Markers | | % C | % W | % H |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | total | SD | | | |
| AP-1 | Inter-specific (cultivated x wild) | G40022 | G40186 | 100 | 25 | 11 | 31.6 | 22.8 | 45.6 |
| AP-2 | Inter-specific (wild x cultivated) | G40186 | G40022 | 100 | 25 | 13 | 24.1 | 29.9 | 38.8 |
| AT-1 | Inter-varietal (cultivated x wild) | G40022 | G40240 | 46 | 20 | 3 | 25.4 | 17.8 | 56.8 |
| AT-2 | Inter-varietal (wild x cultivated) | G40240 | G40022 | 46 | 20 | 5 | 21.8 | 34.6 | 43.6 |

# Viability of Seed of Reciprocal Interspecific Crosses between *Phaseolus vulgaris* L. and *Phaseolus acutifolius* A. Gray

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Tepary beans are a potential source of heat, drought, common bacterial blight, root rot and insect resistance not available in common bean. Reciprocal crosses between *P. vulgaris* and *P. acutifolius* have met with limited success due to $F_1$ hybrid sterility and postzygotic barriers (Coyne, 1964; Rabakoarihanta et al.,1980; Prendota et al., 1982; Parker and Michaels, 1986). Embryos often fail to form seed and abort within 16-25 days. Embryo rescue is used to produce viable seedlings. Hybrid embryos that survive rescue may develop abnormalities such as stunting and wilt (Parker and Michaels, 1986). Honma (1956) reported a fertile Great Northern x *P. acutifolius* $F_1$ hybrid and was able to produce a $F_2$ segregating for bacterial blight resistance. The Great Northern varieties 'Nebraska 1', 'Jules', 'Tara', 'Star', 'Valley' and 'Emerson' are direct descendants from this cross (Hucl and Scoles, 1985). This initial success has not been duplicated since. Interspecific hybrids that reach maturity and flower have indehiscent anthers that do not shed their pollen. Pollen fertility is usually absent or very low (0-8%)( Rabakoarihanta et al.,1982). Female interspecific gametophytes are fertile but zygotes abort early during their development (Mok et al., 1978; Prendota et al., 1982). Hybrid sterility is most likely caused by the incompatible epistatic effect between dominant complementary genes controlling gametophyte development (Dobzhansky 1936; Muller 1942). The following research is an attempt to produce interspecific hybrids between tepary and common beans without embryo rescue.

Seeds for this study were obtained from the tepary seed collection at the tropical agricultural station in Puerto Rico (TARS) and from the common bean seed collection at the horticultural sciences department, University of Florida. Parental lines included the tepary lines PI 321637-s and PI 321638-s and common bean lines Ica Pijao, Sierra and Regalfin. Plants were grown in greenhouses, during the fall and winter months (September-April). Temperature was controlled by ventilation or heating when necessary. Plants were fertilized weekly with a N-P-K liquid fertilizer and pests were controlled as needed. Crosses were performed using the methods developed by Buishand (1956). The viability of hybrid seed was tested by germinating the seeds on a moist filter paper in petri dishes.

$F_1$ hybrid progeny from *P. vulgaris x P. acutifolius* and *P. acutifolius x P. vulgaris crosses*. Interspecific crosses between *P. vulgaris* and *P. acutifolius* parents aborted at 26-34 days post-pollination. Two types of aborted seeds were observed on *P. vulgaris* cytoplasm. Aborted seed using Ica pijao as the female parent were either pale brown or black. Both types were shrunken in appearance with the brown seeds being the smaller of the two. Only 10-20% of the black $F_1$ seeds were viable. Plants from these seeds flowered in approximately two months and continued to do so for 3 months. The reciprocal cross on tepary cytoplasm produced very few viable seeds (table 1), the seed that did form were not shrunken and were either rounded or kidney shaped. These seeds were all viable and produced mature flowering plants in approximately 45 days.

**Table 1**. Number of aborted seed, viable seed and mature plants from seed obtained from interspecific crosses between *P. vulgaris* L. lines Ica Pijao, Sierra and Regalfin and *P. acutifolius* L. lines PI 321637-s and PI 321638-
**s**

| Crosses | Number of crosses | Number of pods | Number of aborted seed | Number of seed | Mature plants |
|---|---|---|---|---|---|
| **PI 321637-s x Ica pijao** | **50** | **12** | **34** | **1** | **0** |
| **PI 321637-s x Sierra** | **50** | **14** | **33** | **4** | **3** |
| **PI 321637-s x Regalfin** | **50** | **6** | **15** | **2** | **2** |
| **PI 321638-s x Ica pijao** | **50** | **19** | **37** | **11** | **6** |
| **PI 321638-s x Sierra** | **50** | **15** | **29** | **0** | **0** |
| **PI 321638-s x Regalfin** | **50** | **7** | **14** | **0** | **0** |
| **Sierra x PI 321637-s** | **10** | **10** | **34** | **0** | **0** |
| **Regalfin x PI 321637-s** | **10** | **7** | **21** | **0** | **0** |
| **Ica pijao x PI 321637-s** | **10** | **10** | **12** | **28** | **3** |
| **Ica pijao x PI 321638-s** | **10** | **10** | **14** | **28** | **6** |
| **Sierra x PI 321638-s** | **10** | **10** | **26** | **0** | **0** |
| **Regalfin x PI 321638-s** | **10** | **10** | **19** | **0** | **0** |

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# Timing of Seed Coat Colour Development in Black Beans

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## Introduction

Common bean has a wide array of seed coat patterns and colours. Appearance and seed coat condition determine the quality and value of the bean crop, particularly colour uniformity and stability. For some black bean varieties grown in western Canada, the seed coat colour does not fully develop until the pod is mature. This may be the result of genotype, or environment, or both. In some cases, harvest timing based on plant maturity instead of seed maturity causes seed coat colour variability. Some beans in crop samples may have a gray or purple tinge, resulting in economic losses due to colour discounts of 5-10% of the crop value. In this experiment, we examined the timing of pigment deposition in the seed coat in relation to pod maturity to determine if there is genetic variation for timing of seed coat colour development. If this trait is under genetic control, we will be able to breed for earlier colour development to improve full expression of seed coat colour at plant maturity.

## Materials and Methods

Five cultivars of black bean (CDC Expresso, CDC Jet, CDC Nighthawk, AC Black Diamond and T39) were grown in pots under greenhouse conditions in the fall of 2002. A complete set of cultivars was seeded each week for 3 weeks at the rate of 6 seeds per pot. After emergence, seedlings were thinned to 3 uniform plants per pot. Pots were randomly repositioned on benches at each watering to ensure each pot received the same amount of light as the next pot.

Flowers were tagged and dated as they opened. Tagged pods of each cultivar were removed on three separate occasions to sample a range of maturities from 5 days after flowering (DAF) to seed maturity. At each harvest, pods and seeds were examined for colour development using both visual and histochemical methods. Visual observations of the developing bean seeds and pods were used to determine the number of days from flowering to initial observable colour development and to determine the rate of seed coat colour development. Observations of pod development stage were used to determine the relationship between pod maturity and stage of seed coat colour development.

A small portion of the seed coat from around the hilum or from the opposite side of the seed was removed and placed in a microtitre plate. The tissue was then stained with methanol-HCl (Lees et al., 1997, Takeoka et al., 1997), which causes anthocyanins to stain red in less than 5 minutes.

## Results and Discussion

CDC Expresso was the first variety to flower and had the shortest time to initial colour development (Table 1). Full colour development, however took the longest in this variety (Table 1). When AC Black Diamond started to produce colour it proceeded very rapidly and had a fully developed black seed before all the other varieties (Table 1). T39 is later maturing than the other four varieties. It had a longer vegetative period than the others but once it started to flower it had a very similar flowering to seed coat colour development interval as the other beans and was as quick as AC Black Diamond to complete colour development (Table 1).

For all cultivars, colour development started at the micropyle and progressed from the hilum outwards. Immature seed coat segments treated with methanol-HCl only stained when there was visual evidence of colour in the seed coat. Mature seed coats treated with methanol-

HCl rapidly turned pink indicating the presence of anthocyanins. Segments of uncoloured seed coat adjacent to the hilum did not stain red, suggesting that the onset of anthocyanin deposition coincides with visual observation of colour.

Table 1. Timing of flower and seed coat colour development for five black bean cultivars under greenhouse conditions.

| Cultivar | Days to flower | Days from flowering to initial seed coat colour development | Days from initial to full seed coat development | Total days from flowering to full seed coat colour development | Total days from planting to full seed coat colour development |
|---|---|---|---|---|---|
| CDC Expresso | 39 | 24 | 16 | 40 | 79 |
| CDC Jet | 44 | 27 | 12 | 39 | 83 |
| CDC Nighthawk | 41 | 30 | 11 | 41 | 82 |
| AC Black Diamond | 42 | 26 | 9 | 35 | 77 |
| T39 | 48 | 28 | 9 | 37 | 85 |

CDC Nighthawk took the longest time to initiate colour development but only took two days longer than AC Black Diamond to complete colour development once initiated (Table 1). Seed coats of AC Black Diamond were completely black when physiologically mature at maximum size and high moisture level. Seed coat colour of T39, CDC Jet and CDC Expresso was not fully developed until seeds started drying down. For CDC Nighthawk, full seed coat colour was not developed until seeds were dry.

The pods of AC Black Diamond were still green while seed coat colour was developing, and the seed coats were fully coloured by the time the pods turned brown. In contrast, pods of CDC Nighthawk were turning brown before the seed coats were even grey. This points out that pod maturity is an unreliable indicator of seed coat maturity for some black bean cultivars. A range of 4 days for the flowering-first colour development period was observed. A much greater range of 7 days was observed for the period from first colour to full colour. Our observations suggest that days to flower has no effect on seed coat colour development and that days to seed coat colour development and total days to full seed coat colour development are independent traits.

## Conclusions

This preliminary look at the timing of seed coat colour development in black beans suggests that it may be under genetic control. In addition, some evidence exists that the period from flowering to first colour and from first seed coat colour to full seed coat colour may be independent traits. Simultaneous selection for early colour development and rapid colour development may be possible even within this small group of genotypes. Under greenhouse conditions, selection for early colour development in black beans can be done by opening the pods developed from the first flowers 20-30 days after flowering and looking for colour around the hilum. Plants that deposit colour quickly can be identified by opening pods as they turn brown, approximately 35-40 days after flowering.

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# GENETIC INHERITANCE OF ORANGE CORONA CHARACTER IN COMMON BEAN SEEDS OF COMMERCIAL CARIOCA GROUP

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**INTRODUCTION:** The common bean (*Phaseolus vulgaris* L.) is a normal component of a daily Brazilian diet and it constitutes a main source of proteins. The commercial carioca group, which exhibits cream colored seedcoat with light brown stripes, is the most consumed bean type and sometimes it shows in the seeds an orange corona around the hilum ring. Cultivars that show this character have a commercial restriction, with a low price in the bean's market. This way, bean's farmers refuse to cultivate bean varieties with the orange corona. Several researches about the genetic control of seedcoat color are found in the literature. Prakken (1970, 1972) revised and made a synopsis about the genetic inheritance of seedcoat color of bean. The author concluded that eight loci (*P*, *C*, *D*, *J*, *G*, *B*, *V* and *Rk*) control the seedcoat color and a complex epistatic interations occur between these genes. Disagreements are found in literature about the genetic control of corona color. However, the number of genes that controls the corona character is smaller than the number of genes that controls the seedcoat color of bean (Mendonça et al., 1998). The presence of corona around the hilum ring is controlled by the gene *Cor*, but the different colors of corona are controlled by genes *B*, *D* and *G* (Mendonça et al., 1998). Because the economic importance of orange corona character, the purpose of this research was to elucidate the genetic control of this characteristic in the commercial carioca group to give support to the breeding programs.

**MATERIAL AND METHODS:** Nine crosses between cultivars belong to commercial carioca group (IAPAR 14/Carioca, IAPAR 14/Rudá, IAPAR 14/FT-Paulistinha, IAPAR 57/Carioca, IAPAR 57/Rudá, IAPAR 57/Maichaki, Aporé/Carioca, Aporé/Rudá and Aporé/Maichaki) that differ only in the presence of orange corona, were done at Instituto Agronômico do Paraná (IAPAR), located in Londrina City, Paraná, Brazil. The $F_2$, $BC_1P_1F_1$ and $BC_1P_2F_1$ progenies were obtained from $F_1$ progenies derived from crosses between contrasting parents for orange corona character. A random sample of 300 seeds of the $F_2$ generation and 90 seeds of each backcross were sown in the experimental field of IAPAR, Londrina City. Then, a sample of 150 plants of $F_2$ population and 15 plants of backcross populations were selected at random. The progenies were evaluated for the presence or absence of orange corona. A progeny test of the selected individual plants was made and 50 seeds of each plant of the $F_3$, $BC_1P_1F_2$ and $BC_1P_2F_2$ were sown in the Experimental Station of IAPAR, located at Ponta Grossa City, Paraná, Brazil. In the physiologic maturation stage a pod of each plant was harvested, which seeds ($F_4$, $BC_1P_1F_3$ and $BC_1P_2F_3$) were evaluated for the presence of orange corona. The phenotypic evaluation for the presence of orange corona were done in the subsequent generation because the seedcoat is a tissue of maternal origin, then, the character is observed in the seeds of the next generation. The observed phenotypic proportions in the seven generations of each crossing were compared by a

chi-square test to expected proportions that agrees with the segregation of a dominant gene controlling the orange corona character. To verify the possibility that only a genetic hypothesis explains the segregations observed in each generation of the nine crosses a homogeneity chi-square was done.

**RESULTS AND DISCUSSION:** The observed phenotypic proportions in the $F_2$, $BC_1P_1F_1$ and $BC_1P_2F_1$ generations fitted well with the hypothesis that a gene with complete dominance controls the orange corona character in the bean carioca seeds. The homogeneity chi-square tests for each above generations showed that the data are in according to the expected proportions. In four crosses (IAPAR14/Carioca, IAPAR 14/FT-Paulistinha, IAPAR 57/Carioca and Aporé/Rudá) all seven generations showed a segregation pattern that agrees with the hypothesis of a dominant gene. However, in the crosses IAPAR 14/Rudá, IAPAR 57/Rudá, IAPAR 57/Maichaki and Aporé/Maichaki the chi-square was significant for the $F_3$ and $BC_1P_2F_2$, showing a distortion of the expected proportions. Then, with the results obtained in this research is possible to conclude that the orange corona character in the carioca bean group is controlled by two dominant genes (*Cor* and *G*). Because the gene *Cor* is in homozigosis in this commercial group, the observed segregations are corresponding to the action of the gene *G*. The parent without orange corona has the genotype *bbddCorCorgg* and the parent with orange corona has the genotype *bbddCorCorGG*. These conclusions are in line with the results obtained by Leakey (1988), Mendonça et al. (1998) and Bassett et al. (2002).

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# INHERITANCE OF THE CARTRIDGE BUFF MICROPYLE STRIPE EXPRESSED IN THE GENETIC STOCK, *p* $BC_3$ 5-593, OF COMMON BEAN MAINTAINED AT PULLMAN, WA

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The Florida dry bean breeding line 5-593 has been used for nearly 20 years as a recurrent parent for the construction of a large number of genetic stocks. Those stocks have one or more genes (usually recessive) controlling expression of seed coat color, pattern, or shininess backcrossed (usually to $BC_3$) into 5-593. The seed coat genotype of 5-593 is described in detail in a companion paper in this volume of the BIC Annual Report (vol. 46, 2003). The genetic stock of interest here has the gene *p*, which usually expresses an entirely white seed coat in most genetic backgrounds. The *p* gene used to construct *p* $BC_3$ 5-593 was derived from the snap bean variety 'Triumph', which has a seed coat that is entirely white, including the micropyle stripe zone of the seed. Surprisingly, the genotype *p*/*p* is not able to block expression of cartridge buff color in the micropyle stripe zone of the seed due to some background genotype provided by 5-593. This paper presents inheritance data for the cartridge buff micropyle stripe.

In 1987, 'Triumph' was crossed to 5-593 and selection was made (Spring 1988) in the $F_2$ progeny for plants with *p* white seeds. In Fall 1988, *p* $F_3$ 5-593 plants were backcrossed to 5-593, and in Fall 1989, a serial backcross was made to 5-593. The *p* $BC_2$-$F_1$ plants were grown in the greenhouse in Winter 1990, and eight $BC_2$-$F_2$ progenies were grown in the field in Spring 1990. Selection was made for plants with *p* white seeds, but no data were recorded on segregation for the cartridge buff micropyle stripe (BMS) trait in any of the above breeding work. In 1994, the third backcross of *p* to 5-593 was accomplished, but again, no data were recorded on BMS segregation. The primary reason that this trait received so little early attention is that it has low expression (variable expressivity) under field conditions, but expresses well under greenhouse culture.

In Winter 2000, the cross *p* $BC_3$ 5-593 x 'Triumph' was made, and the $F_1$ progeny were grown in the field in Spring 2000. The $F_2$ seed produced on those $F_1$ plants were completely white, which is consistent with the hypothesis that BMS is a recessive trait. In Winter 2001, 28 $F_2$ progeny were grown in the greenhouse (Table 1). Segregation for a spontaneous chlorophyll mutation (systemic, no sectoring) was observed in addition to the segregation for seed coat color. The chlorophyll mutant produced small but fertile plants that yielded about 1/4 the normal seed crop when grown in the greenhouse. This chlorophyll mutant is a seedling lethal when grown in the field. The $F_2$ data (Table 1) are consistent with the hypotheses that each of the BMS and chlorophyll traits are controlled by a single recessive gene.

The 28 $F_2$ progeny from the cross *p* $BC_3$ 5-593 x *p* Triumph (snap bean) were progeny tested in $F_3$. The seven $F_2$ parents with the chlorotic mutation and white seed were true breeding for the mutation and died in early seedling development stage (data not shown). No data were recorded for segregation for the chlorophyll mutation in the remaining $F_3$ progenies. The five plants with BMS did not appear to breed true (Table 2). However, the $F_3$ plants with BMS had such a weak level of expression of the trait that one must suspect that the "All white" $F_3$ plants observed in those progenies most probably carry the genotype for the trait but failed to visibly express it. In a similar way, the observed segregation in $F_3$ from "All white" $F_2$ parents probably gives a very unreliable test for the underlying genotypes (Table 2). Such variable and weak expression of BMS under field conditions makes it nearly impossible to obtain a rigorous $F_3$ test

of the hypothesis of a single recessive gene being responsible for control of BMS expression. The only conclusion possible is that the $F_1$ and $F_2$ observations suggest that a single recessive gene controls the BMS trait. Similarly, the $F_1$, $F_2$, and the $F_3$ progeny test of the seven chlorotic mutant $F_2$ parents suggest that a single recessive gene controls the spontaneous mutant for a seedling lethal type of chlorosis (without sectoring). Because the $F_3$ progeny tests did not lead to satisfactory results, no gene symbol will be proposed either for the BMS trait or for the spontaneous, seedling-lethal chlorophyll mutant.

The BMS trait is not valuable as a seed coat pattern marker gene because of its low expression level under field conditions. However, an attempt to determine its inheritance is important because the putative gene involved is able to overcome the usually complete suppression of seed coat color development determined by the genotype *p*/*p*.

Table 1. Segregation for cartridge buff micropyle stripe (BMS) and a spontaneous chlorophyll mutant in the $F_2$ from the cross *p* $BC_3$ 5-593 x *p* Triumph (snap bean).[z]

| White seed<br>Normal plant | White seed<br>Chlorophyll mutant | BMS seed<br>Normal plant | BMS seed<br>Chlorophyll mutant |
|---|---|---|---|
| 16 | 7 | 5 | 0 |

[z]For the data 16, 5, 7, 0, the $\chi^2$ (9:3:3:1) = 2.349, $P = 0.50$.

Table 2. Segregation for cartridge buff micropyle stripe (BMS) in the $F_3$ generation from the cross *p* $BC_3$ 5-593 x *p* Triumph (snap bean).

| | | Segregation for seed coat pattern observed in $F_3$ | | |
|---|---|---|---|---|
| No. of $F_3$ plots | Phenotype of $F_2$ parent | All white seed | White seed with BMS trait[z] | Ratio of BMS plants to total |
| 10 | All white seed | 354 | | |
| 6 | All white seed | 239 | 8 | 0.032 |
| 3 | White seed with BMS trait | 51 | 46 | 0.474 |
| 2 | White seed with BMS trait | 60 | 5 | 0.077 |

[z]The BMS trait has weak expression under field conditions such that sampling the seed from a single pod and selecting a representative seed from that pod will frequently result in an all white seed being selected from a plant carrying the BMS genotype.

# THE SEED COAT COLOR GENOTYPE OF 5-593, THE RECURRENT PARENT FOR MANY GENETIC STOCKS OF COMMON BEAN MAINTAINED AS PI LINES AT PULLMAN, WA

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In previous BIC Annual Reports (37:244-245, 1994 and 41:127-127, 1998) I have given experimental evidence for the genotype at most of the various seed coat genes presented below for Florida dry bean breeding line 5-593. To achieve the brevity needed for the 2-page space limit, all citations of refereed journal papers supporting th general summary given below are omitted. Fifteen genes control expression of seed coat color, pattern, and shininess, and they are presented in Table 1. The existence of the fibula arcs gene *Fib* is based on unpublished data (Bassett), and the genotype at *Fib* and *Cl* in 5-593 is also based on unpublished data of Bassett, except for his preliminary report on the fibula arcs factor (BIC 44:171-172, 2001). Eight seed coat genes have a multiple allele series: *C* (a very large allele series); *P* (5 recessive alleles); *V*, *Rk*, and *Bip* (each with 3 recessive alleles); and *T*, *Z*, and *J* (each with 2 recessive alleles).

Black is the primary wild-type seed coat color of common bean, and 5-593 has black, shiny, unpatterned seed coats and purple flowers. Seed coat patterns in cartridge buff (controlled at *C* and expressed by $c^{u}$ for the entire seed coat) provide much of the camouflage to defend bean seeds from animal predators. For 12 of the genes in the table below, the wild type allele is dominant, whereas the recessive alleles at those 12 loci change unpatterned, non-shiny, black seeds to other colors, patterns, and degrees of luster. The three exceptions are: *C*, for which dominant pattern alleles exist (e.g., $C^{st}$); *R*, which expresses dominant red (oxblood); and *Fin*, which restricts partly colored seed coat patterns as a dominant gene effect. The large number of possible seed coat colors that can be expressed is achieved by a complex system of epistasis.

There are four genes that control pattern in bean seed coats. By far the most important is *C*, which controls a range of patterns so great, and with finely graded differences, that a true catalog of the allele series is not feasible to construct. The gene *T* potentiates the very large series of partly colored seed coat patterns existing in nature, where the size and shape of the colored zone (the remainder always being white) is controlled by interactions among four other genes: *Z*, *Bip*, *J*, and *Fib*. The gene *P* has only recently been discovered to have a true seed coat pattern function. With *T P C Z* , the *J* gene (with genotype *j*/*j*) expresses what is generically termed the "margo" pattern (described in Table 1), but the possible variation of color distribution and hues is amazing because of the inherent variable expressivity of *j*. No true Mendelian classification of seed coat phenotypes is possible in a progeny segregating at *J* because the actual genotypic class boundaries are imperceptible, i.e., the hues of the various genotype classes form a continuous color spectrum from one class to the next. The author has observed complex dotting patterns with *T P C j V*, reminding him of transposable element patterns in maize kernels.

The seed coat genes are mostly independent, but there are exceptions. Two gene pairs have weak linkage, viz., *T* and *cl* (with 36 cM) and *B* and *Rk* (with 27 cM). The genes *J* and *Bip* are located in linkage group B10, with their corresponding STS markers 27.7 cM apart. Three genes have close linkage, viz., nearly unbreakable linkage for [*C R*] and very close linkage for *C* with *Gy*, where the *Gy* STS marker was linked to *C* at a distance of 2.7 cM. The genes *T*, *P*, *C*, *Z*, *J*, *G*, *B*, and *V* are known to be in different linkage groups, and those linkage groups were recently demonstrated to be associated with eight different chromosomes.

**Table 1. Seed coat genotype of 5-593 and description of phenotypic effects of various alleles.**

| Genes | Seed coat phenotype effects of the gene and its various alleles |
|---|---|
| *T* | *Totally* colored; *t*/*t* potentiates partly colored seed coat pattern expression. |
| *cl* | *Circumlineatus*; with *t v*, *cl*/*cl* expresses a physical groove in the seed coat surface at the boundary between the white and colored zones of partly colored seeds. |
| *Z* | The *zonal* factor for partly colored seed coat patterns. With *J*, *Z* (formerly *D*) has no effect on hilum ring color; with *T*, *Z* expresses no partly colored seed coat effects. |
| *Bip* | The *bipunctata* factor for partly colored seed coat patterns. With *T* (or *t Z*), *Bip* does not express partly colored seeds. *t z bip* expresses bipunctata pattern; *t Z* $bip^{ana}$ expresses Anasazi pattern; *t z* $bip^{vgt}$ expresses virgata pattern (unpublished data). |
| *Fib* | The *fibula* arcs factor; with *T*, *Fib* has no effect on partly colored patterns, but with *t*/*t*, *Z Fib* expresses expansa with fibula arcs and *z Fib* expresses arcus pattern. |
| *P* | With *V* and either *C J*, *c J*, or *C j*, *p*/*p* expresses white seeds and flowers, $p^{gri}/p^{gri}$ expresses gray white seeds and patterned flowers, $p^{stp}/p^{stp}$ and $p^{hbw}/p^{hbw}$ express different stippled seed patterns and different flower patterns, and $p^{mic}p^{mic}$ expresses white micropyle stripe and no flower pattern. |
| [*C r*] | *C* expresses no seed coat pattern; *r* expresses no oxblood red color, whereas *R* does. |
| *Gy* | With *P* [*C r*] *J g b v* (or *G b v*), *gy*/*gy* expresses strong greenish-yellow seed color. |
| *J* | *J* expresses no seed coat pattern; with *P C*, *j*/*j* expresses margo pattern (loss of color in the corona), immature (pale) seed coat colors, and reduced seed coat shine. With *t*, *j* expresses marginata pattern of partly colored seed coats. |
| *G* | *G* is the yellow (*Gelbe* in German) seed color factor. *P C J G b v* expresses yellow brown seed coats. |
| *B* | *B* is the brown seed color factor. *P C J G B v* expresses mineral brown seed coats and *P C J g B v* expresses grayish brown seed coats. |
| *V* | With *T P*, *V* expresses purple flowers. With *P C J*, *V* expresses various colors: maroon with *g b*, dark brown violet with *G b*, and black with *G B*. |
| *Rk* | *Rk* is the red kidney gene, which does not express red colors with *Rk*. With $c^{u}$ *J*, *rk* or $rk^{cd}$ express pink (light red kidney) seed and $rk^{d}$ expresses garnet brown (dark red kidney) seed color. With *C J*, $rk^{cd}$ expresses garnet brown seed color. With $c^{u}$ *J*, $rk^{p}$ achieves full pink seed expression only when grown under low humidity conditions. |
| *Asp* | *Asper* is the common gene for seed coat shininess; *asp*/*asp* expresses non-shiny (dull) seeds with no change in the hue or color distribution on the seed coat. |

# A Single Dominant Gene Controlling Green Color of the Keel's Tip twisting in Common Bean (*Phaseolus vulgaris* L.)

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In common bean (*Phaseolus vulgaris* L.) there is a difference in the color of the keel's tip twisting - either green (persistent chlorophyll) or white. This character can be used as a differentiation trait when performing expertise for differentiation, uniformity and stability (DUS) of the new common bean cultivars, as well as of the breeding lines, landraces and mutants. It remains stable under various climatic conditions and different agro-technological schemes. The aim of the study was to determine the genetic control over green color of keel's tip twisting.

In 2000, crosses were made between accession with white keel's tip twisting (Abritus, Vulkan and 95-20) and accessions with green keel's tip twisting (Dobroudjansky 7, Dobroudjansly ran, Ludogorie, Obraztsov chiflik 24, Ternovo 13, G 2883, HR 45 and NAB 69) (Figure 1): Ternovo 13/Prelom, Dobroudjansky 7/Abritus, NAB 69/Abritus, Abritus/HR 45, Abritus/G 2883, Ludogorie/Vulkan, Obraztsov chiflik 24/95-20 and 95-20/Dobroudjansky ran. In 2001, back-crosses were made ($BC_1$ and $BC_2$). In 2002, $P_1$, $P_2$, $F_1$, $F_2$, $BC_1$ and $BC_2$ were sown in three replications. Seeds were sown in 3 m long rows. The distance between seeds in the row was 15 cm, and the space between the rows - 40 cm. Evaluation of the keel's tip twisting color was done on freshly opened flowers (Genchev & Kiryakov, 1994), registering color as either green or white.

1 - green (*Kpc*) 2 - white (*kpc*)

**Figure 1.** ***Keel's tip twisting color***

Table 1 presents the data obtained from the six generations $P_1$, $P_2$, $F_1$, $F_2$, $BC_1$ and $BC_2$. All plants from $F_1$ of all eight crosses were with green keel's tip twisting color, as that of Dobroudjansky 7, Dobroudjansky ran, Ludogorie, Obraztsov chiflik 24, Ternovo 13, G 2883, HR 45 and NAB 69.
In $F_2$, segregation in all crosses was nearest to the expected rate 3(green) : 1(white) at P = 0.30 to 0.80.

All plants from the back-crosses with the parent carrying a dominant allele of the gene determining the color of the keel's tip had a green tip. In the back-crosses with the parent carrying a recessive allele, segregation into green and white color was observed at a ratio 1:1 at P = 0.80 to 0.995. Thus, the hypothesis established in $F_2$ that the green color of the keel's tip twisting is controlled by a single dominant gene was confirmed. We suggest to designate this gene with the symbol ***Kpc*** (keel's persistent chlorophyll) for the dominant gene controlling green color and ***kpc*** for the recessive gene controlling white color of the keel's tip twisting.

**Table 1. Segregation Ratios for Green Keel's Tip Twisting Color in Six Segregating Generations derived from Eight Crosses.**

| Cross | Gene-ration | Number of plants | | | | $\chi^2$ | P |
|---|---|---|---|---|---|---|---|
| | | Observed | | Expected | | | |
| | | green | white | green | white | | |
| Ternovo 13/Prelom | $F_1$ | 66 | 0 | | | | |
| | $F_2$ | 242 | 88 | 247 | 83 | 0.4024 | 0.50-0.60 |
| | $BC_2$ | 101 | 95 | 98 | 98 | 0.0612 | 0.80-0.90 |
| Dobroudjansky 7/Abritus | $F_1$ | 57 | 0 | | | | |
| | $F_2$ | 194 | 62 | 192 | 64 | 0.0833 | 0.70-0.80 |
| | $BC_2$ | 72 | 70 | 71 | 71 | 0.0282 | 0.80-0.90 |
| NAB 69/Abritus | $F_1$ | 51 | 0 | | | | |
| | $F_2$ | 252 | 78 | 247 | 83 | 0.4024 | 0.50-0.60 |
| | $BC_2$ | 58 | 60 | 59 | 59 | 0.0339 | 0.80-0.90 |
| Abritus /HR 45 | $F_1$ | 63 | 0 | | | | |
| | $F_2$ | 203 | 71 | 205 | 69 | 0.0775 | 0.70-0.80 |
| | $BC_1$ | 49 | 53 | 51 | 51 | 0.0784 | 0.70-0.80 |
| Абр G 2883 | $F_1$ | 69 | 0 | | | | |
| | $F_2$ | 197 | 67 | 198 | 66 | 0.0202 | 0.80-0.90 |
| | $BC_1$ | 54 | 56 | 55 | 55 | 0.0182 | 0.80-0.90 |
| Ludogorie/Vulkan | $F_1$ | 83 | 0 | | | | |
| | $F_2$ | 270 | 82 | 264 | 88 | 0.5455 | 0.40-0.50 |
| | $BC_2$ | 71 | 71 | 71 | 71 | 0.0000 | >0.995 |
| Obraztsov chiflik 24/95-20 | $F_1$ | 65 | 0 | | | | |
| | $F_2$ | 61 | 16 | 58 | 19 | 0.7316 | 0.30-0.50 |
| | $BC_2$ | 43 | 41 | 42 | 42 | 0.0476 | 0.80-0.90 |
| 95-20/ Dobroudjansky ran | $F_1$ | 72 | 0 | | | | |
| | $F_2$ | 62 | 17 | 59 | 20 | 0.5105 | 0.30-0.50 |
| | $BC_1$ | 55 | 59 | 57 | 57 | 0.1404 | 0.70-0.80 |
| 95-20 | P | 0 | 55 | | | | |
| Abritus | P | 0 | 63 | | | | |
| Vulkan | P | 0 | 55 | | | | |
| Dobroudjansky 7 | P | 47 | 0 | | | | |
| Dobroudjansky ran | P | 52 | 0 | | | | |
| Ludogorie | P | 46 | 0 | | | | |
| Obraztsov chiflik 24 | P | 49 | 0 | | | | |
| Prelom | P | 0 | 57 | | | | |
| Ternovo 13 | P | 69 | 0 | | | | |
| G 2883 | P | 61 | 0 | | | | |
| HR 45 | P | 59 | 0 | | | | |
| NAB 69 | P | 51 | 0 | | | | |

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# BIOLOGICAL MANIFESTATIONS IN COMMON BEAN $M_1$ AND $M_2$ GENERATIONS AFTER TREATMENT OF SEEDS WITH NEU AND EMS

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## Introduction

Instead that experimental mutagenesis was applied for a long time in different crops, there is not still a clear and exact knowledge about mechanisms of mutagens action on treated plant material. These mechanisms cannot still be controlled for obtaining of economically important mutants. Different concentrations (Ciftci et al., 1994; Gautam et al., 1998; Reddy et al., 1998) and times of mutagen treatments (Isasi and Busto, 1982; Rukmanski and Rodriges, 1990) were used, but still the genotype reactions in different generations are interesting to be studied. That is the aim of our investigation.

## Material and Methods

Four years (1990-1994) investigations were conducted. Seeds from Bulgarian white-seeded common bean (*Phaseolus vulgaris* L.) variety Tcher Starozagorski were treated with chemical mutagens ***NEU*** (N-nitroso-N'-ethyl urea) and ***EMS*** (ethylmethane sulphonate). Mutagens were applied in concentrations: NEU - $6{,}2.10^{-3}$ ($LD_{85\text{-}90}$); $3{,}1.10^{-3}$ ($LD_{45\text{-}50}$); $1{,}55.10^{-3}$ M ($LD_{25\text{-}30}$); EMS - $2{,}5.10^{-2}$ ($LD_{85\text{-}90}$); $1{,}25.10^{-2}$ ($LD_{45\text{-}50}$); $6{,}2.10^{-3}$ M ($LD_{25\text{-}30}$) and exposition of treatment - 8 hours. Two controls (treatment with buffers with pH 6,0 and 7,0) were also used. Influences of mutagens on germination, surviving, induction of chlorophyll and morphological changes of plants in $M_1$ and $M_2$ generations were investigated. Bi-factorial ANOVA was used to determine significance of the differences between the treatments. Regression analysis was performed to determine the best fitting curves and their coefficients of determination ($R^2$) and regression (Rx/y and Ry/x), (Socal and Rohlf, 1981).

## Results and Discussion

Correlation and regression analyses were conducted for evaluation of dependences between biological traits germination-surviving, chlorophyll-morphological changes of plants in $M_1$ and $M_2$ generations. The established correlation coefficients, of the two dependences, are high and statistically significant at the highest level ($P_{0,1\%}$). The found regression coefficients are presented in equations on Figure 1. They describe the connections between surviving in dependence of germination and chlorophyll changes in dependence of morphological ones.

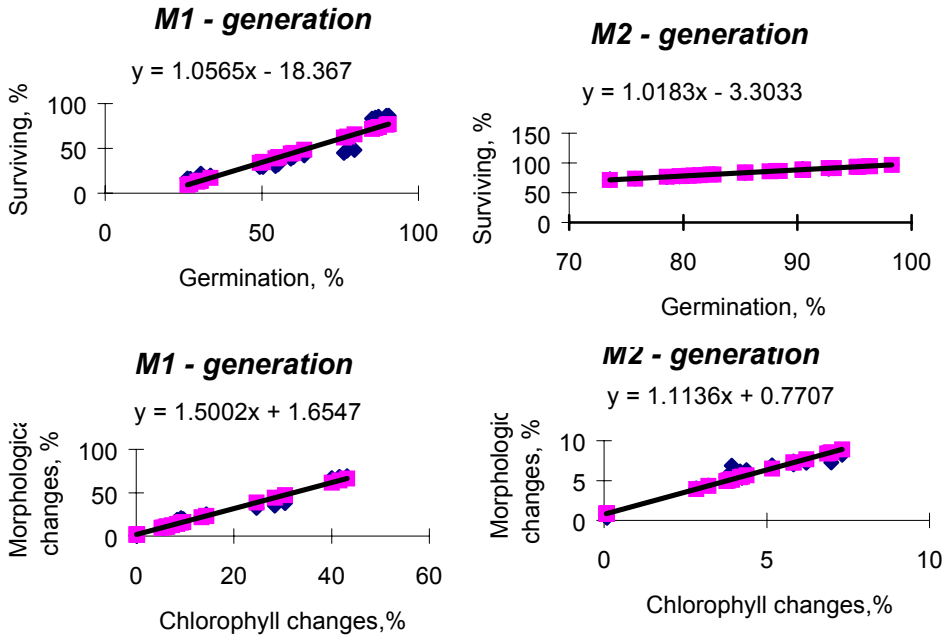


***Fig.1.*** Regression dependences between germination and surviving; chlorophyll and morphological changes in $M_1$ and $M_2$ generations

We studied also the influence of meteorological factors on presented in this study biological traits of the plants in $M_1$ and $M_2$ generations (Svetleva and Kouzmova, 2003). The points of distributions, showing on graphics (Fig. 1), construct lines by the equation $y = a + bx$. Bi-factorial dispersion analysis was conducted, where data from the two generations ($M_1$ $M_2$) and eight treatments were included in the total complex of studied traits for the period of investigations. Application of that analysis permit to check the significance of differences between studied traits, in the two generations, independent of the treatments and to find the influence of the treatments independent of the studied generation, as well as the interactions between them.

It was found that the percentage of germination in $M_2$ generation was significant higher than that one in $M_1$. Perhaps that is in relation with the find lesser influence of the mutagenic treatment in $M_2$ generation.

Evaluating the influence of the treatments, independent of the generation impress with the fact, that the percentage of the germination plants is the highest in the two control variants and there is not significant differences between them. The variants of treatment with the lowest mutagenic concentrations ($1{,}55 \cdot 10^{-3}$ M NEU - $b_4$ and $6{,}2 \cdot 10^{-3}$ M EMS - $b_8$) are on the second position in this range.

Studying the influence of interaction between two factors – generations and concentrations, it was found that the highest values are in combinations of $M_2$ generation with the two control variants ($M_2b_1$ $M_2b_5$), following by the combination of $M_2$ and the treatment $6{,}2 \cdot 10^{-3}$ M EMS ($M_2b_8$).

The same dependence was found studying the results concerning the relations between presented traits and surviving. It can be well understand because there are high positive correlations between germination and surviving in the two generations ($r = 0{,}929$ in $M_1$ and $r = 0{,}998$ in $M_2$). Coefficients of determination are also high ($R^2 = 0{,}863$ in $M_1$ and $R^2 = 0{,}995$ in $M_2$).

Chlorophyll spots and morphological changes of shape and number of leaf parts were induced as a result of treatment with mutagen factors in $M_1$ generation. These changes were significantly lesser in $M_2$. They were more as physiological reactions of plants to the mutagenic treatment, than genetically determined but there was positive relation between percentage of changes and the concentration of applied mutagen. The lowest concentrations of the two mutagens induce fewer changes. The percentage of chlorophyll and morphological changes is significantly proved at highest level in $M_1$ generation, independently of the mutagenic treatments influence. Following the significance of the differences between treatments, it can be seen that significantly the smallest percentage of chlorophyll and morphological changes is in control variants. The highest values of these traits are found after application of the two mutagens at the highest concentrations ($6{,}2 \cdot 10^{-3}$ M NEU and $2{,}5 \cdot 10^{-2}$M EMS) and their combinations with $M_1$ generation. It was also found high positive correlations between chlorophyll and morphological changes in the two generations ($r = 0{,}984$ in $M_1$ and $r = 0{,}976$ in $M_2$) and coefficients of determination are also high ($R^2 = 0{,}968$ in $M_1$ and $R^2 = 0{,}956$ in $M_2$).

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# DEPENDENCE OF SOME PHENOLOGICAL AND BIOLOGICAL MANIFESTATIONS IN $M_1$ AND $M_2$ GENERATIONS OF FRENCH BEAN CULTIVAR TCHER STAROZAGORSKI FROM METEOROLOGICAL CONDITIONS

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## INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is important legume in many countries in the world. Experimental mutagenesis was applied as a method for creation of bigger genetic diversity and obtaining of new lines or cultivars (Stoyanova et al., 1999; Svetleva et al., 1999; Svetleva and Mehandzhiev, 1999). Some authors (Ciftci et al., 1994a,b; Stoyanova and Milkov, 1995) investigated the effect of mutagen treatment on plant reactions in different generations or described different mutation types (Park and Buttery, 1994; 1997). Hershkovich (1984) made evaluation of agro climatic resources and agro climatic deviation of Bulgaria into districts. The change of agro climatic resources, in the frame of global climatic changes, will impose new criteria in development of agricultural crops in special districts of the country. Important task of breeding programs in the last years become creation of new cultivars with resistance to water deficient and temperature stress. Creation of new cultivars with that resistance needs of application of better methods and breeding technologies (Mehandziev and Todorova, 2002). The meteorological conditions can influence on the effect of mutagen treatments and that is the reason why we set as a mean goal of our investigations, to study the influence of main agrometeorological factors (mean air temperature and amount of rainfall) on phenological development of French bean and specific peculiarities in plant reactions in $M_1$ and $M_2$ generations. The dependences between studied parameters were also object of this study.

## MATERIAL AND METHODS

Our investigations were conducted in the period 1990-1994 on the area of city Plovdiv in Bulgaria. Calibrated seeds of French bean, cultivar Tcher Starozagorski were treated with chemical mutagens with exposition of 8 hours. The mutagenic factors were applied in the next concentrations: *NEU* – 0,0062; 0,0031; 0,00155M and *EMS* – 0,025; 0,0125; 0,0062 M. Influence of mutagenic treatment on phenological development, germination, surviving, fertility and sterility of plants, as well as chlorophyll and morphological changes of leaves in $M_1$ and $M_2$ generations of French bean, in dependence of agrometeorological conditions, were studied. Data were arranged in special complex. The relation between studied parameters was established statistically by special complex of programs constructed for processing of phenological, biometrical and meteorological data, integrated in Exel (Kouzmova, 2002).

## RESULTS AND DISCUSSIONS

Influence of mutagenic treatment was stronger in $M_1$ generation and it was less expressed in $M_2$. Studied phenological and biological manifestations were in dependence of applied mutagenic concentrations. Percentage of germination, survived and fertile plants decrease, while sterile plants, as well as plants with chlorophyll and morphological changes of leaves increased with increasing of mutagenic concentrations of the two applied mutagens. The extension of phenological periods and vegetation period also become longer with increasing of concentrations. Influence of NEU was stronger, on studied parameters, comparing to that one of EMS. Exclusively tight dependence, of studied traits, on amount of rainfall and mean air temperatures was established in $M_1$ and $M_2$ generations during the period sowing - germination but there was not found differences between variants of mutagenic treatments. Correlation coefficients between studied traits and mean temperatures or amount of rainfall were high (more than 0,9). The period flowering - beginning of fruit formation, as well as vegetation period were very sensitive to the influence of the outside conditions and obtained correlation dependences for them were expressed in different ways for different variants of mutagenic treatments. The periods flowering - full maturity and beginning of maturity - full maturity, were in the closest dependence on the amount of rainfall and variants of

mutagenic treatments (coefficient of correlation r > 0,7). Plant fertility and sterility in $M_1$ generation were in very tight dependence on the amount of rainfall and mean of air temperatures in the period flowering - beginning of fruit formation while the amount of rainfall only, was more important for plants in the period of their maturity. That dependence was expressed almost for all variants of mutagenic treatments. The appearance of chlorophyll changes (chlorophyll spots) on leaf surface was in tight dependence on the amount of rainfall in the period beginning of maturity - full maturity for all variants of treatment. The same dependence was found with the mean of air temperatures in the period flowering - beginning of fruit formation. Morphological changes of leaves, in $M_1$ generation, were in tight dependence on the amount of rainfall in the period germination - beginning of flowering (coefficient of correlation r > 0,6). These changes were in tight dependence on the mean of air temperatures in the periods: germination – full flowering and beginning of flowering – beginning of fruit formation (coefficient of correlation r > 0,7). A tight dependence of morphological changes on amount of rainfall was established in the period beginning of maturity – full maturity for all variants of mutagenic treatments. The optimal air temperature in the period germination – flowering for the area of Plovdiv is 20-22$^{o}$ C, concerning investigations of Gurova (1967), and the extension of that period decrease with increasing of temperatures. Phaseolus vulgaris L. has long flowering period (especially plants of II$^{nd}$ or III$^{rd}$ architectural type). This fact determine the special effect of meteorological factors not only on plant development but also on the formation and development of pods and seeds. It was found (Stoyanova, 1959) that the hill of common bean flowers is between 8 up to 30%, while the hill of pods is between 40 up to 82%. The same author repots that the hill of flowers increased at high air temperatures and low relative air humidity, while the hill of pods increased at low soil humidity. It was found, from our investigation, that the meteorological conditions (amount of rainfall and mean of air temperatures), for the studied period, influenced specifically prolongation of phenological periods. They influenced also plant fertility and sterility, as well as appearance of chlorophyll and morphological changes of leaves induced by chemical mutagens NEU and EMS. Very tight dependences, expressed in different phenological periods, were established between studied meteorological factors, plant generations and applied variants of mutagenic treatments. The influence of mutagenic treatments was not found only in the period sowing - germination. Studied biological manifestations of plants in $M_1$ and $M_2$ generations are in tight dependence on meteorological factors in different phenological periods.

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# GENOTYPE RESPONSE OF BEANS (*Phaseolus Vulgaris L.*)TO GAMMA-IRRADIATION STRESS

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## Introduction

During the last years new, synthetic nature pollutants have occurred. They are connected with the application of the nuclear energy for economic and military purposes, with the construction of nuclear power stations, and the use of satellites. In spite of the high protection degree of the systems, in case of working nuclear power stations or accidents, a large amount of nuclides is dumped into the environment, which results in the increase of the radiation background. All this generates dangerous biological consequences and puts forward the problem of application of more radioresistant genotypes in agriculture. Nofal at all, 1995, studied the radio sensitivity of three bean genotypes.

The objective of the research was to study the effect of gamma rays on growth and leaf gas exchange in bean plants.

## Material and Methods

The experiment was carried out with three bean genotypes Dobrodjanski 2 (Dj 2), Dobrudjanski 3 (Dj 3) and Ruse 17 (Rs 17) . The plants were grown as substrate crops (peril) in plastic pots (0,5 l). There were four plants in each pot and they were watered twice a week with a Hoagland solution (at about 50 ml/per pot), and in the remaining days they were watered with distilled water, so as to maintain the soil to field capacity. The plants were grown under the following conditions: light intensity 200 $\mu$ moll $m^{-2}s^{-1}$ (PAR), photo period 14 h (light) 10 h (darkness), temperature of 18±2/22±2 $^{o}$C day/night, and relative humidity of the air 60-70%. 14 days after the emergence the plants were divided into two groups - control and irradiated with 5 Gy gamma-rays $^{60}$Co. Prior to and 10 days after the irradiation the plant dry weight and leaf area (LA) were estimated. Based on these values, the plant growth analysis parameters were determined following the classical approach (Beadle, 1993). Leaf gas-exchange was estimated as well.

## Results and Discussion

The results in Table 1 show that gamma-irradiation with 5 Gy, causes stress in the plants, as a result of which the growth parameters were inhibited. Relative growth rate (RGR) was reduced more considerably in the plants of genotype Dj 3. RGR was determined by two parameters – net assimilation rate (NAR) and leaf area ratio (LAR) (Hall, D., and S.Long, 1993)

NAR was inhibited to the greatest extent in genotype Dj-3 (31%). NAR was determined by net photosynthetic rate, dark respiration intensity, and relative ratio of non-photosynthetic organs – roots.

It is a well-known fact that in case of gamma-irradiation the roots are the plant organs, which are most seriously affected (Stoeva, 2000). This disturbs the water supply of the cells and induces water deficiency.

The distribution of the biomass in the plant organs of the three genotypes differs considerably. In genotype Dj - 3 the inhibition of RWR is with 10 % greater than that in the Dj - 2.

LAR is determined by two components - leaf weight ratio (LWR) and specific leaf area (SLA). In the three studied genotypes, SLA decreased to a greater extent as compared to that in LWR. According to us, the lower LAR values were due to the enlarged leaf dry tissue, as a result of the decreased cell water-content and the reduced leaf area in the irradiated plants.

The analysis of the results of the NAR inhibition gives us a reason to presume that the process of photosynthesis was disturbed in the plants subjected to gamma-irradiation stress. On the other hand, the

changes in LAR and SLA indicate disturbances in transpiration rate, a fact that motivates us to conduct studies in this respect.

The data indicate that the photosynthesis rate was inhibited with 29-32% after the irradiation. The results with regard to the transpiration intensity show a similar tendency. In case of irradiation of the plants with 5 Gy the transpiration is inhibited by 34% in the genotype Dj – 2, and by 44% in the genotype Rs - 17. This is the result of the suppressed growth of the root system and RWR, and hence the water supply and stomato cells closure. In the plants of the first genotype, the stomato conductivity ($g_s$) is reduced by 25 %, in the second genotype –by 28%, and in the third one - by 28 %. The stomato closure is a well-known reaction of the plants in case of water deficiency, aiming to diminish the loss of water. This undoubtedly has a negative effect on the photosynthesis rate, mainly as a result of the restricted access of $CO_2$ to the mesophyll cells (Chaves, M.1991).

Table 1.Growth parameters and leaf gas-exchange in young bean plants

| Indexes | Dj – 2 (0 Gy) | Dj - 2 (5 Gy) | Dj – 3 (0 Gy) | Dj – 3 (5 Gy) | Rs – 17 (0 Gy) | Rs – 17 (5 Gy) |
|---|---|---|---|---|---|---|
| RGR | 0.088±0.002 | 0.064±0.003 | 0.086±0.003 | 0.059±0.002* | 0.091±0.001 | 0.065±0.001* |
| NAR | 0.385±0.032 | 0.290±0.018* | 0.366±0.022 | 0.254±0.019* | 0.368±0.031 | 0.262±0.028* |
| LAR | 0.286±0.015 | 0.245±0.018* | 0.258±0.021 | 0.188±0.015 | 0.315±0.019 | 0.225±0.022* |
| SLA | 0.699±0.041 | 0.540±0.011*** | 0.720±0.036 | 0.580±0.021*** | 0.735±0.018 | 0.615±0.031 |
| RWR | 0.268±0.018 | 0.225±0.022 | 0.288±0.030 | 0.211±0.029* | 0.230±0.021 | 0.185±0.020 |
| LWR | 0.356±0.020 | 0.295±0.018** | 0.362±0.012 | 0.315±0.034 | 0.415±0.026 | 0.350±0.019* |
| A | 6.45±0.20 | 4.70±0.19*** | 7.15±0.10 | 4.65±0.20** | 6.18±0.18 | 4.28±0.11*** |
| E | 3.18±0.12 | 2.11±0.18* | 3.48±0.05 | 2.01±0.12** | 3.15±0.11 | 1.95±0.19*** |
| $g_s$ | 0.12±0.002 | 0.09±0.003 | 0.11±0.002 | 0.08±0.001 | 0.11±0.003 | 0.08±0.004 |

RGR-relative growth rate (g $g^{-1}$ $day^{-1}$); LAR-leaf area ratio ($cm^2$ $mg^{-1}$); NAR- net assimilation rate ( mg $cm^{-2}$ $day^{-1}$); SLA-specific leaf area ($cm^2$ $g^{-1}$); LWR-leaf weight ratio (g $g^{-1}$); RWR-root weight ratio (g $g^{-1}$); A-photosynthesis rate (μmol $CO_2$ $m^{-2}$ s-1), E-transpiration intensity(mmol $H_20$ $m^{-2}s^{-1}$) and $g_S$ -stomatal conductance (mol $m^{-2}s^{-1}$) . Means of 3 separate experiments ± S.E. (n=15). *p<0.1 **p<0.01 ***p<0.001.

On the basis of the obtained results we are able to determine genotype Dj-2 as more radio resistant than the other two genotypes.

The model research can be applied as a test for radiosensitive genotypes, which in turn can be recommended for growing in regions facing a real danger of increased radiation background.

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# GENETIC PROGRESS IN COMMON BEAN AFTER FOUR CYCLES OF RECURRENT SELECTION

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The objective of this study was to estimate the genetic progress in a recurrent selection program with common bean that started in 1990 at the Universidade Federal de Lavras. The program was designed to develop new inbred lines with carioca grain type and high yield.

The base population was obtained from the following parents: Bat 477; IAPAR 14; FT 84-29; Jalo; A 252; A 77; Ojo de Liebre; ESAL 645; Pintado; Carioca. The parents were crossed in diallel scheme to obtain the bi-parental hybrids which were then crossed to generate double hybrids. One hundred and fifty seeds from the $F_2$ generation of each double hybrid with ideal grain type were joined to form the original Cycle I (CI) population ($S_0$ generation). The breeding program followed the methodology schematically shown in Figure 1, until Cycle IV (CIV) lines were obtained.

A total of 20 inbred lines, five best lines from each selection cycle were selected to assess the recurrent selection efficiency. These 20 lines and the control Pérola cultivar were sown in July 2002 in Ijaci, MG, Brazil (latitude 21°13´S, 915 altitude) for assessment. A randomized complete block design with five replications was used, with plots formed by two four-meter long rows. The between row spacing was 45cm and 15 seeds were sown per linear meter of row.

Data collected were yield in grams/plot and grain type in a scale of scores ranging from 1 to 5, where: 1 – typical carioca grain, cream colored with light brown stripes, pale base, without corona, mean weight of 100 seeds of 22 to 24 g, grains not flattened and 5 – cream colored grain with dark brown stripes, dark base, with corona, mean weight of 100 seeds less than 22g, flattened grains. These data were submitted to analysis of variance. The family means in each cycle were used to estimate the progress from selection by the least squares method.

The mean yield of the best five lines in each cycle increased with selection. The estimated coefficient of linear regression was positive and different from zero (b=77.7 g/plot and $R^2$=94.7%). This result showed that the genetic progress was 77.7 g/plot per cycle, which corresponds to 7.4% of the mean yield of the lines in the first cycle.

The grain type scores also improved with selection. The estimated coefficient of linear regression was b= -0.32, which indicated progress from selection of 10.5% over the means of the families in the original cycle.

It must be emphasized that the recurrent selection method allowed the introduction of lines from other programs for recombination. This allowed a much more dynamic process that capitalized over the genetic progress obtained in other programs. Therefore, the progress from selection in both characteristics was in part due to the lines introduced for recombination at each cycle. However, the greatest progress proportion, without any doubt, should be attributed to the recurrent selection program.

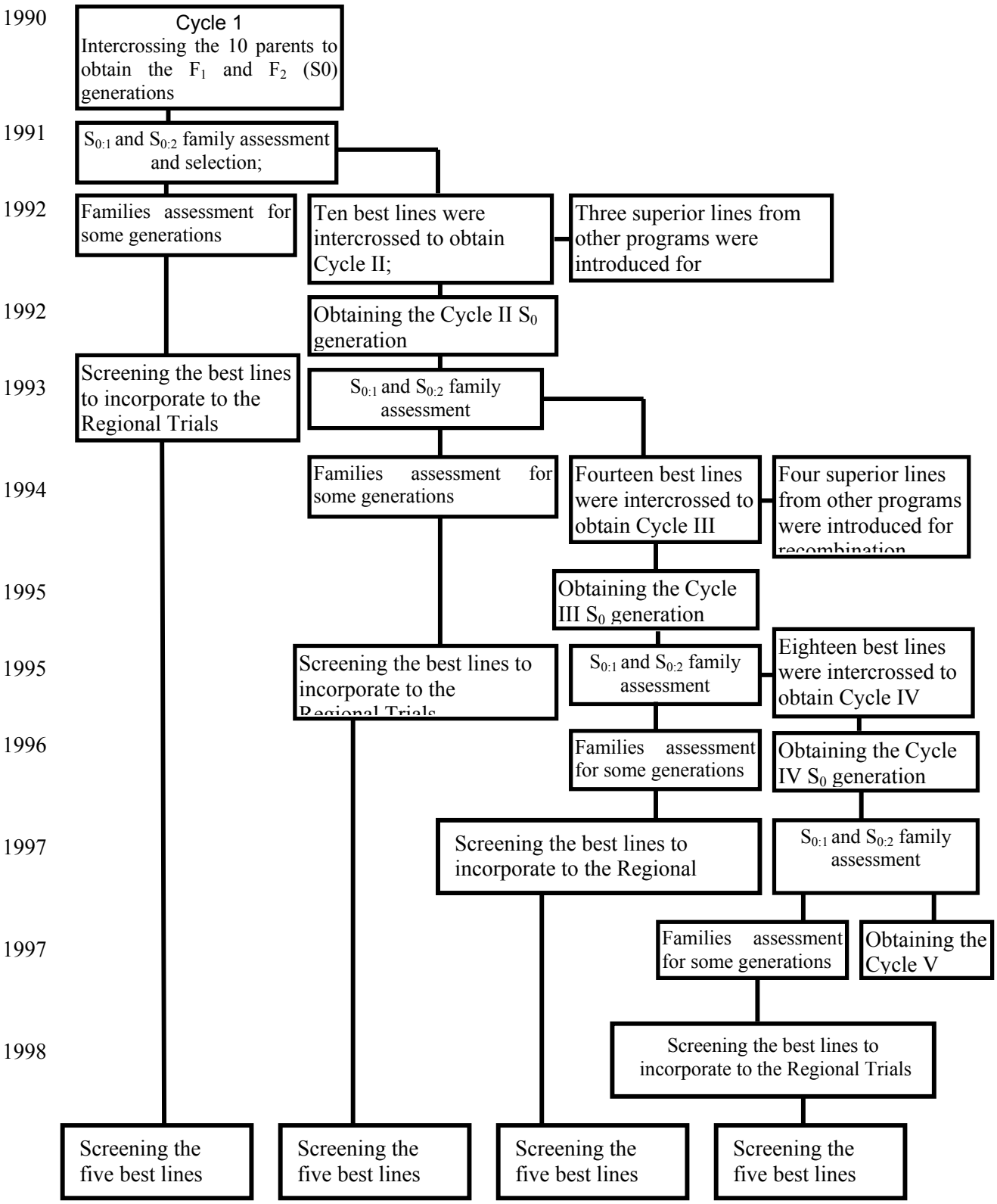


Figure 1. Recurrent selection method used to breed common bean.

# Development of additional BAC libraries in common bean

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BAC (bacterial artificial chromosome) libraries are large-insert that have been very useful in cloning large portions of genomes in a wide range of crops (Vanhouten and Mackenzie 1999; Allouis et al. 2001; Gindullis et al. 2001; Tomkins et al. 2001; Wang et al. 2001a; Gentzbittel et al. 2002; Tomkins et al. 2002). Insert sizes are generally from 100-150 kbp. Availability of such libraries allows researchers to develop a physical map of the genome of choice by developing contigs of overlapping clones (Tao et al. 2001). In turn, this physical map can be related to the genetic map by locating existing genetic markers on the contigs and, conversely, locating markers obtained from analysis of the contigs onto the genetic map (Lewers et al. 2002). Contigs become a starting point for positional cloning of specific genes (Wang et al. 2001b; Xu et al. 2001). A correlation of physical and genetic maps can accelerate the discovery of genes underlying phenotypes of agronomic interest (Liu et al. 2001). It can also help in identifying more closely linked markers for marker-assisted selection (MAS). Furthermore, BAC clones - because of their size - can be used as probes in *in situ* hybridizations of chromosomes (Kim et al. 2002). In common bean (*Phaseolus vulgaris*), BAC libraries have been developed previously by (Vanhouten and Mackenzie 1999) for genotype Sprite FR (an Andean cultivar) and (Kami and Gepts 2000) for genotype BAT 93, a Mesoamerican breeding line.

A compelling aspect of the biology of common bean is the existence of information regarding its ancestry and phylogeny (Fig. 1) (Gepts 1998; Delgado-Salinas et al. 1999). Domesticated common bean consists of two major geographic gene pools, Andean and Mesoamerican, originating from independent domestications in the southern Andes and Mesoamerica. In turn, the wild common bean gene pools in these two areas are derived from a common ancestor located in the Andes Mountains from Ecuador and northern Peru.(Kami et al. 1995). Several *Phaseolus* species, some of which have also been domesticated, are closely related to common bean (Fig. 1). These include the runner bean (*P. coccineus*), the year bean (*P. polyanthus*, a hybrid between a proto-*P. vulgaris* and *P. coccineus*), and the tepary bean (*P. acutifolius*). All the preceding species belong to the *P. vulgaris* clade or lineage within the genus *Phaseolus*. A more distantly related species is lima bean (*Phaseolus lunatus*), which belongs to a different clade altogether (Delgado-Salinas et al. 1999).

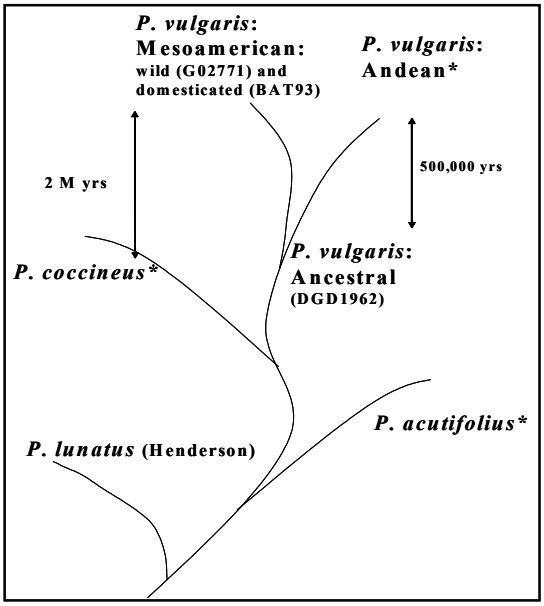


**Figure 1**. Phylogenetic and genealogical relationships among domesticated *Phaseolus* species [summarized from Llaca et al. 1994; Kami et al. 1995; Schmit et al. 1993; Delgado-Salinas et al. 1999). * Libraries in these taxa exist or should be developed to complete the model.

To analyze micro-evolutionary changes in genome structure, four BAC libraries are currently being developed in four different *Phaseolus* genotypes. Specifically, we want to follow the evolution of the APA complex locus on linkage group 4 (Gepts 1999). APA is a family of closely related seed proteins; Alpha-amylase inhibitor, Phytohemagglutinin and Arcelin . Phytohemagglutinin (PHA) proteins are widespread among all legumes, including *Phaseolus*. In the *P. vulgaris* lineage, the alpha-amylase inhibitor subfamily (αAI) appeared by duplication and divergence. Finally, in the Mesoamerican branch of *P. vulgaris*, some wild beans show a third subfamily, that of the arcelins (ARL). Consequently, the four BAC libraries are being developed in selected genotypes that encompass the entire evolution of the APA proteins in the *Phaseolus* genus. The four genotypes include *P. lunatus* cv. Henderson ($PHA^+\alpha AI^-ARL^-$), *P. vulgaris* wild DGD1962 ($PHA^+\alpha AI^+ARL^-$), *P. vulgaris* Mesoamerican domesticated BAT93 ($PHA^+\alpha AI^+ARL^-$), and *P. vulgaris* Mesoamerican wild G-2771 ($PHA^+\alpha AI^+ARL^+$). These complement the BAC library developed in cv. Sprite of Andean origin (Vanhouten and Mackenzie 1999).

For each of the libraries, high molecular weight DNA was obtained after nuclei isolation (Kuehl 1964). Following partial digestion with *Hin*dIII and electroelution (Strong et al. 1997), the DNA was ligated into the pIndigoBAC5 vector (Epicenter Technologies) and transformed into electrocompetent *E. coli* cells (Strain DH10B ElectroMax

cells, Life Technologies). Clones have been distributed in 384-well plates and arrayed on high-density membranes. The libraries have the following characteristics (Table 1).

Table 1. Main characteristics of *P. vulgaris* BAC libraries developed in this project

| Library | Clones | Average Size | Genomes | Empties[a] | Chloroplast | | APA-containing clones | Phaseolin-containing clones |
|---|---|---|---|---|---|---|---|---|
| | | | | % | total # | % | total # | total # |
| BAT93 | 36,864 | 110 | 5.7 | 11% | 21 | 0.05 | 5 | 6 |
| DGD1962 | 52,608 | 105 | 8.7 | 1.40% | 200 | 0.4 | 10 | 11 |
| G-2771 | 55,296 | 139 | 12.1 | 0.50% | 49 | 0.08 | 38 | 14 |
| Henderson | tbd[b] | ~130* | tbd | 0%* | tbd | tbd | tbd | tbd |

[a] Based on a sample of 200-400 clones per library
[b] tbd: to be determined
* Based on a preliminary sample of 40 clones

Based on a conservatively large genome size of 637 Mbp (Bennett et al. 2000; Aramuganathan and Earl 1991), the genome coverage ranges from 6x to 12x. There was generally a low frequency of empty and chloroplast DNA clones. The larger number of BAC clones identified after hybridization with a APA sequence in the G2771 library may be due to the presence of all three subfamilies, in comparison to the BAT93 and DGD1962 libraries, which were developed in genotypes that only contain two subfamilies. We will deposit these libraries in genomics centers at Clemson or Arizona.

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# Towards Cloning the *Co-4²* Locus Using a Bean BAC Library

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## Introduction

The *Co-4²* locus was first identified in the Mexican landrace G2333 that also carries two others anthracnose resistance genes (Young et al. 1998). *Co-4²* is one of the most valuable anthracnose resistance genes as it controls 97% of all identified races of the bean pathogen *Colletotrichum lindemuthianum*. This locus has been genetically characterized and tagged with several molecular markers (Young et al. 1998, Melotto and Kelly 2001). The availability of those markers prompted us to screen a bean BAC library to isolate the genomic region harboring this gene. In this study, our aim was to identify BAC clones that carry the *Co-4²* gene using linked markers in order to facilitate its cloning and molecular characterization.

## Material and Methods

The BAC library used for this study was custom made by Bio S&T, Montreal, Canada. Young leaves from 10 seedlings of the bean genotype G2333, were used as DNA source for the library construction. High molecular weight DNA was cloned into *Hin*d III-cut pBeloBAC11 vector. After transforming DH10B *E. coli* cells, recombinant colonies were selected on solid LB medium with 12.5ug/ml chloramphenicol. Twenty-eight BAC plasmids were digested with *Not* I and visualized through PFGE (Pulse Field Gel Electrophoresis) to determine the average insert size and the genome coverage represented in the library.

The *COK-4* gene, previously located at the *Co-4²* locus (Melotto and Kelly 2001) was used as probe to screen bulks of 20 BAC clones. Bulked clones were cultured together, plated on solid medium, colony lifted, and subjected to hybridization using the ECL system of labeling and detection as suggested by the manufacturer (Amersham Pharmacia Biotech). Positive bulks were selected and the 20 clones were grown individually to identify the one carrying the *COK-4* gene.

## Results and Discussion

The average insert size of the library was determined as 125kb (Figure 1). Considering that the genome size of common bean is between 450-600 Mb and the library collection contains 24,960 BAC clones, we estimate that this library covers 5-7 times the bean genome. The whole BAC library was screened with the *COK-4* probe and three bulks were identified as positives. Figure 2A shows a strong hybridization signal of one bulk containing a *COK-4* homologous BAC clone. The 60 clones represented in the three bulks were grown individually and were again subjected to hybridization with the *COK-4* probe. Three individual clones named P18J13, P23N24, and P45F17 were selected (Figure 2B).

The identified BAC clones will be tested with *Co-4²* flanking markers and partially sequenced for the identification of resistance gene candidates. Use of BAC libraries has been a valuable tool for cloning genes from other crops (Wang et al. 2002) with scarce genomics resources like common bean.

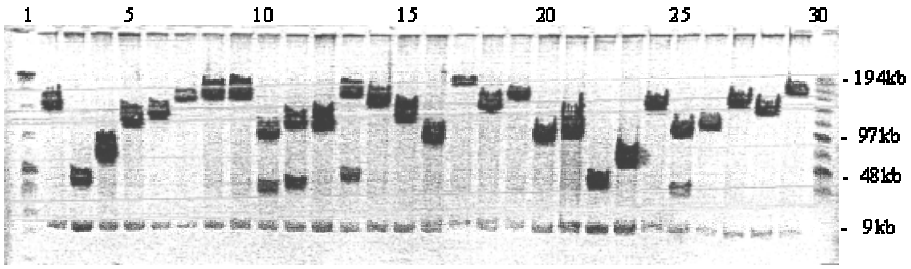


Figure 1. Pulse field gel electrophoresis of 28 BAC clones digested with *Not* I. Lanes: 1 and 30 = low range molecular weight marker (BioRad), 2-29 = random BAC clones. The lower band across all lanes is the pBeloBAC11 vector.

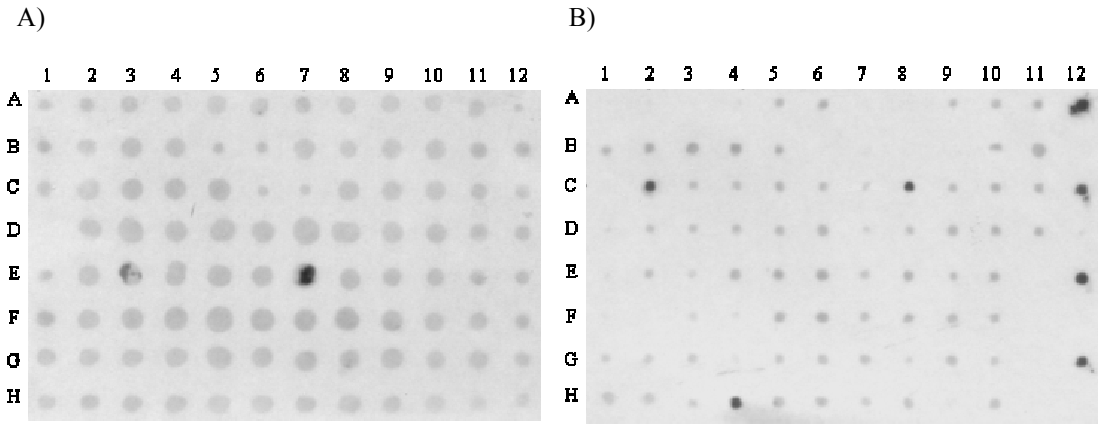


Figure 2. A) X-ray film showing hybridization signal at the position E7 of the 96-well microplate. This plate contains 20 BAC clones per well. B) X-ray film showing the identification of three BAC clones homologous to the *COK-4* gene at the wells C2 (P18J13), H4 (P23N24), and C8 (P45F17). Wells A12, C12, E12, and G12 have the *COK-4* probe as positive control.

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# VARIATION OF BOWMAN-BIRK INHIBITOR REACTIVE SITE LOOPS IN COMMON BEAN GENOTYPES

## Preliminary results.

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Differences in trypsin inhibitor (TI) level among common bean (*Phaseolus vulgaris* L.) genotypes are described in literature. Recently, Piergiovanni and Pignone (2003), studying the antitryptic activity of 21 Italian landraces of common bean, observed a different response (variation or constancy of TI level) to the year-to-year variation of mean temperature and rainfall quantity. The reasons of TI variation are not completely understood but could be related to the presence of inhibitor isoforms. Wu and Whitaker (1990), isolated and purified by affinity chromatography four isoinhibitors from red kidney bean (*P. vulgaris* L. var. Linden). Isoinhibitors have been found also in other species. There are two possible origins for such forms, either genetic polymorphism (Domoney et al., 1993) or differences in post-translational processing (Wilson and Chen 1983).

This study was undertaken to ascertain if a different array of isoinhibitors is expressed by genotypes showing a different response to the year-to-year climatic variations. Three common bean landraces 'Gialletto', 'Tuvagliedda' and 'Ciuoto' selected among those investigated by Piergiovanni and Pignone (2003), were analysed. 'Tuvagliedda' was characterised by a positive correlation between TI level and rainfall quantity, 'Gialletto' showed no significant correlation between these parameters, while TI level of 'Ciuoto' was not influenced by year-to-year variations.

Genomic DNA from the above landraces was extracted from dry half seed. TI sequences were amplified by PCR using two specific primers designed on the partial gene sequence encoding for the Bowman-Birk type inhibitor (BBI). The amplified product of about 300 bp was gel purified and cloned in p-GEM-T plasmid. Several clones for each landrace, were sequenced with an automated sequencer. A FASTA search of these clones against the EMBL Nucleotide Sequence Database revealed a high similarity with the nucleotide sequence of BBI.

All the deduced amino acid sequences analysed showed two independent reactive sites one for trypsin and the other one for chymotrypsin. The reactive site for trypsin showed only the residues Lys-Ser at the position $P_1$-$P_1$', as defined by Schecter and Berger (1967), while Leu or Phe were detected at the $P_1$ position of chymotrypsin reactive site. More differences among the BBI amino acid sequences were detected by comparing the binding loops relative to trypsin and chymotrypsin, constituted by a nine residue disulphide-linked motif. In 'Tuvagliedda' two different residues, Ile or Arg, were detected in the $P_2$' position of binding loop toward trypsin, while only $P_2$'-Ile was observed in 'Gialletto' and 'Ciuoto' sequences. Regarding to the chymotrypsin loop it was found an association between the residues in $P_1$ and $P_6$' positions. $P_1$-Leu was always associated with $P_6$'-Val, while $P_6$'-Ile was detected only in the presence of $P_1$-Phe. Further differences located outside the protease binding loops were observed but their role on the activity of full-length BBI should be marginal. In fact, it is known that the inhibition widely depends upon the interaction between the reactive site loop motif and the active site of

the target protease. Gariani et al. (1999) observed remarkable differences of trypsin inhibition and BBI rate of hydrolysis in relation to the residue at the $P_2$' position of reactive-site loop.

The highest trypsin inhibition was observed with Ile at the $P_2$' position and the presence of different residues reduces the antitryptic activity (Gariani et al. 1999). Consequently, the two types of BBI sequences detected in the tested landraces, having different residues at the $P_2$' position, are characterised by a different activity. Similarly, it is known that chymotrypsin has a higher affinity towards amino acids having aromatic residues. Therefore, it could be expected that the two variants at the $P_1$ position (Leu or Phe) observed in this study, are able to change the inhibition capacity. The residue Phe best satisfy the specificity requirements of chymotrypsin.

These preliminary results revealed differences in canonical site loop of both trypsin and chymotrypsin which might affect the activity of common bean BBI. The presence in 'Tuvagliedda' of two different inhibitors with different activity could be related to the uncommon trend of TI variation showed by this landrace over the years. To confirm the above hypothesis other genotypes, showing a different TI variation in relation to climate changes, are going to be analysed.

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# EVALUATION OF DRY BEAN RECOMBINANT-INBRED-LINES FOR AGRONOMIC PERFORMANCE AND CULINARY QUALITY

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## INTRODUCTION.

Major efforts of dry bean breeders are concentrated on the increase of yield and its stability over a wide range of environments. However, consumer preferences and processors' requirements need equal consideration to yield. Seed size, shape, color, and culinary traits related to preparation for consumption, such as water uptake and cooking time, are important parameters, which influence the acceptability of cultivars by consumers. A cultivar of poor quality is likely to be rejected by both consumers and processors, regardless of how agronomically superior it may be. Several studies have indicated genetic variability in dry bean for culinary traits (Castellanos et al.,1995; Shellie and Hosfield,1991; Elia et al. 1997; Boros 2001). The significant variation found for the evaluated traits indicated that they can be changed by selection. The objective of this study was to evaluate the agronomic performance and to assess the seed quality parameters existing among RILs and parental forms.

## MATERIALS AND METHODS.

Twenty-one $F_{2:8}$ advanced recombinant inbred lines from cross Prosna x Nida and the parental forms were evaluated. The parental forms showed contrasting cooking time among the registered high yielding cultivars. They differed in earliness and susceptibility to bean bacteriosis, with Nida cultivars being early maturing and tolerant to halo blight. Field trials were planted at two locations. The experiment was arranged in the randomized complete block design in four replication. The determination of seed physical traits, water absorption and cooking time were done in the laboratory. The seed physical traits and the percentage of testa in seed are an average of 10 seeds in 3 replications. The percentage of water absorption of the entries was determined on the basis of replicated samples of 50 seeds. Seeds were 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 and Varriano-Marston, 1981). The cooking time was calculated as the time from initial cooking until the time when 80% of pins penetrated seeds in the cooker. Analysis were done in three replications.

## RESULTS.

Parental cultivars differed in respect of earliness, TSW, water absorption and cooking time. The mean values for particular traits of RILs were not different from that of parental forms. However wider variation among RILs was observed (table 1 and table 2), suggesting possible transgressive segregation. The differences in water uptake influenced seeds hydration properties, texture, palatability, and cooking time were partially associated with structure of the epicuticular wax layer of seed coat related to the chemistry of the *Asp* and *asp* allele as it was demonstrated in the studies by Bushey et.al. (2002). Cooking time exhibited a wide range from 17 to 28 min that, in general, is rated as medium to good cooking quality compared to dry bean cultivars cultivated in Poland (Boros 2001). Three lines, F 5348, F 5322 and F 5404 were identified that have shorter or equal cooking time,

**Table 1. Agronomic traits of parental cultivars and bean RILs**

| Entry | Vegetation days | Plant height cm | Yield dt $ha^{-1}$ | Protein yield dt $ha^{-1}$ |
|---|---|---|---|---|
| Prosna | 104.8 | 38.0 | 29.90 | 6.33 |
| Nida | 99.1 | 36.0 | 31.44 | 6.47 |
| RILs | 101.2 | 36.6 | 29.98 | 6.20 |
| Range | 98.8 - 104.6 | 34 - 40 | 24.27 - 33.68 | 5.10 - 7.22 |
| CV | 1.5 | 10.5 | 8.05 | 9.67 |
| LSD | 1.5 | 5 | 2.39 | 0.59 |

that were also earlier and had slightly improved yield comparing to the maternal parent. The best yielding lines exhibit colored seeds. The relation between cooking time and water absorption ( r= - 0.60 ) has indicated that, to some degree, the RILs with higher water absorption were faster to cook. Our data, similar to the earlier reports (Castellanos et al.,1995; Shellie and Hosfield,1991 and Elia et al.,1997), also showed that the accessions with white seed coats and higher water absorption cooked comparatively faster than Nida and lines that with color seed coats. The longest cooking time may be caused by tannin content in the testa. The data on cooking time were similar to those of Elia et al. (1997), who found that low tannin beans cooked faster than beans with high tannin.

**Table 2. Seed quality traits of parental bean cultivars and RILs**

| Entry | TSW. G | Testa % | Protein % | Absorption % | Cooking time min |
|---|---|---|---|---|---|
| Prosna | 401 | 7.59 | 21.7 | 103.9 | 18.72 |
| Nida | 383 | 7.20 | 21.4 | 98.0 | 27.19 |
| RILs | 404 | 7.56 | 21.4 | 99.9 | 22.86 |
| Range | 333 - 479 | 6.82 - 8.18 | 20.0 - 22.3 | 92.5 - 106.1 | 16.65 - 28.06 |
| CV | 1,6 | 2.65 | 4.7 | 1.1 | 4.12 |
| LSD | 10.5 | 0.33 | 1.4 | 0.54 | 1.55 |

The intermating late maturing high yielding and fast-cooking cv. Prosna with early maturing high yielding color seeded cv. Nida provided recombinants with improved yield, earliness and fast-cooking. Further testing is planned to investigate these lines in adverse conditions and their reaction to diseases.

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# EVALUATION OF SUGAR BEANS TEXTURE USING THE SHEAR PRESS AND SENSORY ANALYSIS

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## Introduction

Sugar-cooked beans have potential in the U.S., given the recent consumer interest in "healthy" foods, and the increasing ethnic population. Beans are good sources of fiber and folate and a lower fat alternative to animal proteins. Texture, or firmness of the cooked/ready-to-eat beans, is an important marketing attribute. Typically a shear press is used to determine bean texture and optimum processing conditions, such as temperature and time. Considerable time and money can be saved in product and process development if shear press results can be used to predict consumer panel results. Because batch processes are commercially impractical and economically unfeasible, U.S. manufacturers typically require continuous processes. Therefore, the objective of this study was to determine if mechanical shear press measurements from sugar beans processed under two different conditions can accurately represent results from a discrimination sensory panel.

## Methods

*I. Simulation of a batch dynamic temperature (control) process with a continuous isothermal (treatment) process.*

Whole dry Michigan Cranberry bush beans (*Phaseolus vulgaris)* were used. A control was cooked per a standard process, given below. Six different preliminary treatments were cooked to determine the isothermal cooking time required to obtain the same shear-press measurement as the control. The process flow for the control sample was: 1) soak beans for 12 hr in water at 23°C containing less than 0.1% (w/w) sodium bicarbonate and sodium polyphosphate; 2) heat beans in a steam-jacketed kettle at 5ºC/min from 23-98ºC in 15 min. (dynamic temperature blanch); 3) blanch beans in a separate kettle at 98ºC [containing above additives] for 8 minutes; 3) Sugar-cook in 50% sugar/water solution at 70°C for 45 minutes. The treatment process differed in two ways only: 1) The soak was at initial temperature of 77ºC; and 2) Steps 2 and 3 were combined into one isothermal blanch at 98ºC for 9, 10, 11, 12 14, or 16 min. Bean texture was measured after the sugar-cook using a Kramer shear press. Maximum force at a shear press speed of 4 was measured. Texture measurements were expressed in units of Newtons per reference weight of 20.3 beans. The control was run in triplicate and the treatments in duplicate, all on different days. For each run, five samples were measured for control and treatment. The treatment blanch time (11min) for sensory analysis was chosen by equating the control and treatment texture measurements.

II. Sensory evaluation

Bean samples from the control and treatment were presented to a sensory panel that was not aware of any cooking differences. The 11-min cook treatment sample was run again on the day of the test. Bean texture after the sugar cook was measured using a Kramer Shear Press at 23°C per above methods.

An untrained panel of anonymous panelists was recruited from the university community to evaluate the two samples. 97 panelists completed the first evaluation. The second (repeat) evaluation was completed by 95 of the same panels (two panelists completed only one evaluation), for a total of 192 evaluations. Each person received a complimentary ice cream coupon as an incentive. Testing was performed under controlled conditions in the Department of Food Science and Human Nutrition's sensory evaluation laboratory. Questionnaires were prepared and provided to the panelists using SIMS Sensory Evaluation Software [Simms2000-version 3.3.]. A Directional Difference test (Meilgaard et al 1999) was

used to determine if panelists could determine which sample was firmer in texture. Each panelist was presented two samples and asked to chew each sample, and decide which sample was firmer than the other. This was conducted as a forced choice method, i.e. panelists were not given the option to choose "no difference." Sensory data were analyzed using SAS program for a two-tailed test.

## Results and Discussion

*I. Simulation.* Texture measurements for control and the six preliminary treatments are shown in Figure 1. Treatment firmness shows a clear decreasing trend with blanch time. We chose 11 min blanch time (Fig. 1, 11-min mean + std. dev. = control mean) as the simulation treatment that would give a texture nearly equal to the control.

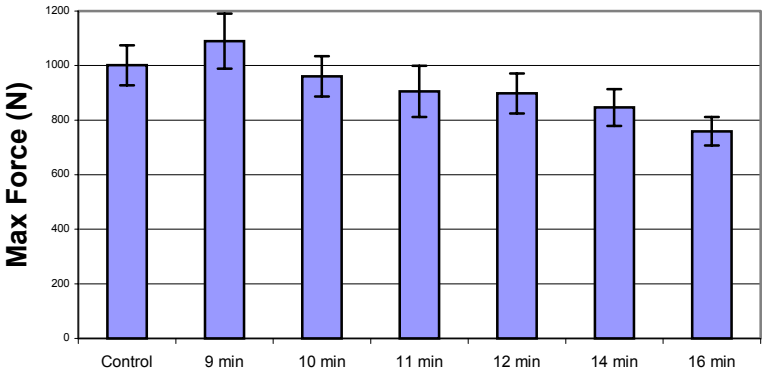


Figure 1. Average of maximum force from shear press tests on control and treatment beans after sugar cook. Speed of shear press = 4, bean sample size = 20.3 ± 0.2 g.

*II. Sensory.* The mean ± standard deviation of force measurements were 581 ± 48.4 and 654 ± 92.4 N for control and treatment samples, respectively. These two means were not significantly different at 95% confidence using a t-test.

Sensory results (Table 1) showed that the panelists could not detect a firmness difference between the control and treatment samples. These results indicate that texture-shear press measurements can confirm the sensory panel results when attempting to produce products with identical firmness.

Table 1. Sensory panel results for sugar bean firmness

| Replication # | Number of panelists choosing the control sample as firmer | Number of panelists choosing the treatment sample as firmer | Total number of panelists | Critical Value* |
|---|---|---|---|---|
| 1 | 51 | 46 | 97 | 59 |
| 2 | 47 | 48 | 95 | 59 |

*Minimum number of correct responses required for significance at α level of 0.05

Therefore, the shear press data may be used to predict the consumer's ability to discriminate between texture of value-added sugar beans. This outcome is significant, showing that money and time can be saved by using quick, objective texture measurements.

Future testing could establish how different the texture press data need to be before a sensory panel could detect a difference. Additional work may involve evaluation on other Michigan bean varieties for developing similar value-added snack products.

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## ACKNOWLEDGEMENTS

Appreciation is extended to Agri Analysts, LLC for providing beans and partial funding for this study, and to Maureen Beck, Jeff Moore, and Mavis Tan for assistance in evaluation of samples.

# COOKING TIME OF BEAN MATERIALS IN MALAWI

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## Introduction

In a 1992 study of 176 farming families in Dedza and Ntchisi districts in Malawi, cooking time was found to be the most important factor (80%) to consider when choosing beans for consumption. Taste was rated second (69%) in this study, flavor third (64%), and broth thickness fourth (63%). Families are most concerned about long cooking time because it results in higher firewood consumption, an increasingly scarce and expensive resource.

The B/C CRSP programme has been evaluating cooking time of breeding lines and varieties in Malawi since 1997. Previous studies have shown that cooking time varies with water composition, and tap water or borehole water that contain divalent cations result in longer cooking time than deionized water. Divalent cations such as calcium are bound by pectic substances within the middle lamellae of the bean cotyledon, forming calcium pectates that are insoluble and resist cell separation during cooking. In our cooking time studies, we use deionised water as a standard type of water so that we can compare our results to other regions and laboratories. Composition of tap (prevalent in urban and peri-urban areas) and borehole water (prevalent in rural villages) varies widely from region to region and it is therefore impossible to standardize cooking time evaluations using these types of water. This paper highlights some results of our cooking time evaluations, and a more complete paper can be viewed in the East Africa Bean Workshop Proceedings, http://eastafricacrsp.wsu.edu.

## Materials and Methods

Bean vareties and breeding lines were evaluated for cooking time using a Mattson bean cooker at the Foods Laboratory at Bunda College of Agriculture from September 2000 to March 2001. Cooking time was evaluated using three types of cooking water (deionised, tap and borehole water) and 26 varieties in a completely randomized design with three replications. Fifty beans of each material were soaked overnight (16 hours) in deionised, tap, or borehole water, and 25 soaked beans were then cooked in the same type of water that was used for soaking. The data was analyzed using Anova in the MSTAT statistical package.

## Results and Discussion

There were significant differences in cooking time among bean materials within the three types of cooking water (Table 1). Cooking time was fastest in deionised water (range 53-101 minutes, mean 73 minutes), second fastest in tap water (range 61-132 minutes, mean 87 minutes), and slowest in borehole water (range 88-231 minutes, mean 140 minutes). The mean increases in cooking time in tap and borehole water as compared to deionised water were 14 and 67 minutes, respectively. Only six materials had cooking time in tap water that was faster than or equal to cooking time in deionised water – AND 656, Sugar 47, DC 184-35, Fitomeko, Bwenzilaana and Sugar 59.

B/C CRSP-released varieties Kalima and 2-10 were two of the fastest cooking materials in all three types of water, whereas the Malawian crosses (3J/2, 2N/2, 15P/8 and 2G/2) were all slow cooking in borehole water. Kalima, DC 95-170 and 2-10 may be suitable for a wide variety of areas that include both hard water (high calcium and divalent cation levels) and soft water (low calcium and divalent cation levels) because the increase in cooking time due to type of

cooking water was minimal. Other bean materials such as 3J/2, IZ 226-1, DC 86-191, 15P/8, DC 86-250, Kanzama, 2G/2, Sugar 47 and ZPV 906 would be fast cooking only in areas with soft water but would be very slow cooking in areas with hard water.

Based on this information it is necessary to test the cooking time of bean breeding lines using several types of water before the line can be classified as fast cooking. Breeding lines should be evaluated so that cooking time can be taken into consideration before a line is considered for release. Differences due to type of cooking water need to be further investigated to allow breeders to determine the characteristics to select for faster cooking time.

**Table 1.** Cooking time (minutes) of 26 dry bean varieties and breeding lines in three types of cooking water (deionised, tap, and bore hole water) in 2001.

| | Types of Soaking and Cooking Water[x] | | | Differences in Cooking Time | | |
|---|---|---|---|---|---|---|
| **Variety** | **Deionised (D)** | **Tap (T)** | **Borehole (B)** | **T - D** | **B – D** | **B – T** |
| 3J/2 | 53 | 83 | 187 | 30 | 134 | 104 |
| Kalima | 57 | 64 | 96 | 7 | 39 | 32 |
| IZ 226-1 | 60 | 78 | 203 | 18 | 143 | 125 |
| Sugar 59 | 62 | 62 | 146 | 0 | 84 | 84 |
| DC 86-191 | 62 | 100 | 162 | 38 | 100 | 62 |
| DC 86-244 | 62 | 90 | 147 | 28 | 85 | 57 |
| 2-10 | 63 | 67 | 88 | 4 | 25 | 21 |
| 15 P/8 | 64 | 98 | 192 | 34 | 128 | 94 |
| AI 97 | 65 | 94 | 155 | 29 | 90 | 61 |
| DC 96-95 | 65 | 79 | 150 | 14 | 85 | 71 |
| ZPV 292 | 66 | 132 | 148 | 66 | 82 | 16 |
| And 278 | 67 | 108 | 145 | 41 | 78 | 37 |
| Enseleni | 67 | 90 | 161 | 23 | 94 | 71 |
| Bwenzilana | 69 | 61 | 128 | -8 | 59 | 67 |
| DC 86-250 | 72 | 94 | 231 | 22 | 159 | 137 |
| Fitomeko | 73 | 67 | 128 | -8 | 55 | 61 |
| Kanzama | 74 | 79 | 209 | 5 | 135 | 130 |
| Sugar 56 | 76 | 91 | 145 | 15 | 69 | 54 |
| 2G/2 | 83 | 91 | 197 | 8 | 114 | 106 |
| DC 184-35 | 86 | 75 | 102 | -11 | 16 | 27 |
| 2N/2 | 86 | 93 | 155 | 7 | 69 | 62 |
| Sugar 47 | 86 | 75 | 180 | -11 | 94 | 105 |
| DC 95-170 | 88 | 112 | 94 | 24 | 6 | -18 |
| PC 512 –B4 | 95 | 100 | 121 | 5 | 26 | 21 |
| ZPV 906 | 99 | 114 | 184 | 15 | 85 | 70 |
| AND 656 | 101 | 74 | 151 | -27 | 50 | 77 |
| **Grand Mean** | **73** | **87** | **140** | **14** | **67** | **53** |

[x] **LSD (0.01) = 13.2**

# TOTAL SOLUBLE AMINO ACIDS AND PROTEIN CONTENT OF LANDRACE COMMON BEAN (*Phaseolus vulgaris* L.) CULTIVARS COLLECTED IN PARANÁ STATE, BRAZIL.

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## Introduction

Common bean is one important economic and social agricultural product in Brazil (Ramalho et al., 1993). The state of Paraná has been contributing with 18.5 % of Brazilian bean production. However, high variability can be observed in traditional common bean populations that are also known as landrace cultivars. This germplasm is commercialized throughout the state in the common bean producing regions, mainly in street markets. The genetic variability and agronomic qualities of these materials should be more accurately studied after collection and should be preserved in a germplasm bank (Alberini, 2001). The objective of this study is to provide information regarding genetic diversity through the evaluation of seed nutritional quality of landrace *Phaseolus vulgaris* L. cultivars collected in state of Paraná, southern Brazil.

## Material and Methods

The 25 accessions of landrace dry bean were evaluated in the field in the State of Paraná, southern Brazil, during summer 2001/02, using a complete randomized block design with four replications. After harvesting, 100 g of seeds from each cultivar were collected from each replicate. Thereafter, 5 g were macerated using mortar and pestle, and the samples were lyophilized for 30 hours. Total soluble amino acids and proteins were extracted from 2 g of the lyophilized material, using the method described by Bielski and Turner (1966). Total soluble amino acids were determined according to Moore and Stein (1948) and the total soluble protein according to Bradford (1976), using triplicates for each replicate. The data were submitted to analysis of variance according to Banzatto and Kronka (1995), and the averages were compared by the method of Scott & Knott (1974).

## Results and Discussion

Total soluble amino acids and protein content varied among the cultivars, indicating that there is genetic variability in these materials (Table 1). Cultivars Navy-UEM, Preto 3, Roxinho and Carioca 4 showed the highest total soluble amino acids content, when compared to Carioca 6 which content was 1163 $\mu mol.g^{-1}$. Meanwhile, cultivars Jalo, Pintado, Bolinha, Jalo Vermelho, Roxinho, Preto 3 and Carioca Pintado 2 showed protein content about three times higher than cultivar Carioca Claro. It is important to emphasize that cultivars Navy-UEM, Preto 3, Roxinho and Bolinha showed the highest contents of both soluble total amino acids and protein (Table 1). These results are in agreement with the idea that substantial variability can be found in the *P. vulgaris* germplasm pool for protein fraction in the seed (Shellie-Dessert and Bliss, 1991, quoted by Hosfield, 2000). It is concluded from this study that genetic variability observed in total soluble amino acids and protein content can be used in a dry bean breeding program.

Table 1- Average of total soluble amino acids and protein content in dry bean seeds from different landrace cultivars. Paraná, Southern Brazil, 2001/2002.

| *Cultivars* | *Total soluble amino acids* * | *Total soluble protein** |
|---|---|---|
| | μmol.g$^{-1}$ ** | (%)** |
| Carioca 1 | 1185 c | 15.1 d |
| Carioca 2 | 1587 b | 20.9 c |
| Carioca 3 | 1363 c | 13.7 d |
| Carioca 4 | 1661 b | 20.9 c |
| Carioca 5 | 1519 c | 21.3 c |
| Carioca 6 | 1163 c | 13.0 d |
| Carioca Claro | 1522 c | 11.5 d |
| Carioca Pitoko | 1370 c | 16.6 d |
| Carioca Preto | 1367 c | 27.3 b |
| Carioca Pintado 1 | 1428 c | 18.1 c |
| Carioca Pintado 2 | 1387 c | 30.3 a |
| Preto 1 | 1616 b | 24.5 b |
| Preto 2 | 1655 b | 19.0 c |
| Preto 3 | 1807 b | 30.3 a |
| Preto 4 | 1645 b | 13.7 d |
| Navy-UEM | 2120 a | 24.5 b |
| Rosinha | 1414 c | 22.4 c |
| Jalo Pintado | 1397 c | 36.0 a |
| Jalo Pardo | 1615 b | 25.2 b |
| Jalo Vermelho | 1480 c | 33.9 a |
| Jalo Mulato | 1463 c | 22.4 c |
| Bolinha | 1608 b | 34.7 a |
| Pardo | 1448 c | 26.9 b |
| Roxinho | 1775 b | 32.4 a |
| Iapar 31 | 1556 b | 23.0 c |
| Average | 1526 | 23.1 |
| Coefficient of variation (%) | 14 | 16 |

* Means in a column with different letters are significantly different (P≤0.05) to Scott Knott test. Means represent triplicates for each replicate, N= 12.

** Calculated considering grain with 13% seed moisture.

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# PHASEOLIN CHARACTERIZATION OF CARIBBEAN COMMON BEAN GERMPLASM.

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**Introduction:** The introduction of beans to the Caribbean was postulated to have occurred from northern South America along the "Arawak arc" of Leeward Islands long before the Spanish Conquest. The Caribbean was also know to have been influenced by the pre-Colombian cultures of Central America. Therefore, the Caribbean was a transition zone between the two regions and was likely to have had a mix of bean germplasm even before the time of the colonies. Later, as a trading center and way station for the Europeans, the Caribbean likely received new crops and varieties from all over Latin America. This rich heritage makes the Caribbean a probable center of secondary diversity for beans. Caribbean nations and societies meanwhile have undergone rapid changes in the past fifty years which have led to the abandonment of agriculture in many places there. Where agriculture holds on, such as the interior of Hispaniola (Dominican Republic and Haiti) farm-size is small and land pressure is intense leading to environmental degradation and emigration from rural areas. Given all this it is interesting to document and preserve the genetic diversity of beans that are still left in the Caribbean. The University of Puerto Rico and IDIAF have collected traditional bean types at local markets or from farmers, most of which were classified as "Andean" because of their seed size and color class. The objective of this work was to characterize the phaseolin alleles found in the collected germplasm and to determine the genepool to which the traditional varieties belong.

**Methodology**: A total of 68 entries of common bean genotypes were genotyped for their phaseolin pattern. These included 43 traditional varieties (or selections thereof) from the Caribbean (23 from Puerto Rico collected by L. Durán and J. Beaver, 18 from Dominican Republic collected by J.C. Nín and 1 each from Haiti and Jamaica, from the University of Puerto Rico germplasm bank); 6 bred lines from CIAT, 15 advanced lines from the University of Puerto Rico; as well as 4 modern varieties from Colombia (ICA Palmar), Peru (Blanco Laran) and the United States (Montcalm, Redhawk). Total seed proteins were extracted from 0.10 g of peeled, finely-ground, oven-dried seed by a standard extraction technique used at the Genetic Resource Unit at CIAT. One microliter each of the protein extracts were separated with 6% separation / 12% stacking SDS-PAGE (polyacrylamide) mini-gels run for 50 minutes and stained with Coomasie Blue dye. The phaseolin pattern was compared to known standards provided by O. Toro of the Genetic Resource Unit of CIAT.

**Results and Discussion**: Only three phaseolin patterns were found among the Caribbean landraces: the most common being the "T" allele typical of many bush Andean beans. The "S" allele typical of Mesoamerican beans was the second most common allele while a third pattern, the "C" variant, was found for only a single traditional variety from Puerto Rico (Naranjito I). No additional diversity was observed at this locus for the germplasm studied and the "C" pattern is thought to be a hybrid of "S" and "T" phaseolins. In both the Dominican Republic and Puerto Rico the "T" phaseolin was more common than the "S" phaseolin, however, it was notable that

Puerto Rico had a greater percentage of "T" phaseolin than the Dominican Republic (Table 1). Perhaps this could be explained by Puerto Rico's closer proximity to South America along the suspected route of introduction of Andean germplasm, which typically has "T" phaseolin, through the Leeward island chain into the central Caribbean than the Dominican Republic. Conversely, the Mesoamerican type "S" phaseolin may be more common in the Dominican Republic than in Puerto Rico because of its proximity to Cuba, which may have acted as a bridge to Mexico and Central America, probable sources of Mesoamerican beans with "S" alleles. Indeed, two previous studies showed that Cuba had a mixture of "S", "Sb" and "T" phaseolins, with the Mesoamerican types predominating (Castinieras et al., 1994 Plant Genetic Resource Newsletter 99: 25-28; Lioi et al, 1990; Biologisches Zentralblatt 109: 231-233).

Another observation was that phaseolin allele has typically been associated with seed size in traditional varieties in Latin America with "S" types generally being small seeded and "T" types generally being large-seeded, however, in this study, the genotypes all of which were large seeded had both phaseolin patterns, suggesting that hybridization and recombination between phaseolin type and seed size had occurred in some of these Caribbean "Andean" genotypes. Therefore we may postulate a hybrid Mesoamerican-Andean origin for the Caribbean "Andean" seed classes despite their larger seed size. In the Caribbean, seed size of landraces including the red mottled (Dominican Pompadour), pink striped (Jamaican Miss Kelly, Puerto Rican Colorado de Pais) and red speckled (Haitian Pompadour) types are often intermediate between Mesoamerican and Andean types in seed size as well as growth habit, leaf size and other phenotypic traits. This provides evidence of further mixing of the genepools in this region of the Americas.

Meanwhile, phaseolin was not correlated with seed size in the advanced lines and modern varieties analyzed in this study. Similarily to what occurred in the Caribbean, perhaps breeding programs have encouraged the same sort of recombination between phaseolin types and seed size in their advanced lines. The advantages of hybridization between the genepools is evidenced in some of this germplasm which although they have the medium to large seed size of the Andean types, have a greater adaptation to tropical lowland conditions to which the Mesoamerican types are better suited. Hybrid progeny that fit local preferences and had these advantages were probably selected by the farmers in the Caribbean and as such, the germplasm of this region shows promise for breeding efforts that try to adapt Andean beans to warmer climates.

**Table 1. Phaseolin characterization of Caribbean germplasm**

| Germplasm | Total Genotypes | S phaseolin (%) | T phaseolin (%) | C phaseolin (%) |
|---|---|---|---|---|
| Dominican Republic | 18 | 27.7 | 72.2 | 0 |
| Puerto Rico | 23 | 4.3 | 91.3 | 4.3 |
| Modern Varieties / Breeding Lines | 10 | 40 | 60 | 0 |

# GREEN LEAVES OF COMMON BEANS IN HUMAN NUTRITION

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The species *Phaseolus vulgaris* is used worldwide mainly for two purposes: production of dry seeds and, using appropriate cultivars, production of green pods. But in some African countries it has a third purpose: green leaves for human nutrition. In order to study the potential use of common bean green leaves as a new vegetable for the Brazilian people, principally small farmers, six cultivars were used for sensorial test, in which they were compared to collard leaves, a very popular vegetable in Brazil.

For the defoliation of the six cultivars, one leaflet of each trifoliolate leaf was collected, which corresponds to approximately 33% defoliation. For the sensorial evaluation the bean leaves were used entirely, i.e., without nervure elimination, while the collard leaves had its central, thick nervure eliminated. Leaves of both species were reduced to small pieces, spiced with salt and garlic, and cooked slightly as is usually done to collard leaves. Afterward they were furnished to 70 testers (students of the Federal University of Viçosa), who evaluated them using the following hedonic scale: 9 = I liked extremely; 8 = I liked very much; 7 = I liked moderately; 6 = I liked slightly; 5 = indifferent; 4 = I disliked slightly; 3 = I disliked moderately; 2 = I disliked very much; and 1 = I disliked extremely.

The results were analyzed according to a balanced incomplete block design, in which $t = 7$ (total number of analyzed samples), $k = 3$ (number of samples tested by the tester in each session), $r = 3$ (number of times the tester evaluated each sample, i.e., the number of replications), $B = 7$ (number of blocks or session), and $\lambda = 1$ (number of times the samples were tested together at the same session).

There were significant differences ($P<0.01$) among the means of the tested leaves (Table 1). Diacol Calima was the only bean cultivar that differed significantly from the collard, and was classified between the hedonic terms “I liked slightly” and “indifferent”. All the other bean cultivars did nor differ significantly from the collard and were classified between the terms “I liked moderately” and “I liked slightly”. For collard the hedonic term was “I liked moderately”, practically the same used for the majority of the common bean cultivars.

Chemical analysis of the leaves (Table 2) showed a high percentage of fiber in beans, a consequence of nonremoval of their nervures. During the sensorial tests, many testers emphasized the fibrous texture of bean leaves, more perceptible in some cultivars. Those with a higher content of fiber were also considered somewhat bitter. Thus, with the elimination of the leaflet central nervure, bean green leaves may receive a better acceptance. In regard to the contents of carbohydrate, fat and ash, the differences between collard and common bean were small, but the former showed a higher content of protein.

Other studies demonstrated that 33% defoliation does not reduce the seed yield, and allows the production of 0.4 to 1.5 ton/ha of green leaves, depending on the cultivar and fertilization level. The most adequate time for bean defoliation is before flowering.

**Table 1 – Sensorial acceptability of the green leaves from six bean cultivars and collard**

| Product | Mean acceptability* |
|---|---|
| Collard | 6,9 a |
| Bean cv. Pérola | 6.4 ab |
| Bean cv. Ouro Negro | 6.3 ab |
| Bean cv. Manteigão Fosco | 6.0 ab |
| Bean line Vi. 13-8-3 | 5.9 ab |
| Bean cv. Ouro | 5.9 ab |
| Bean cv. Diacol Calima | 5.6 b |

* Means not sharing a letter are significantly different by Tukey's test (5%).

Table 2 – Chemical composition (%) of green leaves of bean and collard

| | Collard | Common bean* |
|---|---|---|
| Water | 8.9 | 4.6 |
| Protein | 28.1 | 19.3 |
| Carbohydrate | 36.2 | 32.2 |
| Fat | 3.3 | 2.8 |
| Ash | 10.5 | 11.0 |
| Fiber | 13.1 | 30.1 |

* Average of two cultivars (Pérola and Ouro Negro).

# Inheritance of Mechanical Damage of Dry Bean Seed

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## Introduction

Integrity of bean seed is important because beans are used for processing for canned goods or consumed by human. Combine harvest and subsequent handling of dry beans induce some mechanical damages (MD) of beans. Mechanically damaged beans with split, cracked, or loosened seed coats are unacceptable to canners, reducing their market value, and also result in poor seedling emergence, reduced seedling vigour or abnormal seedlings. Mechanical damage is affected by various factors such as seed moisture, cultivars, and physical stress from combine and cleaning, etc. Our preliminary studies suggested that seed moisture is critical, especially when seed moisture is lower than 12-13%, and varietal differences among navy beans (Gillard and Park 2002; Park and Rupert 2002). We report further findings on genetic control of mechanical damage and heritability estimates of navy beans.

## Materials and Methods:

**Recombinant inbred populations:** Two crosses between white bean lines susceptible and tolerant to MD are: Cross 1, Envoy/OAC Laser and Cross 2, OAC Speedvale/Vista. The $F_4$ plants of the two recombinant inbred populations (RIL) were grown in plant rows at Harrow in summer of 2000 and $F_{5:4}$ lines were grown at Harrow and St. Thomas in 2001. Plant type and maturity were observed and at maturity plants were pulled manually at moisture close to 18% to avoid any seed coat damage. They were shelled with an Almaco thresher (SPT model LPR) at 350 rpm with concave set wide open to avoid seed coat damage.

**Sample preparation and observation:** Moisture of seed samples was adjusted to 13% and two sub-samples of 100 seed per line were prepared for evaluation in a MD simulation device, pedal machine (manufactured by Agriculex Inc., Guelph). The samples were dropped with a magnetic vibration feeder one at a time through the pedal machine set at a speed of 8.5 mm/sec (equivalent to 395 rpm) to induce seed coat damage. The cracked samples were soaked in red food dye water solution for 30-40 seconds and then scored visually for damage as 0 for whole bean (no damage), 1 for hair line crack, 2 for clearly visible minor crack, 3 for large crack and 4 for split beans. The control samples (untreated seeds) were checked similarly but seed coat crack was very minimal. Mechanical damage index (MDI) was estimated by Sum[MD score x # of seed in each score]/total # of seed x 100.

**Data analysis:** Frequency distribution of MDI was plotted for each location and average MDI of the two locations was also plotted to examine genetic control. Growth type and maturity of the lines were correlated with MDI to examine any association. Heritability of MDI was estimated in standard unit by parent-offspring regression method (Frey 1957).

## Results and Discussion

**Frequency distribution of MDI:** The MDI distributions of RIL (n=132) of the cross 1 tested at both locations were continuous with a slight skewness toward the tolerance side with a range of 15 to 110 at Harrow and 5 to 80 at St. Thomas. This suggests that there were some environmental effects on expression of MD. However, a continuous distribution of MDI of the cross 1 was very similar to that of 2000 trial at Harrow. Frequency distribution (n=127) of MDI of the cross 2 , OAC Speedvale/Vista, was continuos with fairly normal curve at both locations and they were similar to that of 2000 trial at Harrow. MDI of resistant cv OAC Speedvale were 25 at Harrow and 32 at St. Thomas and MDI of susceptible cv Vista was 110 at Harrow and 93 at St. Thomas (Fig. 1). The expression of variation suggests the MD of both crosses was under quantitative genetic control by multiple minor genes and selection for tolerant lines should be possible as the variation was widely distributed with transgressive segregation..

**Correlation between MD and maturity:** Both RI populations were derived from crosses between determinate (type I) and indeterminate (type II) growth types, and between early and late maturing parental lines. Coincidentally, MD tolerant parental lines are early maturing and short determinate bush type, and two susceptible parental lines are late maturing and indeterminate upright vine type. Both of the characteristics were segregating in the populations. Correlation coefficients between MDI and growth type were not significantly different in both crosses. However, average MDI and maturity of the two locations were significantly negatively correlated with r = - 0.343 for cross 1 and r = -0. 308 for cross 2. The significant negative correlations suggest that late maturing lines tend to be more tolerant to MD than those with early maturity. However, this association needs to be further verified in future studies.

**Estimates of Heritability of MDI:** Average MDI of $F_{5:4}$ lines of cross 1 grown at the two locations in 2001 were regressed on $F_4$ lines and its mean heritability estimated by the parent-offspring method of the cross 1 was 0.55. Similar results were also obtained in cross 2 with mean heritability estimate of 0.65.and its scatter diagram is presented in Fig. 2. These results suggest that selection against MD should be moderately effective though it is controlled quantitatively and that breeding for mechanical damage resistance may be possible.

**Acknowledgments:**
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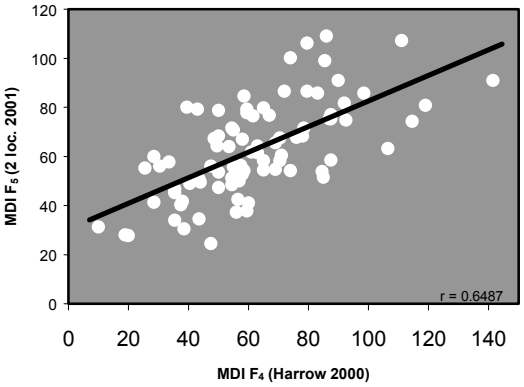


Fig 1. Frequency distribution of mechanical damage index (MDI) of Cross 2, OAC Speedvale/Vista, grown at Harrow and St. Thomas in 2001.

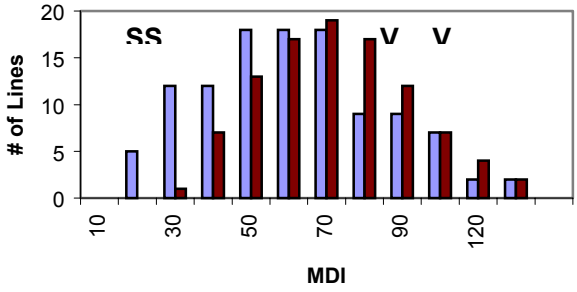


Fig. 2. Parent-offspring correlation of MDI of cross 2, OAC Speedvale/Vista, tested in 2000-2001 (light bar for Harrow, dark bar for St. Thomas).

# RATE AND SITES OF WATER UPTAKE BY BEAN SEED AS AFFECTED BY HIGH TEMPERATURE STRESS

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Among seed-quality factors cooking quality and appearance are the most important factors for consumers to make buying decision. The cooking quality of bean depends on cultivar, cultural practices, stress or no stress environment, handling and storage of bean during harvest and postharvest, and cooking method. The bean seed consists largely of dicotyledone, which contributes to 90-92% of the dry weight and it is responsible for the quality characteristics such as appearance, texture, flavor and nutrient bio-availability of the cooked bean. Seed anatomy influences the water uptake, hence determines the hydration properties of bean, a factor of seed quality. Seed coat accounts for 7-10% of a mature dry bean seed, and it is the entrance of water (Beninger et al., 1988). Important structures on the dorsal of the seed are the hilum, micropyle and raphe. The structures function as the primary sites of water entry into seeds (Korban et al., 1981). Water uptake may occur through the seed coat pores. This mechanism of water entry appears mainly a feature of white navy bean (Adams and Bedford, 1973; Wyatt, 1977).

The entry site and rate of water uptake were verified in two meso-american bean cultivars Pérola (carioca type) and BRS-Valente (black) produced with and without high temperature stress (HTS). To study the principal water entry site during soaking the seed structures were sealed with acrylic glue: 1. micropyle, 2. raphe, 3. hilum, 4. micropyle+raphe+hilum and 5. check (without glue). Prior to the treatment the seed was sieved to standardize the seed size and it was selected for seed without physical damage. Each sample of 20 seeds was weighted before and after applying the glue, maintaining the weight deviation as minimum as possible. After soaking of 4, 7 and 24 hours, the sample was dried and weighted. After weighing the sample it was verified whether the glue was still there and then discarded. Two series of experiments with three replications were conducted. The two experiments gave similar results, hence the two were combined for statistic analysis and the results are shown in Table 1 and Figure 1.

***Table 1***. Seed characteristics of Pérola and BRS-Valente from high temperature stress and non stress site.

| Cultivar | HTS | 100 seed weight (g) | Seed hum. (%) | Seed density (g/liter) | Germination rate (%) | Seed vigor (%) |
|---|---|---|---|---|---|---|
| Pérola | With | 27.5 | 10.68 | 815 | 93 | 61 |
| | Without | 29.1 | 10.65 | 823 | 90 | 61 |
| BRS-Valente | With | 22.3 | 11.31 | 888 | 99 | 83 |
| | Without | 25.4 | 10.59 | 844 | 91 | 62 |

The seed production of Pérola and BRS-Valente under HTS produced lower hundred seed weight but higher germination rate than without HTS (Table 1). Seed vigor of Pérola was low in both environment conditions (61%) and BRS-Valente showed higher seed vigor when produced under HTS than without stress (83 and 61%, respectively). Seed humidity was similar for both environments and cultivars. No differences was found in seed density for Pérola but BRS-Valente seed density was higher under HTS. There was significant higher water absorption rate between Pérola seed derived from HTS than seed from traditional bean growing region, during 4 and 7 hours of soaking and the rate was higher than BRS-Valente from HTS. Pérola with no HTS absorbed water only 46% of its weight after 4 hours of soaking, whereas seed with

high temperature imbibed more than 95% of its weight in the same period of soaking. BRS-Valente showed the same pattern of water uptake but in lower rate. After 7 hours of soaking BRS-Valente absorbed about 80% of its original seed weight and there was small difference in water uptake between seeds from HTS and no HTS. There was no difference in water absorption rate between cultivars and seed origin after 24 hours of soaking. During this period Pérola and BRS-Valente seeds have absorbed water at least the same amount as their original seed weight.

There was no significant difference among sites of water absorption in the two cultivars. The lowest water absorption occurred when all three sites (micropyle, raphe and hilum) were sealed covering the largest area by the sealant, hence impeding water entrance. This indicates that both cultivars absorbed water through the whole seed coat and the role of micropyle, raphe and hilum in water uptake is less important than reported by Korban et al., 1981, but similar as reported by Wyatt (1971) cited in Korban (1981). The two cultivars tested in this experiment were meso-american and absorbed water faster than those temperate zone cultivars reported by Korban et al. (1980). Understanding the difference in water uptake rate and site of bean races, seed storage condition and duration is important for seed quality selection criteria.

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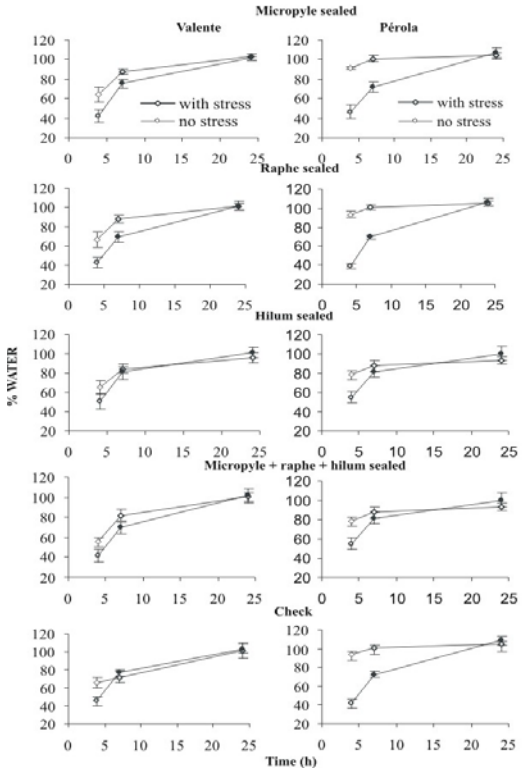


***Figure 1***. Rate and site of water absorption of Pérola and BRS-Valente seed, produced in region with and without high temperature stress.

# BEAN PRODUCTION IN SALINE SOIL IN RELATION TO POPULATION DENSITY

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## INTRODUCTION

Beans are considered salt sensitive, although ayocote (*Phaseolus coccineus* L.) has been classified as intermediate between a moderately sensitive legume species (Lauchli, 1984). Immediate effects of excess salinity include reduced plant growth, especially leaf area, burning of leaf margins, chlorosis and consequent low seed yield.

Management practices may minimize yield reductions under saline conditions (Meiri and Plaut, 1985). A salinity level that causes yield reduction per plant does not necessarily reduce yield on the field level to the same extent, because the field yield is the product of stand density and yield per plant. Conventional planting density in beans was established under nonsaline conditions (Escalante and Kohashi, 1995). A change in the stand density can be obtained by changing inter-row and/or intra-row spacing. A reduced intra-row spacing did not cause plant competition and therefore increased the yield per area (Keren *et al*., 1983). The aim of this study was evaluate the biomass and seed yield production of beans in relation to cultivar and population density under saline conditions.

## MATERIALS AND METHODS

The study was conducted in Montecillo, Mex. (19 °N, 98 °W and 2250 m of altitude) of dry climate (Bs) during the rainy season. Three cultivars of bush bean *Phaseolus vulgaris* L. Bayomex, Criollo of indeterminate type, and Canario 107 of determinate type and one cultivar of *P. coccineus* L. "Ayocote" of indeterminate type, were sown at a population density of 6.25 (180 x 25 cm) and 12.50 (40 x 25 cm) plants $m^{-2}$ on June 19, 2000 in a dry clay soil with pH 8 to 8.7, EC 7 to 14 dS $m^{-1}$ and a percentage of exchangeable sodium of 9.73 to 37.0. When the soil is moist from rain, the EC is reduced to 2 dS $m^{-1}$. The Ayocote and Criollo varieties are cultivated by farmers in nonsaline regions. The origin of Bayomex and Canario 107 is the INIFAP (Agricultural Research Center). All experiments were fertilized with 100-100-00 NPK. The experimental design was a split plot with four replicates. At physiological maturity (final harvest) we evaluated: total biomass, harvest index (seed yield / total biomass) and seed yield (8% humidity) and its components.

## RESULTS AND DISCUSSION

The cultivars showed differences in time to physiological maturity. It occurred at 80 and 100 days after sowing (das) para Canario and Bayomex, respectively; and at 120 das for Ayocote and Criollo. The total biomass, harvest index, and seed yield and its components showed significant differences among the cultivars, population densities and the interaction cultivar * population densities (Table 1). The highest values were shown for Bayomex and the lowest for Canario. The increase of population density increased biomass and seed yield. Bayomex at high density (12.5 plants $m^{-2}$) gave the highest total biomass (398.7 g $m^{-2}$), harvest index (0.61) and seed yield (243.3 g $m^{-2}$); Canario at low density (6.25 plants $m^{-2}$) gave the lowest values with 64 g $m^{-2}$,

0.45; and 28.6 g $m^{-2}$, respectively. The changes in the seed yield were related with changes in its components (Table 1).

These results suggest that it possible to increase the production of beans in this region by the management of population density, particularly reduced inter row spacing, under saline conditions.

Table 1. Biomass, harvest index and seed yield and its components for *Phaseolus vulgaris* L. and *P. coccineus* L., at physiological maturity, in relation to cultivar and population density. Montecillo Mex. MEXICO. Summer 2000.

| Treatments | | Dry Weight 100 Seeds (g) | Number ($m^{-2}$) of: | | | | Dry weight (g $m^{-2}$) of: | | | | Biomass | Harvest index |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| V | D | | Normal Seeds | Pods with seed | Nodes | Racemes | Pericarp | Stem | Normal seeds | Empty seeds | | |
| Bayomex | 6.25 | 26.2c | 432.9bc | 118.0b | 200.1c | 106c | 38.9bc | 53.3 | 115.0bc | 0.74 | 208.0bc | 0.53ab |
| | 12.50 | 27.3c | 877.6a | 211.4a | 462.3a | 222.5a | 77.6a | 76.9 | 243.3a | 0.92 | 398.7a | 0.61a |
| Ayocote | 6.25 | 53.2b | 225.2d | 102.3b | 150.0cd | 104.8c | 55.9b | 112.3 | 120.0bc | 0.60 | 288.8bc | 0.41bc |
| | 12.50 | 65.6a | 230.5d | 107.5b | 308.8b | 108.4c | 48.8b | 115.2 | 151.2b | 0.89 | 316.1b | 0.48bc |
| Canario | 6.25 | 19.4c | 157.7d | 41.3c | 89.6d | 46.1d | 15.4d | 18.5 | 28.6d | 1.45 | 64.0d | .45bcd |
| | 12.50 | 23.0c | 310.8bcd | 104.6b | 209.7bc | 100.4c | 37.3bc | 35.0 | 73.2cd | 2.59 | 148.1cd | 0.49bc |
| Criollo | 6.25 | 23.2c | 271.1cd | 97.9b | 173.4cd | 95.4c | 25.1cd | 75.4 | 62.6cd | 0.83 | 164.0cd | 0.36d |
| | 12.50 | 23.7c | 453.1b | 139.7b | 497.8a | 148.3b | 42.4bc | 97.4 | 109.5bc | 0.94 | 250.2bc | 0.43cd |
| V | | ***(5.0) | ***(107.7) | ***(30.2) | ***(65.1) | ***(18.8) | ***(12.7) | ***(23.4) | ***(37.2) | *(0.9) | ***(65.7) | ***(0.06) |
| D | | NS | ***(57.7) | ***(16.2) | ***(34.9) | ***(10.1) | ***(6.8) | **(12.5) | ***(19.9) | NS | ***(35.2) | ***(0.03) |
| V*D | | ***(8.4) | ***(179) | ***(50) | ***(108) | ***(31) | ***(21) | NS | ***(62) | NS | ***(109) | *(0.09) |

D= Population density (plants $m^{-2}$). In the column values with different letters are statistically different; *P≤0.05, ** P≤0.01,*** P≤0.001, NS=No significant; Tukey 0.05 inside parentheses. V = cultivar.

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# BIOMASS AND SEED YIELD OF BEANS IN SODIC-SALINE SOIL

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## INTRODUCTION

In Mexico the soil of arid and semi-arid regions, contain different types of salts. The soil of the dry lake bed of Texcoco, Mexico, contains high concentrations of sodium salts and they limit bean growth; in the majority of cases the production is low or absent, because highly beans are sensitive to salts (Subbarao and Johansen, 1994). Studies of beans in this region have shown that cv. Negro Precoz and Ayocote had the highest seed yield and were related to changes in the number of pods and racemes (Escalante *et al*., 1998). The objective of this study was seek bean cultivars that show the highest biomass and seed yield and determine their relation with yield components in this region.

## MATERIALS AND METHODS

The study was conducted in Montecillo, Mexico (19°N, 98°W and 2250 m of altitude) of dry climate (Bs) during the rainy season. Three cultivars of bush bean *Phaseolus vulgaris* L., Bayomex (BA) and Criollo (CR) of indeterminate type; Canario 107 of determinate type and one cultivar of *P. coccineus* L.; Ayocote (AY) of indeterminate type, were sown on June 19, 2000 at 12.50 (40*25 cm) plants $m^{-2}$ in a dry clay soil with a pH 8 to 8.7, EC of 7 to 14 dS $m^{-1}$ and the percentage of exchangeable sodium of 9.73 to 37.0. When the soil is moist from rain, the EC is reduced to 2 dS $m^{-1}$. The Criollo and Ayocote varieties are cultivate by farmers in nonsaline regions. The experimental design was a randomized block with 4 replicates. All experiments were fertilized with 100-100-00 N,P,K. 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

The beans cultivars showed different biomass and seed yield. Bayomex gave the highest biomass (398.7 g $m^{-2}$), seed yield (243.3 g $m^{-2}$) and harvest index (0.61) and the lowest was Canario 107, with 148.1 g $m^{-2}$, 73.2 g $m^{-2}$ and 0.41, respectively. The changes in the seed yield were related with changes in its components (Table 1). The seed yield of Ayocote and Criollo (122.2 and 109.5 g $m^{-2}$, respectively) was similar to that of summer 1997 in saline soil (Escalante *et al*., 1998). The differences in the growth and yield of the cultivars of bean under salinity conditions suggest an alternative for increasing the seed yield of beans in this region.

Table 1. Seed yield and yield components of *Phaseolus vulgaris* L. and *P. coccineus* L., to physiological maturity. Montecillo Mex. Summer 2000. Population density 12.5 plants $m^{-2}$ and 100-100-00 NPK $ha^{-1}$.

| **Treatments** | Dry Weight 100 Seeds **(g)** | **Number ($m^{-2}$) of:** | | | | **Dry weight (g $m^{-2}$) of:** | | | | Biomass | **Harvest index** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cultivar | | Normal Seeds | Pods with Normal seed | Nodes | Racemes | Pericarp | Stem | Normal seeds | Empty seeds | | |
| Bayomex | 27.3b | 877.6a | 211.4a | 462.3a | 222.5a | 77.6[a] | 76.9 | 243.3a | 0.92 | 398.7a | 0.61a |
| Ayocote | 65.6a | 230.5d | 107.5b | 308.8b | 108.4c | 48.8b | 115.2 | 122.2bc | 0.89 | 287.1b | 0.41cd |
| Canario | 23.0c | 310.8bcd | 104.6b | 209.7bc | 100.4c | 37.3bc | 35.0 | 73.2cd | 2.59 | 148.1cd | 0.49bc |
| Criollo | 23.7c | 453.1b | 139.7b | 497.8a | 148.3b | 42.4bc | 97.4 | 109.5bc | 0.94 | 250.2bc | 0.43cd |
| **PROB. F** | *****(5.0)** | *****(107.7)** | *****(30.2)** | *****(65.1)** | *****(18.8)** | *****(12.7)** | *****(23.4)** | *****(37.2)** | ***(0.9)** | *****(65.7)** | *****(0.06)** |

In the column values with different letters are statistically different. ***$P \leq 0.001$. Tukey 0.05 inside parentheses.

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# BIOMASS ALLOCATION AND YIELD IN DROUGHT-STRESSED COMMON BEAN UNDER DIFFERENTIAL RHIZOSPHERE CONFINEMENT

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**Introduction.** Variations in biomass allocation among shoot and roots are related to plant interaction with different environmental factors. In the Mexican highlands soil exploration volume by roots is restricted by shallow soils, while in the tropical lowlands no restriction is observed. Variations in soil volume available for root exploration allow in the understanding of root traits influence on drought tolerance mechanisms. A restriction in soil volume available for root exploration in plants reduces shoot and root growth, but the mechanism by which root confinement causes reduction of shoot growth has not been identified (Masoni *et al*., 1997). The objective of the present work was to assess the effect of differential rhizosphere confinement on yield and biomass accumulation in shoots and roots of drought-stressed common bean.

**Material and Methods.** Two experiments were established under glasshouse conditions. The first included irrigation and intermittent drought treatments and four varieties. Two of them from the Nueva Granada race, G4523 (drought-tolerant) and Rayado Rojo (drought-susceptible) with determinate growth habit. The other two from the Durango race, Pinto Villa (drought-tolerant) and Bayo Madero (drought-susceptible) exhibit indeterminate growth habit. For the second experiment only Pinto Villa and G4523 were included, and a terminal drought treatment was added to those tested in the first experiment. Experiments were established in January 28$^{th}$ and July 1$^{st}$ 2002, under a completely random factorial design with split plot and four replications. The experimental unit consisted of one-meter length PVC tube with an average stand of 12 plants. Tubes of three diameters were used (10, 15 and 20 cm) filled with 11, 25 and 36 kg of Haplic Feozem soil type, respectively. Tube segments were laid out in horizontal position, sealed in both extremes and a 6 cm longitudinal groove was made in the upper part. Three 0.5 cm in diameter holes were drilled at the bottom to drain the excess of water.

Under intermittent drought watering was suspended at 18 days after planting (DAP) in the first experiment, and at 29 DAP in the second. In a first drying cycle the plants were allowed to reach the point where most of the leaves exhibited the permanent wilting condition. Afterwards, in both experiments plants were re-watered during a week at the beginning of the reproductive period. Thereafter they were left to mature without further irrigation. Two soil drying cycles were applied before the plants reached maturity or premature death. Terminal drought consisted in the suspension of irrigation at 50 and 49 DAP, in the first and the second experiment, respectively. Biomass was sampled at preflowering (33 DAP) and at physiological maturity (81 DAP) in the first experiment, and in the second only at physiological maturity (84 DAP). In each experimental unit plants were dissected by organ (root, stem, lamina, petiole, pericarp and seed). Plant samples were dried in a forced air oven at 70 °C for 72 h to assess dry weight. Seed yield, days to flowering (DF) and to physiological maturity (DPM) were also recorded.

**Results and Discussion.** In both experiments the drought-tolerant cultivar Pinto Villa showed the highest grain yield, as compared to the other varieties, in all the moisture treatments and tube diameters. This was due to the earliness to flowering (Table 1) observed in Pinto Villa possibly related with the early photoassimilate translocation to the seeds. This cultivar also showed significant modifications in days to maturity in response to drought. This phenological adjustment contributed to lessen the impact of drought stress on yield of Pinto Villa. Biomass accumulation was similar among cultivars for the same tube diameter and moisture condition, while significant differences were observed for biomass production and allocation among moisture treatments and tube diameters. The drought effect was a non uniform

reduction of dry matter among the different plant organs, perhaps due to a differential priority for photoassimilate allocation. Differences in biomass distribution seems to be related to the early strenght of pod set and seed filling (Table 1).

Table 1. Means for days to flowering and to physiological maturity for two glasshouse experiments.

| | Days to Flowering | | | Days to Physiological Maturity | | |
|---|---|---|---|---|---|---|
| Cultivar | I[1] | ID | TD | I | ID | TD |
| | Experiment 1 | | | | | |
| Pinto Villa | 37 | 38 | NT[2] | 82 | 76 | NT |
| General Mean | 41 | 43 | NT | 86 | 75[3] | NT |
| Maximum | 45 | 48 | NT | 88 | 77 | NT |
| | Experiment 2 | | | | | |
| Pinto Villa | 36 | 35 | 36 | 82 | 80 | 73 |
| General Mean | 42 | 43 | 43 | 88 | 83 | 79 |
| Maximum | 48 | 51 | 50 | 94 | 87 | 86 |

[1]I= irrigated; ID= intermittent drought and TD= terminal drought. [2]Treatment not tested; [3]Low value due to premature death of determinate cultivars.

In general, biomass increased according to increments in tube diameter in each moisture condition in both experiments. Differential response was observed among cultivars for root biomass between experiments and moisture conditions. A significant and negative relationship was observed for seed yield and root biomass in G4523 and Pinto Villa (Figure 2). A strong competition for assimilates seems to be established among plant organs, mainly between seeds and roots, related to specie or plant survival, respectively. Earliness to flowering permitted to drought-tolerant cultivars to exert an early demand for assimilates and seems to be related to differences in biomass allocation among plant organs.

Variations in soil volume available to roots provoked a differential response on yield and biomass production and allocation for all the cultivars and moisture conditions. Capability for modifications in biomass production and allocation patterns, in combination with earliness to flowering and maturity adjustment favours the adaptation of dry bean under drought conditions. Intermittent drought caused the highest reductions in biomass accumulation and increased the proportion of dry weight allocated in roots.

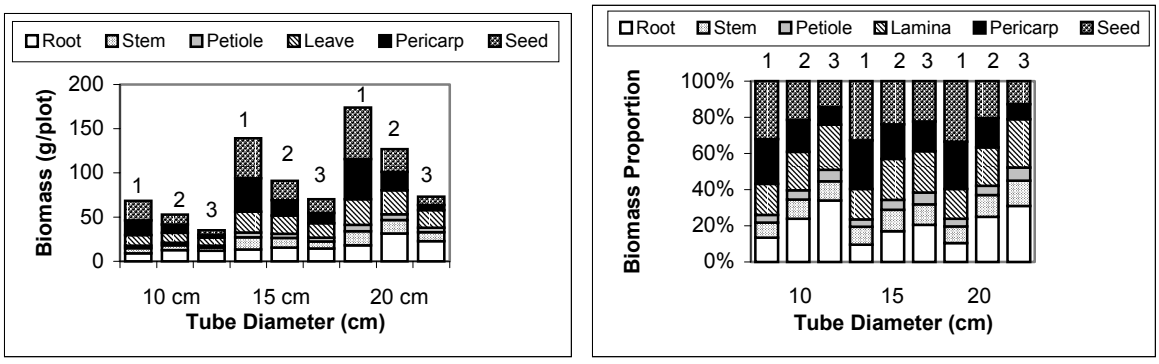


Figure 2. Biomass allocation in plant organs observed in dry bean cv. Pinto Villa grown in different tube diameters and moisture conditions (1= Irrigated, 2= Terminal drought and 3= Intermittent drought).

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# PLANT WATER STATUS IN DROUGHT-STRESSED COMMON BEAN UNDER DIFFERENTIAL RHIZOSPHERE CONFINEMENT

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**Introduction**. In Mexico dry bean (*Phaseolus vulgaris* L.) is mainly produced in regions that typically experience intermittent or terminal drought during the cropping season. Breeding for resistance to intermittent drought is the focus of improvement programs in the highlands and terminal drought in the lowland tropics. In addition to yield, other criteria for line selection under drought are needed. The importance of traits related to internal plant water status and turgidity maintenance and its effect on plant growth or survival is widely recognized (Karyudi and Fletcher, 2002). Inconsistent response on plant water status was observed possibly due to variations in root growth and its environmental interaction (Aiken and Smucker, 1996). The objective of the present work was to assess the effect of differential rhizosphere confinement on plant water status in genotypes of common bean grown under drought stress.

**Material and Methods.** An experiment was established under glasshouse conditions, in July 1st in 2002. Two drought-tolerant cultivars were included, G4523 (Determinate bush Type I) from the Nueva Granada race and Pinto Villa (Indeterminate prostate Type III) from the Durango race. The experiment was planted under a completely randomized factorial design with a split plot arrangement and four replications. The experimental unit consisted of one m length PVC tube. Tubes of three diameters were used (10, 15 and 20 cm) which were filled with 11, 25 and 36 kg of Haplic Feozem soil type, respectively. Tube segments were laid out in horizontal position, sealed in both extremes and a 6 cm groove was made in the upper part. Three 0.5 cm holes were drilled at the bottom to drain the excess water. Planting was made in order to allow plant emergence across the groove and a stand of 12 plants per unit was established.

Moisture treatments included irrigation (I) as a control, intermittent (ID) and terminal drought (TD). Under ID watering was suspended twice during the cycle. The first drying cycle was applied at 25 days after planting (DAP) and the second during the reproductive period (49 DAP). During the first drying period, soil moisture descended until the permanent wilting point was reached; thereafter, irrigation was applied. Two soil drying cycles were applied until the maturity or premature death of the plants, was observed. TD consisted of the definitive suspension of irrigation at 49 DAP. The control treatment was irrigated until physiological maturity in such a way that soil moisture was maintained above 80 %.

In each experimental unit, Relative Water Content (RWC) was determined at 06:00 and 14:00 h. Determinations were performed 12 days after drought treatment initiation (37 DAP) in a central leaflet of a young fully expanded leaf at basal and apical canopy positions. Leaflets were excised, covered with aluminum foil, stored in a portable freezer and taken to the laboratory. From each leaflet six discs, one cm in diameter, were punched and their fresh weight (FW) was determined. Thereafter, the discs were floated for four hours on de-mineralizated water at 25 °C under a photosynthetically active radiation of 15 µmol $m^{-2}$ $s^{-1}$, supplied by fluorescent light. After this hydration process, the leaf portions were taken out, excess water was eliminated and then weighted (turgid weight; TW). The leaf discs were then oven-dried at 70 °C for 72 h to determine the dry weight (DW). RWC was estimated according to Matin *et al*. (1989). The remaining portions of the leaflets were used to determine osmotic potential (OP) with a 5520 Wescor Vapro Osmometer. Before determinations, plant tissue were covered with aluminum foil and frozen in liquid nitrogen. Leaf water potential (LWP) was also recorded, at the same hours and plant positions, with the pressure chamber technique.

**Results and Discussion**. Inconsistent data were observed for all the evaluated traits, in most of the plant position and sampling hour combinations. Therefore, more experimental units, moisture treatments or contrasting cultivars were needed due to a dynamism registered on plant water status. Pinto Villa showed the highest values for RWC in all the evaluated conditions, perhaps due to its plant traits as the lower stomatal index in the adaxial surface, in comparison with susceptible cultivars (Aguirre *et al*., 1999). Under drought at 06:00 h, G4523 exhibited a consistent reduction in RWC according to a decrease in tube diameter (Figure 1). Results showed larger effects in G4523 due to the reduction in soil volume available to roots, in comparison to Pinto Villa. Similar results were observed at 14:00 h, in which a 60 % reduction in RWC was registered in G4523. That value seems to be lethal for G4523, since turgidity was not regained in some plants, after irrigation.

Values for OP showed similar trends from those observed for RWC. G4523 exhibited constant increments in OP according to the reduction in tube diameter at 6:00 and 14:00 h. A positive and significant relationship was observed for RWC and OP, for both varieties across moisture treatments and tube diameters. Regression values were higher at the predawn readings. Results suggest that increments in OP are needed to maintain high RWC values or that the reduction in cellular water content promoted increments in cell solutes (Hopkins, 1999), since reductions in osmotic potential seems to be related to a decrease in RWC. Variable responses were observed for LWP in both cultivars. Pinto Villa showed the lowest values for LWP at both plant positions and reading hours. This trait in combination with high values for RWC and a lower decrease in OP suggest that this cultivar maintained an adequate internal water status.

**Conclusions**. Variations in rhizosphere confinement caused differential responses for plant water status among dry bean cultivars. A combination of shoot traits allows drought tolerant cultivars to maintain an adequate internal water status as reductions of osmotic and leaf water potential occur. Differences in plant water status were observed between basal and apical plant positions, across tube diameters and moisture treatments.

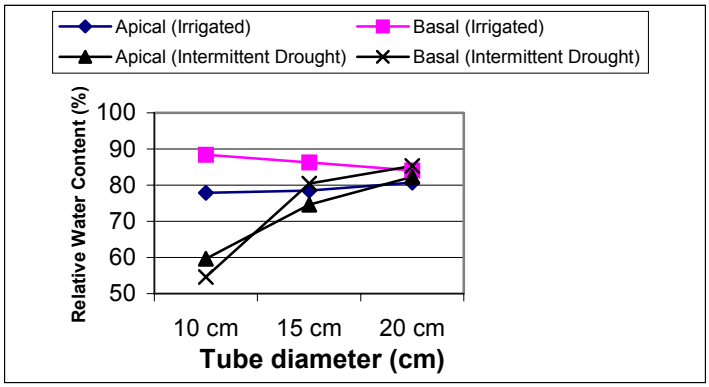


Figure 1. Relative water content observed for G4523 at 06:00 h in different tube diameters and moisture conditions.

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# BREEDING FOR DROUGHT RESISTANCE IN DRY BEAN IN BULGARIA

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## Introduction

At least 40 % of dry bean (*Phaseolus vulgaris* L.) annual production in big region Dobrich is limited by moisture stress (drought; Petrova, Mitranov,Todorov, 1986). Dry bean in Bulgaria usually is not irrigated and in 2001 the lowest yield was 5,2 kg/ha. In favorable years, like 1979 the maximum of yield was 33,7 kg/ha. (table1).The objective of this study was to confirm the drought resistance of some crosses and to select the best genotypes.

**Table 1. Mean yields from varietal trials with dry bean during 1978-2002 .**

| Year groups by dry climate | Kg/ha | Individual year |
|---|---|---|
| I. Favorable years - over 25 kg/ha | 33,7-29,7-26,5-31,8-26,8-27,3-28,3-26,0 | 1979,1982,1983,1986,1987, 1989,1991,1998. |
| II. Normal years -20-25kg/ha | 22,3-21,1-22,3-21,4-21,5-23,2-21,8-24,2 | 1988,1992,1993,1995,1997, 1999. |
| III. Dry years - 15-20 kg/ha | 17,5-15,9-18,6 | 1984,1865,1994 |
| IV. Very dry years -10-15 kg/ha | 13,9-14,0-11,0 | 1990,1996,2002. |
| V. Extremely dry years-less than 10 kg/ha | 7,2-5,2 | 2000,2001 |

## Material and Methods

A comparative varietal trial was carried in the field of DAI-General Toshevo during 1999-2001. The year 2001was chosen as a standard of drought for the last 23 years (table 1). This is the year with minimum yield of 5,2 kg/ha. The maximum productivity was 33,7 kg/ha in 1979. Relatively dry years with productivity lower than 16,0 kg/ha were 1985, 1990, 1996 and 2002. The year 2001 had the lowest rainfall during the vegetation period (table 2).

**Table 2. Precipitation in the vegetation period of dry bean (mm)**

| Years/ months | April mm | May mm | June mm | July mm | Total mm | Winter Store mm |
|---|---|---|---|---|---|---|
| 1999 | 40,9 | 33,2 | 122,2 | 35,3 | 246 | 255,5 |
| 2000 | 45,8 | 42,1 | 40,4 | 6,7 | 153 | 210,6 |
| 2001 | 18,4 | 28,9 | 35,0 | 4,8 | 106 | 145,2 |

For characters showing significant differences an injury index (Blum,1988) was calculated:

% of injury (depression) = [(C- T)/C]*100; C= Control, T=Treatment Means

## Discussion

The first step of this study was to determine the negative influence of water stress on the field yields in dry bean.(*P. vulgaris* L.). In this case it is determined by depression coefficient, that express the degree of injury of all reproductive system. The depression coefficient for yields in kg/ha is ranged 39,3-71,0 % (table 3).

**Table 3. Influence of drought on yields of some indeterminate crosses in dry bean**.

| **Crosses** | **Kg/ha 2001** | **Kg/ha 1999** | **±D Kg/ha** | **Depression, %** |
|---|---|---|---|---|
| Vulkan x Astor | 8,9 | 24,2 | 15,2 | 62,8 |
| Astor x Bianco INIA | 7,9 | 14,9 | 7,0 | 47,0 |
| Avans x Chapi 11 | 7,4 | 12,2 | 4,8 | 39,3 |
| Gambit x Vulkan | 6,4 | 17,3 | 10,9 | 63,0 |
| Ipanema x Rousse 13 | 8,0 | 22,5 | 14,5 | 64,4 |
| Turnovo 13 x Astor | 5,1 | 16,4 | 11,3 | 68,9 |
| Slavena x Dobrodja 7 | 7,2 | 22,3 | 15,0 | 67,3 |
| Rico 23 x Prima | 8,6 | 25,7 | 17,1 | 66,5 |
| Gracia x Arestuben | 9,4 | 17,0 | 7,6 | 44,7 |
| P.Tetovac x Vulkan | 7,6 | 22,0 | 14,4 | 65,4 |
| A56 x Gracia | 5,8 | 19,2 | 13,4 | 70,2 |
| Vulkan x Lacer | 8,3 | 23,2 | 14,9 | 64,2 |
| Burgas x Ruen | 5,8 | 20,0 | 14,2 | 71,0 |
| Pindac x Vulkan | 8,3 | 26,2 | 17,8 | 67,7 |
| Gracia x Debute | 8,1 | 25,8 | 17,7 | 68,6 |
| Avans x Zagor | 9,6 | 18,7 | 9,2 | 48,9 |
| Tetovac x Vulkan | 8,1 | 16,3 | 8,1 | 50,3 |
| Rio Grande x Avans | 8,3 | 23,6 | 15,3 | 64,8 |
| *Average* | - | - | - | **65.2** |

Good resistance for water stress was found in the genotypes Astor x Blanco INIA, Gracia x Arestuben and Avans x Chapi 11. Lower resistance was found in Bourgas x Ruen (71,0 %). Average resistance of all genotypes was 65,21 %.

In the future we will study the influence of water stress on number of pods, number of seeds/plant and 1000 seeds weight.

**Conclusions**: The severe drought in 2001 provided the possibility for effective selection of resistant lines in a natural challenging background.

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# POSSIBILITIES FOR SELECTION OF GARDEN BEAN (PHASEOLUS VULGARIS L.) GENOTYPES TOLERANT TO HIGH TEMPERATURE. I. CHANGES IN CHLOROPHYLL FLUORESCENCE PARAMETERS

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## Introduction

Breeding of new varieties with higher temperature tolerance is one of the most promising ways for an increase of the bean productivity and make it cultivation more sustainable (Palomares et al., 1992; Genchev, 1995; Kleiner and Frett, 1996).

Under stress conditions, the photosynthetic apparatus (PSA) is one of the most sensitive components. The high temperature (HT) strongly influences parameters of the PS2 fluorescence emissions, therefore they may successfully used as criteria of assessment to stress tolerance (Goltsev et al., 1994; Briantais et al., 1996).

In the Institute of Horticulture and Canned Foods, Plovdiv is under way a breeding program in garden bean for searching of initial material tolerant to high temperature. Our preliminary investigations indicated that the changes in chlorophyll fluorescence parameters could be used as reliable criteria for temperature stress (Petkova et al., 2002).

Within the frames of this program the objective of our investigation was to establish the effect of high temperature on the PSA temperature stability of two garden bean accessions by changes in chlorophyll fluorescence parameters.

## Materials and methods

During 2000 - 2001 two pot experiments took place. Two accessions of garden bean - cv. *Oreol* (bred in the Maritsa Vegetable Crops Research Institute, Plovdiv) and line *87201231* (from the gene pool of the Institute of Plant Genetic Resources, Sadovo), preliminary characterized as tolerant to high temperature were used. The plants were grown in a 5 l pots on soil-peat substrate in glasshouse at 25/18$^{o}$C day/night±1$^{o}$C.

The PSA temperature stability of investigated accessions were evaluated by changes in the chlorophyll fluorescence parameters $F_o$, $F_m$ and $F_v$ and their ratios at high temperature (35 and 40$^{o}$C) compared with controls (22$^{o}$C) measured by Plant Efficiency Analyzer MK2 (PEA) (Hansatech, UK). Whole plants were treated during the anthesis in thermostate, with duration of 90 min. Fluorescence parameters were registered in 30 replications, on intact, dark-adapted (for 30 min) fully developed leaves, illuminated with actinic light (>650 nm) with photon flux 1500 μmol.m$^{-2}$.sec$^{-1}$ for 15 s.The data were statistically processed by the common MS Excel software.

## Results

The measured values of $F_0$, $F_v$ and $F_m$ and their ratios were compared in the control and HT experienced plants (Table 1). It is established that initial fluorescence level ($F_0$) increases under temperature stress (Schreiber and Berry, 1987; Briantais et al., 1996) and describe a loss of the excitation energy during its transfer from the pigment bed to RC of PS2 (Yordanov et al., 1997). Investigated accessions differed significantly by $F_0$ only in 40$^{o}$C treatment.

Under mild stress the $F_v/F_0$ ratio, which is considered as an indicator for the electron transport chain state and effectiveness, remained practically unchanged in cv. *Oreol,* while in line *87201231* it decreased by 2,39% compared to the control. However, under a 40$^{0}$C temperature stress, the ratio $F_v/F_0$ decreased by 13,15% and 8.1% in cv.*Oreol* and line *87201231,* respectively to the controls.The ratio $F_v/F_m$, characterizing the potential effectiveness of PS2 have not been influenced under applied HT and its

values maintained in normal limits (Bolhar-Nordenkampf et al., 1989).The core complex stability, expressed by the ratio $F_0$(control)/$F_0$(HT), showed the similar tendency.

Table 1. Chlorophyll fluorescence parameters in bean plants treated with high temperature ($35^oC$ and $40^oC$ for 90 min). Values represent the means of two experiments ± SD, n = 30.

| Variants | Fo | Fv/Fo | Fv/Fm |
|---|---|---|---|
| cv. *OREOL* | | | |
| Control | 0.0420 ± 0.004 | 5.788 ± 0.654 | 0.850 ± 0.013 |
| $35^o$ C | 0.0410 ± 0.001 | 5.842 ± 0.119 | 0.854 ± 0.025 |
| Control | 0.0407 ± 0.002 | 6.052 ± 0.200 | 0.852 ± 0.013 |
| $40^o$ C | 0.0458 ± 0.003 | 5.256 ± 0.267 | 0.840 ± 0.020 |
| Line *87201231* | | | |
| Control | 0.0405 ± 0.002 | 6.068 ± 0.314 | 0.858 ± 0.006 |
| $35^o$ C | 0.0415 ± 0.002 | 5.923 ± 0.140 | 0.854 ± 0.004 |
| Control | 0.0402 ± 0.001 | 6.151 ± 0.177 | 0.859 ± 0.003 |
| $40^o$ C | 0.0412 ± 0.001 | 5.653 ± 0.158 | 0.848 ± 0.009 |

## Conclusions

The results obtained showed that:

- The tested bean accessions cv.*Oreo*l and line *87201231* showed a tolerance to $35^oC$ (for 90 min) temperature.
- HT affected the chlorophyll fluorescence parameters only just at $40^oC$ for 90 min which could be used as screen-temperature in breeding for heat-tolerance of garden bean.
- In both bean accessions the HT effect was best expressed by the ratio $F_v/F_0$ (which supported the results of Yordanov et al.,1997 obtained for maize and sunflower plants).

**Abbreviations:** HT-high temperature; Fo-initial, Fv-variable, Fm-maximal chlorophyll fluorescence; PS - photosystem; PSA - photosynthetic apparatus; RC - reaction center(s).

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# POSSIBILITIES FOR SELECTION OF GARDEN BEAN (PHASEOLUS VULGARIS L.) GENOTYPES, TOLERANT TO HIGH TEMPERATURE. II VARIATION OF POLLEN VIABILITY

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## Introduction

The high summer temperatures are one of the important abiotic factors having negative effect on the processes of pollination and fertilization as well as on the garden bean yield. An object of research studies for specialists from different spheres are the disturbances in physiological (Petkova et al., 2001; Konsens et al., 1991) cytological (Nikolova and Poryazov, 1995), molecular (Keeler et al., 1996; Keeler and Kitto, 1998) and other processes in this crop under the influence of the temperature stress.

Preliminary studies, carried out in the Maritsa Vegetable Crops Research Institute, Plovdiv showed that the pollen viability changes can be used as a criterion for breeding of tolerant genotypes, in treatment of garden bean plants with temperatures 30, 35 and 40$^{o}$C.

The aim of the present study was to investigate the possibilities for selection of forms resistant to high temperatures, on the basis of the variation between pollen viability of the different genotypes under temperature stress.

## Material and methods

Plants during the anthesis from two accessions, which have showed some tolerance to temperature stress – cultivar Oreol and accession 87201231 were treated with temperatures 35$^{o}$ and 40$^{o}$C for 90 minutes in the period 2000 – 2001. The influence of these temperatures on the pollen germination ($\bar{x}$ ,%) and on pollen tube elongation ($\bar{l}$ ,μ) in the flowers and flower buds was studied by the humid chamber method (Nikolova and Poryazov, 1995) and through preparation of slides stained with 4% acetocarmine. The pollen viability in non-treated plants was analyzed .for comparison. The variation between studied plants by both traits, determining the pollen viability was established by coefficients of variation (CV).

## Results and discussion

In our investigation we established that the treatment of the bean plant with temperature 35$^{o}$C for 90 min stimulated slightly the pollen germination and pollen tube elongation in the flowers and flower buds of plants from both studied accessions (Table 1). Low depression, in these temperature parameters, was established only in germination of pollen grains in the buds of cv. Oreol - $\bar{x}$ - 24.7%, compared to the control plants - $\bar{x}$ - 35.2%. This temperature caused a decrease in heterogeneity of studied plant population to a certain extend. As a result of this, the variation of both traits in the treated plants was poorer in comparison to the controls.

The increase of the temperature with 5$^{o}$C (40$^{o}$C) at the same treatment duration (90 min) had negative influence on the pollen viability in flowers and buds of plants, from cv. Oreol and accession 87201231. The negative effect of this temperature was determined also from the

significant percentage of flowers and flower buds in which all pollen grains loose viability. The pollen of 48.7% of studied flowers from cv. Oreol and 17.5% - of flowers from accession 87201231 was completely non-vital. Pollen grains did not germinate also in 80.6% and 55.4%, respectively, from studied flower buds of these two accessions. In the control plants there was no pollen germination only in 3.8 and 8.3 per cent from the flowers and in 28.1 and 37.1 per cent from the buds of the studied accessions. This increase in flower and bud quantity, in which the pollen grains did not germinate under influence of temperature stress, reduced the possibilities for fertilization (autogamous and cleistogamous) and the pod set formation. Simultaneously with the negative effect on the pollen viability, the temperature stress at 40°C disclosed the available diversity between the studied plants on the basis of their pollen reaction to this abiotic factor. As a result of this the coefficients of variation in pollen germination were very high (CV varied from 63.2 to 186.8). These strongly expressed differences give a reason to prefer temperature 40°C for treatment of tolerant garden bean accessions, for selection of genotypes tolerant to temperature stress.

**Table 1. Pollen viability (germination and length of the pollen tubes in flowers and flower buds) in the control and in treated with high temperature plants**

| Treatments | Cultivar Oreol | | | | Accession 87201231 | | | |
|---|---|---|---|---|---|---|---|---|
| | in flowers | | in flower buds | | in flowers | | in flower buds | |
| | $\bar{x}$ (%) | $\bar{l}$ (μ) | $\bar{x}$ (%) | $\bar{l}$ (μ) | $\bar{x}$ (%) | $\bar{l}$ (μ) | $\bar{x}$ (%) | $\bar{l}$ (μ) |
| Control plants | 55.3 | 169.3 | 35.2 | 102.7 | 42.1 | 155.7 | 26.1 | 95.5 |
| Plants treated t° 35°C | 58.7 | 177.9 | 24.7 | 110.1 | 50.3 | 190.1 | 29.5 | 117.9 |
| Control plants | 41.9 | 103.3 | 22.0 | 71.2 | 38.3 | 127.7 | 20.5 | 75.3 |
| Plants treated t° 40°C | 25.2 | 54.5 | 8.3 | 20.3 | 26.5 | 101.1 | 12.9 | 54.2 |

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# Common Bean Root Response to Abscisic Acid Treatment

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## Introduction

Identifying and understanding the mechanisms of drought tolerance in common bean (*Phaseolus vulgaris* L.) are major goals of plant physiologists and breeders. Research indicates that rooting depth is one factor affecting drought tolerance in common bean (White et al., 1990). Root architecture may be important for mining minerals, nutrients, and water from the soil (Lynch and van Beem, 1993), and increased abscisic acid (ABA) concentrations have been found in xylem sap from droughted plants (Fort et al., 1997). The objective of this study was to investigate the effects of ABA on root length in common bean.

## Materials and Methods

Two experiments were conducted in an environmentally controlled growth chamber -- a control treatment with plants grown in half-strength Hoagland's nutrient solution and an ABA treatment with plants grown in half-strength Hoagland's nutrient solution + $10^{-6}$M ABA [*cis-trans*, ± ABA, Sigma]. Both studies utilized a split plot design with days after transplant (14, 21, and 28 DAT) as the main plot, genotype as the subplot, and four replications. Eight common bean genotypes were grown: BAT 477 [nodulating (nod)], PR9603-22, DOR 364 [non-nodulating (nn)] XAN 176, BAT 477 (nn), SEA5, 8-42-M-2, and DOR 364 (nod). The WinRhizo root imaging program (WinRhizo, Regent Instruments Inc.) was utilized to determine root length according to 10 diameter classes ranging from 0.1 to >4.5 mm.

## Results and Discussion

Root length was significantly higher in the ABA than in the control treatment for all root classes except root class 9 at 21 DAT (Table 1). At 28 DAT, ABA increased total root length (TRL) and growth in root classes 2, 3, 7, and 9 (Table 1). At 21 DAT, the genotype XAN 176 had a significantly higher (P # 0.05) TRL than all genotypes in the control treatment except SEA5 and the genotype SEA5 had a significantly higher (P # 0.10) TRL than all other genotypes in the ABA treatment except PR9603-22, DOR 364 (nn), and 8-42-M-2 (Table 2). At 28 DAT in the ABA treatment, the genotype 8-42-M-2 had a significantly higher (P # 0.01) TRL than all other genotypes except DOR 364 (nn) (Table 2).

ABA increased TRL at 21 and 28 DAT and increased the production of finer roots with greater than 97% of the roots occurring in root classes 1 and 2 (Table 1). Such an occurrence during a moisture deficit would increase the root absorptive surface area, thereby permitting the plant to obtain more soil moisture. ABA induced significantly greater root length in the drought susceptible check (8-42-M-2) than in the drought resistant check (BAT 477). Additional research is needed in this important area.

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**Table 1**. Total root length (TRL) and root length (cm) of eight common bean genotypes germinated in a germination chamber for 4 d at $25^0$C and transplanted to an environmentally controlled growth chamber at $23/20^0$C day/night temperatures and a 15 h photoperiod. Grown under control conditions in a half-strength Hoagland's solution or in $10^{-6}$ M ABA solution. Roots were sampled at 14, 21, and 28 days after transplanting (DAT) and divided into 10 classes based upon root diameter (0-0.5, 0.51-1.0, 1.01-1.5, 1.51-2.0, 2.01-2.5, 2.51-3.0, 3.01-3.5, 3.51-4.0, 4.01-4.5, and >4.5 mm). n = 32.

| | TRL | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | C9 | C10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **14 DAT** | | | | | | | | | | |
| ABA | 2017 ns | 1437 ns | 533 ns | 35 ns | 6 ns | 1 ns | 0.3 ns | 0.1 ns | 0.09** | 0.05 ns | 3.6 ns |
| Control | 1899 | 1402 | 453 | 32 | 5 | 1 | 0.2 | 0.1 | 0.05 | 0.04 | 3.5 |
| | **21 DAT** | | | | | | | | | | |
| ABA | 3999** | 2880* | 1039*** | 58* | 10** | 2.4* | 0.7* | 0.25* | $0.12^+$ | 0.09 ns | 7.8** |
| Control | 2511 | 1884 | 550 | 40 | 6 | 1.4 | 0.4 | 0.10 | 0.05 | 0.07 | 4.2 |
| | **28 DAT** | | | | | | | | | | |
| ABA | $5776^+$ | 4336 ns | 1313* | $90^+$ | 18 ns | 5 ns | 2 ns | $0.66^+$ | 0.3 ns | 0.10* | 11 ns |
| Control | 3760 | 2835 | 809 | 59 | 12 | 4 | 1 | 0.37 | 0.1 | 0.03 | 8 |

ns Indicates no significant difference among means within a column.
***, **, *, + Indicates significant difference among means within a column at P # 0.001, 0.01, 0.05, and 0.10, respectively, according to DMRT.

**Table 2**. Genotypic differences in TRL (m) at 14, 21, and 28 days after transplanting (DAT) for eight common bean (*Phaseolus vulgaris* L.) genotypes germinated in a germination chamber for 4 d at $25^0$C and transplanted to an environmentally controlled growth chamber at $23/20^0$C day/night temperatures and a 15 h photoperiod. Plants grown under control conditions in a half-strength Hoagland's solution or in half-strength Hoagland's solution plus $10^{-6}$ M ABA solution. N = 4.

| | 14 DAT | | 21 DAT | | 28 DAT | |
|---|---|---|---|---|---|---|
| Genotypes | Control | ABA | Control | ABA | Control | ABA |
| BAT 477 (nod) | 18 ns§ | 15 ns | 20.6 b* | 31.4 c+ | 34 ns | 39.3 d** |
| PR9603-22 | 18 | 17 | 25.1 b | 48.6 ab | 33 | 43.0 cd |
| DOR 364 (nn) | 15 | 20 | 22.2 b | 41.0 abc | 32 | 76.0 ab |
| XAN 176 | 24 | 19 | 37.4 a | 33.6 bc | 43 | 60.4 bcd |
| BAT 477 (nn) | 18 | 21 | 22.4 b | 32.6 c | 45 | 49.4 cd |
| SEA5 | 21 | 23 | 31.6 ab | 51.2 a | 31 | 64.6 bc |
| 8-42-M-2 | 22 | 27 | 21.9 b | 48.8 ab | 26 | 88.2 a |
| DOR 364 (nod) | 17 | 19 | 19.7 b | 32.8 c | 56 | 41.3 cd |
| Mean | 19 | 2.1 | 25.1 | 40.0 | 38 | 57.8 |

ns Indicates no significant difference among means within a column.
**, *, + Indicates significant difference among means within a column at P # 0.01, 0.05, and 0.10, respectively, according to DMRT.

# INDIRECT SCREENING TECHNIQUES FOR DROUGHT RESISTANCE IN DRY BEANS

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**Introduction:** The identification of drought resistance in dry beans (*Phaseolus vulgaris*) requires large-scale field testing over many locations and years. Early generation selection criteria could save valuable resources and accelerate the selection process. In dry beans, the root system contributes more to yield than the shoot (White and Castillo, 1992). Since the association of root data to field data is laborious, a new technique utilizing a pouch system allows for rapid evaluation of root length of numerous genotypes and is relatively inexpensive (Yabba and Foster, 1997). Another screening technique for drought resistance, involving RAPD markers, has been successful in Durango race populations (Schneider et al., 1997). Neither technique has been tested on current MSU drought resistant bean germplasm.

Drought resistant black bean line, B98311, was crossed to the tropical breeding line TLP 19 to create the recombinant inbred line (RIL) population, L88 of 81 individuals. Yield under stress (Yd) and non-stress conditions (Yp) for population L88 was measured in Zamorano, Honduras and Veracruz, Mexico (Frahm et al., 2003). Sufficient yield data was assembled to test the two indirect selection techniques. This study was conducted to evaluate indirect screening techniques for drought resistance using the pouch method to assess root characteristics and the potential of using previously identified RAPD markers associated to drought resistance (Schneider et al., 1997).

**Materials and Methods.** Twenty to thirty seeds per (81) genotype in the $F_{3:4}$ generation were germinated for the pouch study. After four days, seedlings from each genotype were transferred to pouches consisting of a 25.4 cm x 35.6 cm clear plastic bag with 21.0 cm x 37.6 cm germination paper inside. The pouches were placed into a growth chamber with a 23/20˚C day/night temperature and a 15 hr photoperiod. Each sample (pouch) received 360-400 ml of Hoagland's solution throughout the 14-day growth period. The root was excised from the shoot and was put into a 0.1 g/L staining solution of methyl violet. After a 24-hour period of staining, the root samples were scanned into a digital image using WinRhizo™ 4.10b (Régent Instruments Inc., 2000). At 14 days after transplanting, the average root density was 0.03 mm root per $mm^2$ of surface area. A resolution of 300 dpi and the automatic threshold for WinRhizo™ were used.

RAPD markers associated to drought resistance (Schneider et al., 1997) were screened across the 81 RILs of population L88. PROC GLM and CORR analyses were used to calculate LSD, CV and Pearson correlation coefficients (SAS Institute, 1999).

**Results and Discussion:** B98311, the drought resistant parent, was greater in length than TLP 19 in every root category measured (Table 1). This evidence coincides with the field observations that B98311 has a vigorous deep-penetrating taproot, while TLP 19 has shown shallow rooting characteristics (Liao et al., 2001; Rubio et al., 2003). Correlations between root length and yield were expected among the RILs as B98311 and TLP 19 varied so drastically in root length traits. The most drought resistant RIL, L88-63, and the most drought susceptible RIL, L88-18, however, showed no significant difference in any root length trait. A stress treatment may need to be included in the pouch method protocol since no root characteristics correlated to yield under drought stress.

The only root categories that L88-63 differed from L88-18 were the larger classes I and J and the number of root tips (Table 1). The I class was the only root class to be significantly correlated to yield under non-stress conditions, r =0.17** (Table 2). Taproot size in bean seedlings can predict the amount of water absorbed during later growth stages. Root tips were significantly correlated to yield under non-stress conditions and geometric mean (GM) in the experiment at Veracruz, r =0.21*** and r =0.27* respectively (Table 2). Root tips are associated with the amount of fine roots. Therefore in non-stress conditions, yield will increase with more root tips. Unfortunately, no root characteristic correlated with yield under drought stress.

Marker F06.970 correlated to yield at Veracruz under both stress (Yd; r =0.28*) and non-stress (Yp; r =0.32**) conditions and the strongest correlation (r =0.52****) was observed with GM. Even though this marker did not correlate to yield in Honduras, additional field data from varying locations are needed to validate the potential of this marker for indirect selection for GM. F06.970 also correlated to only one root characteristic, root tips (r =0.27*). Root tips can "sense" fluctuations of abscisic acid and initiate root elongation in *P. coccineus* (Fleming et al., 1991). Further research needs to be conducted to test these findings and confirm the potential of each technique for the indirect selection for drought resistance in dry beans.

**Table 1.** Total root length (TRL), length according to diameter (A-J) and root tip numbers of the most drought resistant (L88-63) and drought susceptible (L88-18) RILs and their parents.

| Line | TRL | A† | B | C | D | E | F | G | H | I | J | Root Tips |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | centimeters | | | | | | | | | | | Number |
| L88-63 | 2054 | 1645 | 366 | 32 | 5.75 | 1.40 | 0.34 | 0.22 | 0.28 | 0.10 | 2.95 | 1984 |
| L88-18 | 2056 | 1562 | 441 | 42 | 5.93 | 1.68 | 0.48 | 0.24 | 0.26 | 0.08 | 2.41 | 1653 |
| B98311 | 2295 | 1660 | 547 | 62 | 14.74 | 4.30 | 1.99 | 0.77 | 0.32 | 0.19 | 3.56 | 2035 |
| TLP 19 | 1618 | 1174 | 391 | 40 | 8.06 | 1.72 | 0.71 | 0.23 | 0.16 | 0.06 | 2.52 | 1643 |
| Mean | 1959 | 1415 | 475 | 51 | 10.15 | 2.90 | 1.00 | 0.42 | 0.26 | 0.16 | 3.07 | 1937 |
| LSD (0.05) | 688 | 466 | 198 | 39 | 12.62 | 6.15 | 2.29 | 0.88 | 0.57 | 0.31 | 1.66 | 627 |
| CV % | 22 | 20 | 26 | 47 | 77 | 131 | 142 | 131 | 134 | 120 | 34 | 20 |

† Root diameter classes A, B, C, D, E, F, G, H, I, J are 0-0.5, 0.5-1.0, 1.0-1.5, 1.5-2.0, 2.0-2.5, 2.5-3.0, 3.0-3.5, 3.5-4.0, 4.0-4.5, and greater than 4.5 mm, respectively.

**Table 2.** Pearson correlation coefficient values for root tips, the I class and marker F06.970 as related to yield measurements in Zamorano, Honduras and Veracruz, Mexico.

| | ***Zamorano*** | | | ***Veracruz*** | | |
|---|---|---|---|---|---|---|
| | Yd | Yp | GM | Yd | Yp | GM |
| Tips | NS | NS | NS | NS | 0.21*** | 0.27* |
| I Class | NS | 0.17** | NS | NS | NS | NS |
| F06.970 | NS | NS | NS | 0.28* | 0.32** | 0.52**** |

*P<.05, **P<.01, ***P<.001, ****P<.0001; NS - non-significant; Yd, Yp = Yield under stress and nonstress.

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# GRAIN YIELD OF EARLY AND LATE DRY BEAN GENOTYPES UNDER RAINFED CONDITIONS IN AGUASCALIENTES, MEXICO[1]

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**Introduction**. One of the main limiting factors that reduce grain yield of dry beans in the highlands of Mexico is the scarcity of rainfall. In this region, which includes the states of Chihuahua, Durango, Zacatecas and Aguascalientes are sown 1.2 million hectares under rainfed conditions. The average amount of precipitation during the growing season ranges from 300 to 400 mm, and it is frequently that some drought periods from one to three weeks may occur during the crop cycle. When these drought periods occur during the reproductive stage of bean plants seed yield may be reduced considerably. Thus, the plant breeding program is focusing in obtaining dry bean varieties adapted to this condition of intermittent drought. One of the alternatives to face drought stress is by adjusting the growth cycle to escape water stress. This may be achieved with early genotypes, however, the yield potential of these cultivars is generally low. Therefore, the objective of this study was to evaluate the performance of early and late common bean genotypes under rainfed conditions in Aguascalientes, México.

**Materials and Methods**. The study was conducted at the Research Station of Sandovales, Aguascalientes (22° 09' N, 102° 18' W, 2000 masl) during the summer of 2001 and 2002. The experiments were established on June 27 in 2001 and June 14 and July 8 in 2002. The bean genotypes included were: 1) Early cultivars: Pinto Villa, Pinto Zapata and Azufrado Tapatio and 2) Late cultivars: Tlaxcala-62, Flor de Mayo M-38 and Bayo Criollo del Llano. 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. Days to flowering (DF) and days to maturity (DM) were registered on each plot. At harvest, grain yield (GY), weight of 100 seeds (W100S), harvest index (HI) and grain filling rate (GFR) were registered at all experiments. Data were subjected to ANOVA, and LSD test ($p=0.05$) was used for mean comparisons. Linear regression analyses were realized between DF and DM vs GY of bean genotypes using all observations in both years.

**Results and Discussion**. Total precipitation from June to October was 421 and 467 mm in 2001 and 2002, respectively. A drought period of about 20 days occurred in both years; in 2001 was at the second half of August, while in 2002 occurred from the last 10 days of September and the first half of Octuber. This rainfall pattern influenced the response of bean genotypes in each experiment. However, clearly late cultivars had lower GY, GFR and HI than early cultivars. Agronomic characteristic of the six dry bean genotypes are showed in Table 1.

Table 1. Agronomic characteristics of six dry bean cultivars evaluated under rainfed conditions at Sandovales, Ags.

| Charact. / Year | Genotype | | | | | |
|---|---|---|---|---|---|---|
| | P. Villa | P. Zapata | Az. Tap. | Tlax. -62 | F M M-38 | B. Cr. Ll |
| | | | 2001 (June 27) | | | |
| DF | 44.0 | 41.5 | 43.3 | 56.0 | 55.8 | 56.0 |
| DM | 89.0 | 86.3 | 89.0 | 109.0 | 98.0 | 100.3 |
| GY (g $m^{-2}$) | 77.8 | 70.3 | 71.9 | 59.7 | 54.7 | 54.6 |
| W100S (g) | 31.8 | 31.3 | 28.5 | 30.0 | 23.5 | 31.5 |
| HI (%) | 36.7 | 47.6 | 44.5 | 28.5 | 39.0 | 36.3 |
| GFR (g $m^{-2}$ $d^{-1}$) | 1.73 | 1.57 | 1.57 | 1.12 | 1.29 | 1.24 |
| | | | 2002 (June 14) | | | |
| DF | 47.0 | 46.0 | 46.3 | 58.3 | 56.3 | 56.5 |
| DM | 87.5 | 86.5 | 87.8 | 115.0 | 94.5 | 96.8 |
| GY (g $m^{-2}$) | 44.0 | 47.5 | 45.4 | 17.1 | 45.0 | 23.1 |
| W100S (g) | 27.9 | 25.5 | 24.8 | 25.5 | 25.0 | 22.1 |
| HI (%) | 33.1 | 42.7 | 55.3 | 15.9 | 53.5 | 27.3 |
| GFR (g $m^{-2}$ $d^{-1}$) | 1.09 | 1.17 | 1.09 | 0.30 | 1.18 | 0.57 |
| | | | 2002 (July 8) | | | |
| DF | 43.7 | 42.0 | 44.0 | 49.3 | 51.0 | 48.7 |
| DM | 83.3 | 81.3 | 83.7 | 97.3 | 90.0 | 90.7 |
| GY (g $m^{-2}$) | 63.1 | 53.1 | 31.3 | 49.1 | 40.2 | 35.6 |
| W100S (g) | 33.7 | 32.2 | 26.6 | 27.1 | 24.7 | 26.8 |
| HI (%) | 54.3 | 55.6 | 46.7 | 37.2 | 39.8 | 25.4 |
| GFR (g $m^{-2}$ $d^{-1}$) | 1.59 | 1.34 | 0.79 | 1.02 | 1.03 | 0.85 |

Average DF and DM for early and late cultivars was 44.2 vs 54.1 days and 86.0 vs 109.5 days, respectively. It was observed a tendency among all cultivars to reduce the growth cycle at late planting dates. Overall mean for GY of early genotypes was about 25% greater than in late genotypes, with Pinto Villa being the cultivar showing the highest GY average.

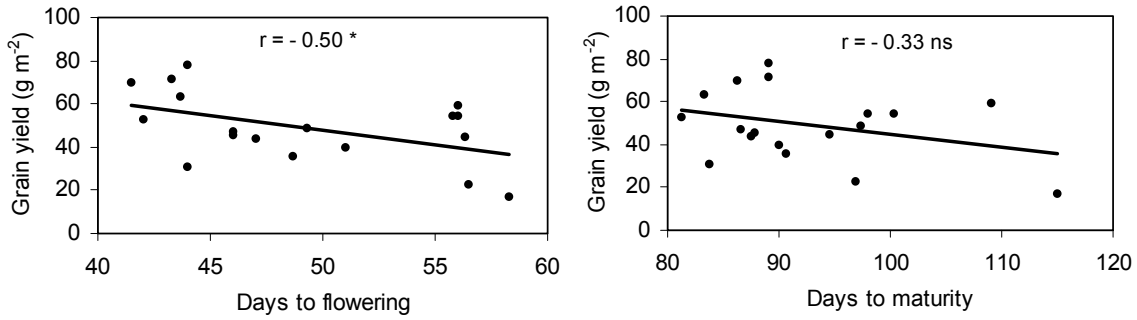


Figure 1. Relationship between DF and DM with GY in dry bean genotypes. Sandovales, Ags.

Regarding to the relationship between DF and DM vs GY it was found a negative association , although it was significant only for DF vs GY (Fig. 1). Thus, both earliness to DF (40-45 d) and DM (85-90 d) must be a characteristic for genotypes to be recommended at highlands to reduce production risks. These results are agree to those reported previously (1, 2).

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# Light Interception in Dry Beans as Related to Leaf Area, Paraheliotropic Leaf Movements and Dry Matter Production.

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## INTRODUCTION

Dry matter accumulation in plants is largely a function of net photosynthesis rates and light interception by the canopy. Monteith, (1977) showed that with an adequate supply of water, the accumulated interception of light by a wide variety of crops, such as barley, potatoes, maize and soybeans was closely related to biomass production. In general, light interception has been shown as a function of leaf area index (LAI), however most of the models linking light interception with dry matter production consider the vegetation canopy as a homogeneous medium with a random leaf orientation distribution (Maddonni, *et al*. 2001). Crops such as dry beans, have some mechanisms of light avoidance that may change the amount of photosynthetic active radiation (PAR) intercepted by the canopy during the day. The objective of this investigation was to study the relationship among light interception, LAI, leaf movements and dry matter production in dry beans under drought and irrigated conditions.

## MATERIALS AND METHODS

A short season navy bean variety was planted in a sandy Spink soil under two water regimes treatments. For the stress treatment, irrigation was halted 30 days after planting (DAP). Light interception was measured with a Licor spatial quantum sensor at 7-days intervals from late vegetative stage to maturity. Diurnal patterns of leaf angle were determined with a protractor attached to a 5-cm level. The angle of 10 randomly selected upper leaves per treatment was measured at 2-hours interval during the day. Leaves with a parallel orientation to the soil surface were considered to have zero angle. Leaf area was measured with a devise (Licor, 1500) at 7-days intervals and the same plants were use to estimate dry matter production

## RESULTS AND DISCUSSION

Leaf area growth rate decreased after the beginning of the drought stress affecting the total amount of light intercepted by the canopy. Light interception for the stress treatment lagged behind the control reaching a difference of more than 50% at 46 DAP (Fig.1). Diurnal changes of paraheliotropic leaf movements were measured for both treatments. Leaf angle in the upper leaves of the stress treatment increased from less than 10 degrees at 7:00am to nearly 60 degrees by noon time (Fig.2a). In the control, a maximum leaf angle of 32 degrees was observed at 13:00pm (EST). These differences in leaf orientation produced a daily pattern of light interception with the lowest values around mid day (Fig.2b). At a LAI of 2.1, the drought treatment was intercepting 23% less PAR as compare to the control due mainly to the paraheliotropic leaf movements of the bean canopy. Toler, *et al.,*(1999) found for maize that the greater interception of PAR was observed in leaves with lower angles.

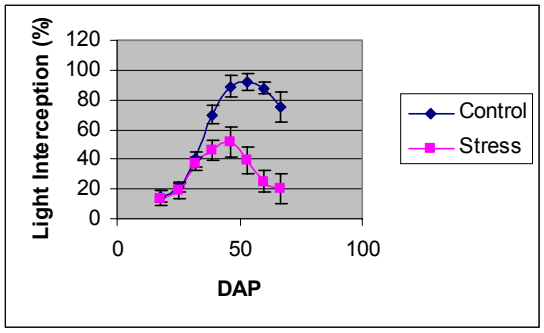


Fig.1. Seasonal Light interception by beans

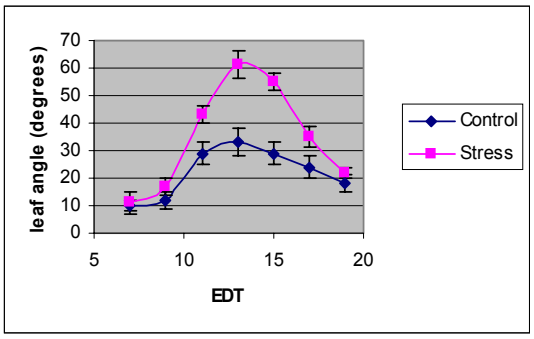


Fig.2a. Diurnal changes in leaf angle

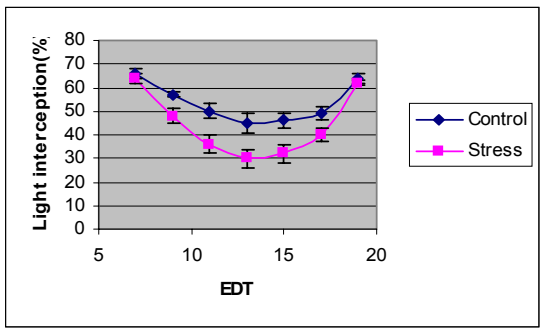


Fig.2b. Diurnal trend of light interception in beans

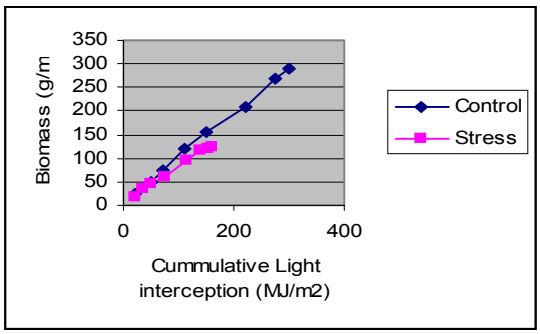


Fig. 3. Cumulative light interception and biomass($g/m^2$) production in beans

The relationship of cumulative intercepted PAR and total biomass production is depicted in Fig.3. At 150 $MJm^{-2}$, biomass production in the stress treatment was 13.5% less than that of the control. Berg and Hsiao, (1986) found that paraheliotropic movements in dry beans may placed significant restrains on photosynthesis and hence on dry matter production. Models need to take into account these light avoidance mechanisms of a bean canopy to improve predictions of biomass production under drought conditions.

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# EFFECT OF FERTILIZATION IN PROTEIN AND TRYPTOPHAN CONTENTS IN THREE BEAN CULTIVARS (*PHASEOLUS VULGARIS L.)*

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**INTRODUCTION:** The state of Paraná in Brazil is one of the biggest national producers. In 1999 it produced 560 thousands tons, there is, 20% of the national production. The bean cultivation in Brazil is still performed mainly in small and median properties, where it is responsible for a great part of the purchase. High productions and productiveness are desirable, however, the nutritional quality is also important. The adequate and balanced supply of nutrients for the bean plant, through chemical fertilization can contribute, increasing both the productiveness and the bean nutritional value (Teixeira et al., 2000).

To achieve higher profits concerning the bean cultivation, as well as to get the quality of the harvested product better, it is necessary, among other factors, the use of both the soil liming and balanced fertilization, trying to offer those nutrients that are not in sufficient quantities in the soil, and select cultivars that are better adapted to the region, mainly aiming better organolaptic and nutritional qualities. This study aimed at evaluating the effect of fertilization considering the nutritional quality of the following bean cultivars: *Aporé, Pérola* e *Rudá* (all of them are *carioca* type).

**MATERIAL AND METHODS:** The study was performed from November/2001 to February/2002 in a nitosoil of the State University of Maringá (UEM) experimental area, in Maringá city, state of Paraná, Brazil.

The cultivars used were: *Aporé*, *Pérola* and *Rudá*. The experimental delineation adopted was the one of blocks at random, with four repetitions, in subdivided plots, inserting the different fertilizations in the plots ($N_1$- not fertilized; $N_2$ – fertilization for the productiveness expected up to 1200 kg/ha, $N_3$ – fertilization for the productiveness expected above 2500 kg/ha). The split-plots embraced the three cultivars.

Fertilization was defined after the soil chemical analysis, and was based on the Recommendations for the Use of Correctives and Fertilizers in Minas Gerais city (5$^{th}$ Approximation – CFSEMG, 1999) related to the levels of productiveness 1 (up to 1.200 kg/ha-$N_2$) and 4 (above 2.500 kg/ha-$N_3$). The soil liming was not necessary (pH H2O=6.0; V=69% e $AI^{+3}$=0). The split-plots were constituted of five lines of bean with five meters of length, with a space of 0.50 m among them. After the harvest the protein and tryptophan contents in the grains were evaluated.

**RESULTS AND DISCUSSION:**

The protein (%) and tryptophan (mg/100 g) contents are shown in the Table 1. According to the findings, we can say that the protein and tryptophan content was distinct among both the different fertilizations and the cultivars evaluated. The highest protein content in the grains was obtained with the $N_3$ (26,94%) fertilization, followed by the $N_2$ (24,67%) fertilization, and the last one without fertilization $N_1$ (22,21%). The *Aporé* cultivar showed the highest protein content (25,58%), not different from the *Pérola* cultivar (24,77%). There was a significant difference between the *Aporé* and *Rudá* (23,47%) cultivars. Silva et al. (1999) studied 17 Brazilian bean cultivars, detecting a proteinic range between 22 and 32%.

Concerning the tryptophan content, it can be verified in the Table 1 that the highest contents occurred in a decreasing order of 0,75; 0,67 and 0,56 mg/100g, for the fertilizations $N_3$, $N_2$ and without fertilization

($N_1$), respectively. However, for the cultivars the tryptophan were 0,71; 0,67 and 0,60 mg/100g, for *Aporé*, *Pérola* and *Rudá*, respectively.

Sgarbieri and Whitaker (1982) showed that the methionine and the tryptophan had low concentrations in 25 bean varieties in Central America, while lysine was observed in all the varieties. The methionine ranged between 0.80 and 1.39% (g. aminoacid/100g of protein), tryptophan between 0.56 and 0.94%, and lysine between 7.22 and 9.22%. The total protein concentration detected in these 25 varieties ranged from 20.1 to 27.9%.

With the $N_3$ fertilization, the *Aporé* cultivar showed more quantity of N and, consequently, of protein and tryptophan (since N is part of its composition), followed by *Pérola* e *Rudá* cultivars.

**Table 1 -** Averages estimated of the protein and tryptophan variables in *Pérola*, *Rudá* and *Aporé* bean cultivars grains, without and with fertilization ($N_1$, $N_2$, $N_3$). UEM, Maringá-PR-BR, 2001.*

| | **PROTEIN (%)[1]** | | | | **TRYPTOPHAN (MG/100g)[2]** | | | |
|---|---|---|---|---|---|---|---|---|
| | **PËROLA** | **RUDÁ** | **APORÉ** | **MEDIA** | **PÉROLA** | **RUDÁ** | **APORÉ** | **MEDIA** |
| **N1** | **22.61** | **20.95** | **23.07** | **22.21c** | **0,57** | **0,52** | **0.60** | **0.56c** |
| N2 | 24.82 | 23.47 | 25.72 | 24.67b | 0.68 | 0.61 | 0.73 | 0.67b |
| N3 | 26,89 | 25.96 | 27.95 | 26.94 a | 0.76 | 0.69 | 0.81 | 0.75A |
| **MÉDIA** | 24.77AB | 23.47B | 25.58[A] | | 0.67AB | 0.60B | 0.71A | |

***** The protein and tryptophan contents were determined at the Agrochemical and Environment Laboratory from the Chemical Department/UEM/PR/BR.

[1] The total N was determined by sulphur acid digestion with salts and catalysers using Kjeldahl semi-micro method; and the gross protein was calculated by the 6.25 conversion factor.

[2]. The tryptophan was determined using OPIENSKA-BLOUTH et al. method (1963), modified by CLEMENTE & PORTELA (1987).

For the cultivar, the equal capital letters on the line indicate that the media are not different among them, by the Duncan test (P>0.05).

For the fertilization, the equal small letters on the column indicate that the media are not different among them, by Duncan test (P>0.05).

$N_1$ = Without fertilization; $N_2$ = fertilization with 20 kg N/ha + 30 kg $P_2O_5$/ha + 20 kg $K_20$ during the plantation, 20kg N/ha in cover; and $N_3$ = fertilization with 40 kg N/ha + 70 kg $P_2O_5$/ha + 20 kg $K_2O$ during the plantation, 60 kg N/ha in the cover.

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# SELECTION FOR LOW SOIL FERTILITY BEAN LINES TOLERANT TO ROOT ROT

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## Introduction

Common bean is the worlds' most important food legume, accounting for about 57% of the worlds' food legume production (CGIAR, 2001). In Africa, production of the crop is constrained by a number of diseases, of which root rots are among the most important. Recently the root rot problem has increased in Eastern and Central Africa particularly in areas of intensive bean production and low soil fertility (Wortman et al., 1998). The disease is severe in soils deficient in P, N, exchangeable bases and AL and Mn toxicity. Genetic resistance is the most effective control strategy for the disease since it is small-scale farmers with limited resources to purchase external inputs grow the crop. Work has been done to identify sources of resistance to root rots and such sources include RWR 719, SCAM 80CM /15, MLB 49 – 89A and RWR 432 (Otsyula et al., 1998). However there is still need to identify more sources of resistance especially in the case of variability of the pathogen. And more to this, some of the available sources of resistance are so far not of acceptable seed types by farmers and other end users. Selection for bean lines tolerant to root rots and low soil fertility is a priority for the regional breeding programmes and started recently. This report presents progress in selection of low soil fertility lines possessing levels of root rot tolerance that have been identified in Uganda.

## Materials and methods

Eighteen genotypes in the BILFA III nursery were grown in a replicated trial in Kachwekano in the second season (October – December) of 1999 at a spot that was very low in fertility. Out of the lines, eight best genotypes namely RWR 2075, RWR 1873, RWR 1946, $C_{30}$-$P_{21}$, G 22501, DB 201/77/1, RWK 10 and G8864 x MASAI were selected based on yield and seed types and were tested further in a preliminary yield trial at Namulonge, Nakabango, and Kachwekano in the first season (April – July) of 2000. Kachwekano, in southwestern Uganda has low soil fertility and is prone to root rot disease. Namulonge and Nakabango sites are of moderate and high soil fertility, respectively. In the first season (March – July) of 2001, the genotypes were tested further in an intermediate yield trial. During this season, very severe root rot disease was experienced at Kachwekano and to measure the extent of root rot damage, data was collected on plant stand count before harvest and mean seed yield. Analysis of variance was performed following PROC GLM SAS procedure (SAS Institute, 1988).

## Results and Discussion

During the second season of 1999, the lines RWR 2075, DB 201/77/1, RWR 1873, RWR 1946, $C_{30}$-$P_{21,}$ G 22501 and G8864 x MASAI were observed with mean seed yields of 1139, 931, 803, 775, 653, 514 and 414 kg/ha, respectively, under low soil fertility. In 2000, mean seed yield of the BILFA selections across sites ranged from 755 to 1075 kg/ha under both low and high soil fertility. During the first season of 2001, the highest yield was observed at Nakabango, where it

ranged from 375 kg/ha to 1050 kg/ha. At Namulonge, yield varied from 217 kg/ha to 733 kg/ha, while plant stand count of between 83 and 129 was recorded. At Kachwekano (with root rot) the average plant stand count ranged between 19 and 129, while the mean grain yield varied from 83 kg/ha to 1567 kg/ha. Across sites, the highest yielding genotypes were RWR 1946, RWR 1873, and RWR 2075, all which had been previously selected from BILFA III. Check varieties NABE 4 and K 131 were also observed with moderate yields of 647 and 756 kg/ha, respectively. This suggests that these genotypes perform well both under low and moderate soil fertility. A low yield at Kachwekano of the remaining genotypes is attributed to the severe root rot disease, which was experienced during the season. Results from this work suggest that the lines RWR 2075, RWR 1946 and RWR 1873 and NABE 4 are probably potential sources of resistance to root rots in Uganda, particularly in the southwestern part of the country where root rot is a major problem. Activities are underway to evaluate the materials further on farmers' fields where root rot problem is prevalent. There is still need to screen these materials with different species of the pathogen in the screen house and other hot spot sites for the disease.

**Acknowledgement**

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# EVALUATION OF NITROGEN FERTILIZATION ON LEAF NITROGEN CONCENTRATION AND BEAN YIELD IN IRRIGATED NO TILL SYSTEM CROPPED ON PLANT RESIDUES[1]

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Brazil is one of the largest producers and world consumers of bean, even so, few farmers use irrigation as normal and recommended practice for elevation of grain production. The bean yield oscillations in the last years, of the main States producers of bean, are explained by the variations of cultivated area, suggesting the little significance of the irrigated area with bean cultivation. The good quality of bean grain is obtained in dry season, time in that the rain allow some production, without the practice of irrigation. However, the risk of production break is very high, because the rain distribution presents casual character. Thus, the medium productivity of bean grain, around 763 kg/ha is very low, when compared with the grain yield above 1891 kg/ha that can be obtained with irrigated crops (Zimmermann et al., 1948). No till system is one of the more advantageous cultural practices of tropical area management, where the crop residue is maintained on the top soil that helps to maintain the free water for a more long period and the erosion is controlled, not only by the action of the crop residue, but mainly for the non soil revolving (Balbino et al., 1996). Other advantages of this system would be, the reduction of the production cost and the simplicity of technical execution, in which could be used mainly for crop succession (Fageria & Ghevi, 1999).

In general way, the nitrogen fertilization, mainly in no till system, constitutes an important factor for productivity increasing. The nitrogen fertilization recommendation for irrigated bean crop vary from 40 to 120 kg/ha of N, while the crop in field absorbs for its complete development amounts around 100 kg/ha of N in dry season crop and in nutrient solution absorbs 200 kg/ha of N (Oliveira et. al, 1996). In that way, not always the applied nitrogen amounts are enough to supply the plant needs. The objective of this research was to evaluate the concentration of leaf N of bean plant under increasing doses of N applied on top soil covered with different plant residues on bean yield in irrigated no till system.

The bean, cv. Pérola, was sowed in the spacing of 0.5 m between lines, with 16 to 18 seeds for lineal meter, using a basic fertilization of 150 kg/ha of NPK in 8:20:20 formulation in irrigated system. The bean sowing was made on crop residues in consortium with brachiaria (21.54 t/ha); sorghum (20.67 t/ha); single brachiaria (19.54 t/ha), rice (8.38 t/ha) and soybean (5.79 t/ha of dry matter) in field conditions. The experimental area covered 192 $m^2$. The doses of nitrogen were 45, 90, 135 and 180 kg/ha.

The leaf samples were taken at flowering stage in all the treatments. Each sample was composed by 10 leaves, that were drought in stove for about 72 hours, at temperatures from 65 to 70 ºC. The samples were grind and sent to laboratory analysis. The largest medium concentration of leaf N in bean plant was obtained when this crop was grown on corn residue + brachiaria that received 180 kg/ha of N, meantime in medium terms, the maximum concentration of leaf N was obtained when the bean was cropped on residue of the soybean crop (Figure 1), reaching 6% of leaf N.

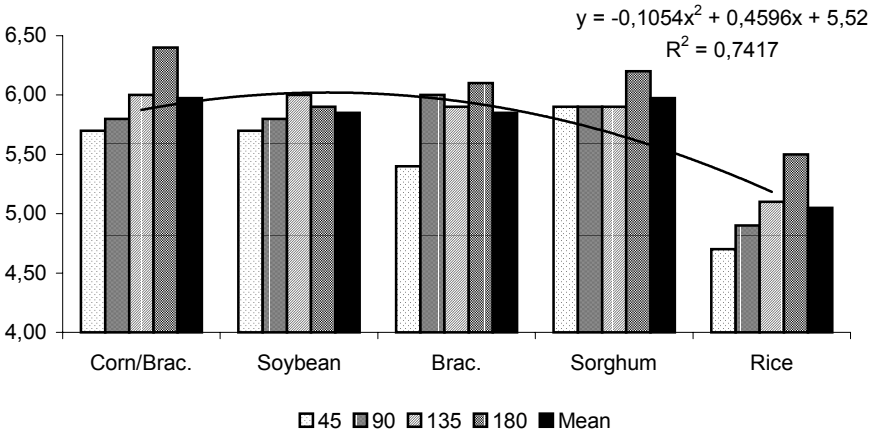


**Figure 1**. Concentrations of leaf N of bean in function of N doses applied on crop residues in irrigated no till system at Santa Fé Farm – Santa Helena de Goiás.

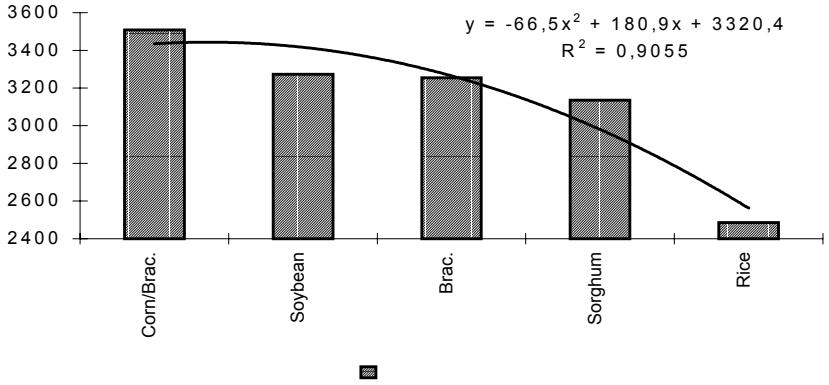


**Figure 2**. Grain yield of bean cropped on plant residue on top soil in irrigated no till system at Santa Fé Farm – Santa Helena de Goiás.

The best grain yield (3 443 kg/ha) was obtained when the bean was cropped on corn-brachiaria residue (Figure 2).

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# BEAN PRODUCTION INFLUENCED BY N APPLICATION IN NO TILL SYSTEM ON DIFFERENT CROP RESIDUES[1]

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Bean grain is an alimentary base of Brazilian population, being cultivated in all national territory, using varied techniques that embraces from subsistence cultivation to technical plantation (irrigation and no till system). No till system is one of the more advantageous practices of crop management in tropical areas in the last ten years. In Brazilian Savannah, that system comes increasing intensively embracing grain productivity and soil conservation. However, the use of crop residue depend on hard studies in relation to plant nutrition, that's why the N fertilization can cause losses in the soil chemical capacity, maintenance of fertilizers response, as well as in the sustainability of the productive system (Fageria & Ghevi 1999).

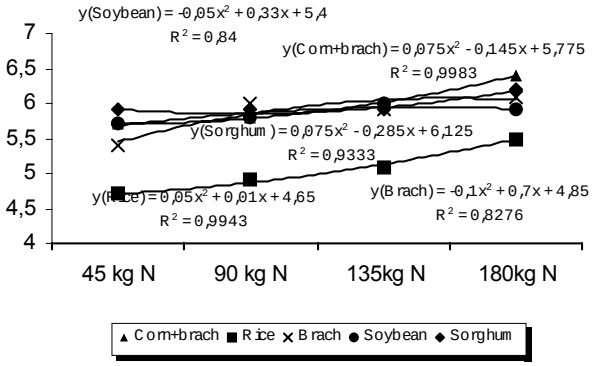
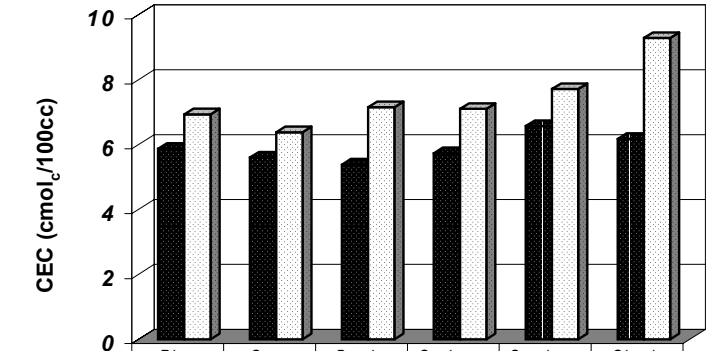



**Figure 1**. Concentration of N in bean leaf, cv. Pérola, cropped in irrigated no till system under increasing doses of N (kg/ha).

In no till system, the plant residue on top soil helps to maintain the free water for a longer time period, that influences the uniform seed germination and provides an increase of water infiltration and reduces the evaporation and soil temperature. In this system there is an economy of electric energy due to the decrease of irrigation frequency. Nutrient imbalances can reduce the productivity of irrigated bean crop in no till system and the most frequent nutrient deficiency occurs in relation to the N, since in this system where the N demand is larger than in traditional system.

The experiment was carried out at Santa Fé Farm, in the municipal district of Santa Helena of Goiás, objecting to obtain information about bean productivity, cv. Pérola, on different crop residues and N doses. The bean was sowed in one oxisol spacing 0.5m between lines, with 16 to 18 seeds/meter, on crop residue of corn, rice, brachiaria, soybean and sorghum using the basic fertilization of 150 kg/ha of NPK of 8:20:20 formulation in soil previously amended. Plant irrigation was made accord to aspersion modality. Four doses of N as ammonium sulfate were studied (45, 90, 135 and 180 kg/ha). Leaf samples were collected at bean flowering stadium, dried in stoves during 72 hours at temperature among 65 and 70ºC, grind and sent for analyses. Soil samples were collected before planting and after harvest time.

The doses of N that allowed the highest leaf N concentrations of corn, rice, sorghum, brachiaria and soybean were 40, 50, 79, 156 and 182 kg N/ha (Figure 1). The low amount of N fertilizer demanded for plants cultivated on corn residue can be explained by N complementation came from corn plant decomposition.




| | Rice | Corn | Brach. | Soybean | Sorghum | Check |
|---|---|---|---|---|---|---|
| Flowering | 5,87 | 5,6 | 5,38 | 5,73 | 6,57 | 6,17 |
| Post-harvest | 6,93 | 6,38 | 7,15 | 7,1 | 7,71 | 9,28 |

**Figure 2** . Cátion Exchange Capacity (CEC) of soil under five crop residues, at flowering stage and after the harvest of bean, cv. Pérola, in the no till system.

Cátion Exchange Capacity (CEC) increased during the plant growth on field, that contributed for Ca increase (from 4.4 to 5.3 cmol$_c$/100cc), K(from 0.46 to 0.63 cmol$_c$/100cc) and H (of 7.8 for 9.45) occurred into the soil. These results reflect the effect of crop residues plus the soil amendment that propitiated high productions and improved the soil fertility. Considering that pH was reduced and basic cations and hydrogen had their concentrations increased, researchers have suggested that through decomposition of soil organic matter, organic radicals are produced and complex the exchangeable cátions and liberate H for soil solution (Franchini et al 2000). The organic matter of soil presented a little increase at post harvest time. Sorghum residues propitiated larger increases of CEC, producing as organic matter as check treatment. These higher CEC and organic matter in check treatment are due to high seed weeds infestation without no crop growing in consortium. High organic matter concentrations observed in areas where sorghum was cropped are due to the high production of organic matter of easy decomposition by this crop.

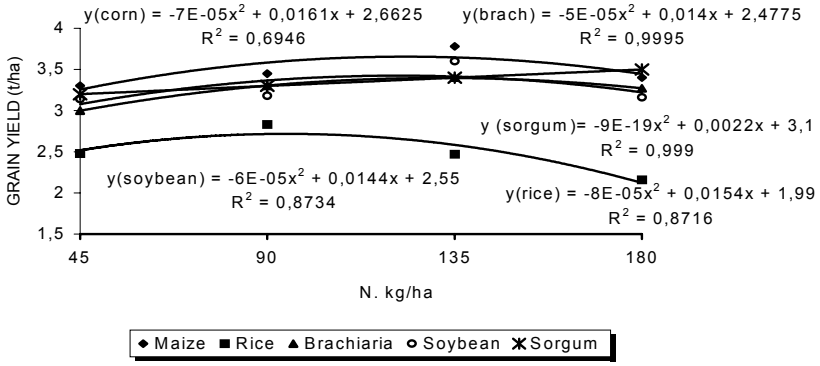



**Figure 3**. Effect of the N fertilization on bean production cropped on different crop residue.

The bean crop produced on residues of rice, braquiária, soybean, sorghum and corn were 2.6; 3.2; 3.3; 3.5 and 3.6 t/ha, respectively (Figure 3).

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# SEED MOLYBDENUM CONTENT AFFECTING COMMON BEAN YIELD

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**Introduction.** Foliar application of molybdenum (Mo) has successfully increased bean yield at Zona da Mata region, State of Minas Gerais, Brazil. Many times Mo amendment have similar effects as nitrogen (N) fertilizer applied as a side dressing. However, many farmers either do not know this technology or have no access to molybdenum fertilizers. One possible solution is to provide farmers with seeds of high Mo content. The objective of this study was to test if it is feasible to produce bean seeds with high Mo content by using seeds harvested from plants fertilized with Mo and then, in a second experiment, verify the effects of sowing these seeds without N as a side dressing.

**Material and Methods.** This field study was divided in two phases. In the first, the effects of high Mo rates on yield and seed Mo content were studied. In the second, seeds with different Mo contents were tested either with or without foliar Mo application. Both trials were sprinkler irrigated.

*First trial*

The trial was conducted during summer-fall at Coimbra, Zona da Mata region, in a soil classified as Red Yellow Podzolic. Soil analysis gave the following results: pH($H_20$)=5.7, P (Mehlich)=12.8 mg $dm^{-3}$, K=54 mg $dm^{-3}$, with 0.0, 2.6, and 0.6 cmol $dm^{-3}$ of Al, Ca, and Mg, respectively. A randomized complete block design with four replications was used. Each plot had six 6m-long rows. Bean cultivar Pérola (carioca type) was sown in rows spaced 0.5 m apart with 12 seeds per meter. Seeds contained 0.119 µg Mo $g^{-1}$ (equivalent to 0.027 µg Mo $seed^{-1}$). The rates of Mo tested are presented in Table 1. Ammonium molybdate was sprayed on foliage with 500 L $ha^{-1}$ of water. All plants received basal N, P, and K at rates of 32, 49, and 53 kg $ha^{-1}$, respectively. Urea application (110 kg $ha^{-1}$) as a side dressing was performed 14 days after emergence (DAE).

*Second trial*

The trial was installed at the end of summer at a distance of 300 m from the previous trial. Soil analysis gave the following results: pH=6.1, P=4.6 mg $dm^{-3}$, K=104 mg $dm^{-3}$, with 0.0, 3.3, and 1.7 cmol $dm^{-3}$ of Al, Ca, and Mg, respectively. Treatments were four seed sources, with or without Mo application (factorial 4 x 2). Seeds harvested from the previous trial (seed sources) with 0.096±0.058, 0.080±0.044, 0.722±0.290, and 1.272±0.579 µg of Mo $seed^{-1}$ were selected. Plants originated from those seeds received either Mo foliar application at 23 DAE or did not (Mo treatments). The trial was laid out on a randomized complete block design with six replications. Each plot had five 5m-long rows with 15 seeds per meter. All plants received basal N, P, and K at rates of 24, 37, and 40 kg $ha^{-1}$, respectively.

**Results and Discussion**. Results of first trial are presented in Table 1. There were no differences in grain yield and 100-seed weight as Mo rates increased from 90 (recommended rate) to 1.440 g $ha^{-1}$, but µg Mo $seed^{-1}$ increased from 0.08 to 1.272. Beans did not respond to Mo application probably because plants received a top-dressing of urea. Split of 1.440 g $ha^{-1}$ of Mo in four times was advantageous to increase Mo content in seed. In the second trial, visible symptoms of N deficiency (yellowing of leaves) were evident at 23 DAE on plants from seeds with Mo content of 0.096 and 0.080 µg. Plants from seeds with higher Mo content were greener, mainly those

from seeds with 1.272 µg Mo seed$^{-1}$. Plants from seeds with low Mo content grew less than those from seeds with 0.722 µg Mo seed$^{-1}$ which, in turn, grew less than plants from seeds with 1.272 µg Mo seed$^{-1}$ (data not presented). However, there was no significant effect of seed sources on Mo in leaves, nodules dry weight, 100-seed weight, and seed Mo content (data not presented). There was interaction between seed Mo content and Mo treatments (Table 2). For plants that received foliar Mo application, bean yield was higher for plants from seeds with the highest Mo content than for plants from seeds with 0.080 and 0.722 µg Mo seed$^{-1}$. For plants not sprayed with Mo, bean yields were higher for plants originating from seeds with 0.722 and 1.272 µg Mo seed$^{-1}$. Table 2 also shows that plants from seeds with low Mo content respond better to Mo foliar application than those from seeds with high Mo content. These results show that it is possible to attain high bean yield without N as a side dressing by combining use of seeds with high Mo content and Mo foliar application.

**TABLE 1. Effects Mo rates applied (split or not) on bean foliage on yield, 100-seed weight and Mo content in seed**

| Molybdenum treatments | Grain yield (kg ha$^{-1}$) | 100-seed weight (g) | Mo content[1] (µg seed$^{-1}$) |
|---|---|---|---|
| Without Mo | 1,945 | 22.5 | 0.096 c* |
| 90 g ha$^{-1}$ at 20 DAE[2] (recommended rate) | 2,019 | 23.0 | 0.080 c |
| 180 g ha$^{-1}$ at 20 DAE | 2,075 | 23.3 | 0.232 bc |
| 360 g ha$^{-1}$ at 20 DAE | 2,097 | 22.8 | 0.481 bc |
| 720 g ha$^{-1}$ at 20 DAE | 2,035 | 22.5 | 0.742 ab |
| 360+360 g ha$^{-1}$ at 15 e 22 DAE | 2,343 | 22.9 | 0.722 ab |
| 1.440 g ha$^{-1}$ at 20 DAE | 2,019 | 22.4 | 0.552 bc |
| 720+720 g ha$^{-1}$ at 15 e 22 DAE | 2,077 | 22.7 | 0.707 b |
| 360+360+360+360 g ha$^{-1}$ at 15, 20, 25 e 30 DAE | 2,162 | 23.1 | 1.272 a |

[1] 100-seed weight was used for this calculation.
[2] DAE = days after plant emergence.
* Average of four replications. Means separation by Tukey test at 5% level.

TABLE 2. Interaction between seed Mo content and foliar Mo treatments on bean yield (kg ha$^{-1}$)

| Mo content (µg seed$^{-1}$) | ***Mo treatments*** | | ***Difference***[3] |
|---|---|---|---|
| | with[1] | without | |
| 0.096 | 2,720 ab[2] | 2.100 b | 620** |
| 0.080 | 2,513 b | 1,772 b | 741** |
| 0.722 | 2,574 b | 2,565 a | 9 ns |
| 1.272 | 3,017 a | 2,624 a | 393 * |

[1] At 23 DAE at a rate of 90 g ha$^{-1}$.
[2] Average of six replications. Means separation by DMRT at 5% level.
[3] ** = significant at 1% level, * = significant at 5% level, ns = not significant.

# CANOPY REFLECTANCE AND YIELD IN COMMON BEAN PLANTS (*Phaseolus vulgaris* L.). I. EFFECT OF NITROGEN

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## Introduction

Remote sensing (spectral reflectance) can be used to assess; leaf area index, absorbed radiation, total chlorophyll, and canopy temperature (Araus *et al.*, 2001). A simple index to measure the contrast in reflectance between different parts of the spectrum is the normalized difference vegetation index (NDVI), which may be used as an indirect assessment of canopy structure such as biomass, photosynthetic potential (total area of leaves and other photosynthetic organs), chlorophyll, etc. (Araus *et al.*, 2001; Gutiérrez, 2002). For that reason, the objective of the present work was to determine whether NDVI may be related to yield parameters in common bean plants under different nitrogen rates (0 and 200 kg $ha^{-1}$).

## Material and Methods

The study was carried out in Montecillo, Mexico (19º19' N, 98º54' W, 2250 masl and a temperate climate) under rainfed conditions (June-September, 2001). Seeds of bean plants (*Phaseolus vulgaris* L.) cv. Flor de Durazno were sown in plots with a density of 25 plants $m^{-2}$ using a randomized complete block design with four replications. Nitrogen (N) rates of 0 and 200 kg $ha^{-1}$ (N0 and N200 respectively) were applied during the sowing. Canopy reflectance was measured with a portable spectroradiometer (FieldSpec, USA) at 14, 25, 32, 39, 50, 84 and 88 days after the sowing (das). Leaf area index, intercepted radiation, biomass and yield components were determined in each treatment.

## Results and Discussion

The results showed that the highest biomass (1003 g $m^{-2}$), seed yield (549 g $m^{-2}$) and harvest index (46%) was increased with 200 kg of N $ha^{-1}$. Pod number, raceme number and seeds $m^{-2}$ were related with the high yield. N0 treatment had low biomass production (932 g $m^{-2}$), seed yield (323 g $m^{-2}$) and harvest index (35%).

NDVI values did not show differences in early stages (vegetative and beginning of reproductive stage). The maximum NDVI values occurred during the flowering-grain filling stage (50-84 das); when the plants began to senescence the NDVI values decreased.

NDVI showed a high relationship with absorbed radiation ($r^2$=0.62-0.80); biomass ($r^2$=0.73-0.98); and leaf area index ($r^2$=0.89-0.91) from early stage (vegetative stage) to pod-filling stage (84 das) (Figure 1a, b, c).

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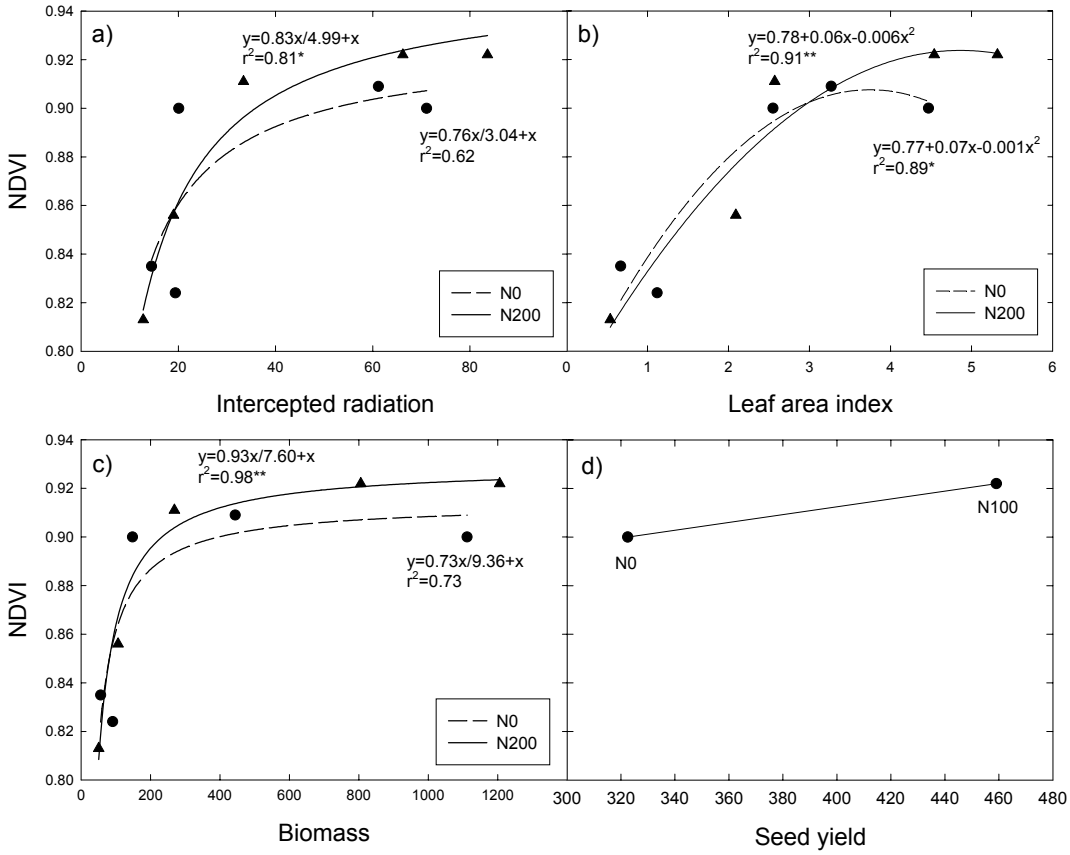


**Figure 1.** Relationship among NDVI, intercepted radiation, leaf area index, biomass and seed yield in common bean plants (*P. vulgaris* L.) under different nitrogen rates. (*) significant at p≤0.05. Montecillo, México.

Yield can be predicted from successive measurements of NDVI during the growing season (Araus *et al.*, 1991). In our study, seed yield showed a high relationship with NDVI of 84 das (pod-filling stage) (Figure 1d).

Conclusions

In conclusion, NDVI estimated seed yield and biomass in bean plants under different N fertilization rates.

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Gutierrez R., M. 2002. La radiometría como estimador del rendimiento en frijol (*Phaseolus vulgaris* L.) y trigo (*Triticum aestivum* L.). Tesis de Maestría. Colegio de Postgraduados. Montecillo, México. 85 p.

# CANOPY REFLECTANCE AND YIELD IN COMMON BEAN PLANTS (Phaseolus vulgaris L.). II. EFFECT OF PHOSPHOROUS

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## Introduction

It is widely recognized that phosphorous (P) directly affects both the rate of crop growth and the quality of grain. Growth and yield of common bean plants (*Phaseolus vulgaris* L.) are increased by P supply (an important element in nodule metabolism) (Blevins, 1995). The spectral reflectance vegetative index (i.e. green normalized difference vegetation index, GNDVI) is particularly useful for assessing physiological parameters (leaf area index, absorbed radiation, total chlorophyll, etc.), and it can be used to estimate canopy biomass and seed yield (Araus *et al.*, 2001; Gutiérrez, 2002). For that reason, the objective of the present work was to determine whether GNDVI can be related to yield in bean plants under different P rates (0, 100 y 200 kg $ha^{-1}$).

## Material and methods

The study was carried out in Montecillo, Mexico (19º19' N, 98º54' W, 2250 of altitude) under rainfed conditions (June-September, 2001). Seeds of bean plants (*Phaseolus vulgaris* L.) cv. Flor de Durazno were sowed in plots with a density of 25 plants $m^{-2}$ using a randomized complete block design with four replications. Different P rates; 0, 100 and 200 kg $ha^{-1}$ (P0, P100 and P200, respectively) were applied during the sowing. Canopy reflectance was measured with a portable spectroradiometer (FieldSpec, USA) at 14, 25, 32, 39, 50, 84 and 88 days after the sowing (das). Leaf area index, intercepted radiation, biomass and yield components were determined in each treatment.

## Results and discussion

The data showed that seed yield was increased with 100 and 200 kg of P $ha^{-1}$ (323 and 468 g $m^{-2}$ respectively), while the P0 had lower seed yield (248 g $m^{-2}$). These changes were related with raceme number, pod number and seeds $m^{-2}$.

Biomass and harvest index were also higher in P100 (932 g $m^{-2}$ and 35% espectively) and P200 (1058 y 44% respectively), compared with P0 (852 g $m^{-2}$ and 29% respectively).

GNDVI values did not show differences in early stages (vegetative and at beginning of reproductive stage). The maximum GNDVI value occurred during the flowering and grain-filling stage (50-84 das) when the plants began to senescence the GNDVI values decreased.

GNDVI showed a high relationship with absorbed radiation ($r^2$=0.62-0.81), biomass ($r^2$=0.73-0.92), and leaf area index ($r^2$=0.85-0.97) from an early stage (vegetative growth) until pod-filling stage (Figure 2a, b, c).

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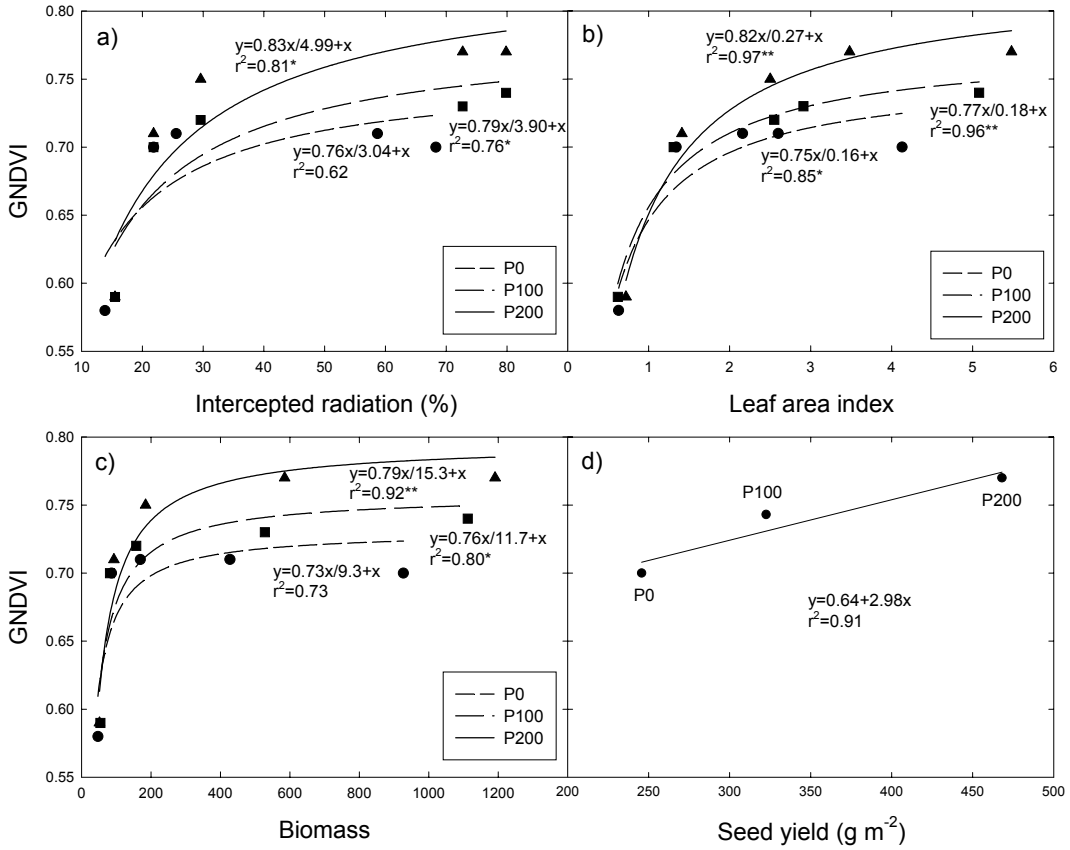


**Figure 1.** Relationship among GNDVI, intercepted radiation, leaf area index, biomass and seed yield in bean plants (*P. vulgaris* L.) under different P rates. (*,**) significant at p≤0.05 and 0.01 respectively. Montecillo, México.

GNDVI at pod-filling stage (84 das) showed a high relationship with seed yield ($r^2$=0.91) (Figure 1d).

## Conclusions

In conclusion, GNDVI provides a good estimate yield in bean plants when influenced by different P fertilization rates.

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# SEASONAL ANALYSIS OF DRY BEAN PRODUCTIVITY FOR DIFFERENT NITROGEN FERTILIZER LEVELS IN PIRACICABA, SAO PAULO, BRAZIL

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Dry bean is one of the most important crops in Brazil. Although yield has increased through the years, problems with fertilization are evident. Farmers have become more receptive to new technologies and have increased the amounts of nitrogen (N) fertilizer that they apply. However, do farmers really need to increase N fertilizer to be able to maintain a sustainable production? The objective of this study was to analyze the seasonal dry bean crop productivity with different N fertilization levels, using a crop simulation model. At the Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ), Sao Paulo State University, Piracicaba, Brazil an experiment with three levels of N fertilizer (T1=0, T2=60 and T3=120 kg $ha^{-1}$) was conducted from April through July 2002 in a Typic Eutrudox soil. Fertilizer was applied at sowing, at $V_3$, and at $R_5$ crop stages. The T2 was divided in three identical applications while 20, 50 and 50 kg $ha^{-1}$ were used in T3. The region's climate is characterized as humid subtropical (Cwa) and during the growing season the average temperature was 20°C and total rainfall was 137mm. The cultivar was IAC-Carioca Tybata, type II (Pompeu et al., 2001) and plots were irrigated to avoid drought stress. The statistical design was a completely randomized block, with 4 replicates. Phenological stages were recorded following the scale proposed by Fernández and Gepts (1985). Growth analysis of roots, stems, leaves and reproductive organs was obtained, as well as the final yield and yield components. The CROPGRO Dry Bean model (Hoogenboom et al., 1994; Boote et al., 1998), included in the DSSAT Version 4.0 (Hoogenboom et al., 1999), was used to predict dry bean yield for 16 years, from 1980 to 1995. Weather data used were obtained from the conventional weather station of ESALQ. For model calibration, data from T2 (60 kg $ha^{-1}$) were used. Genetic coefficients were selected based on the minimum Root Mean Square Error (RMSE). Followin model evaluation, crop yield was predicted, and the seasonal analysis of the DSSAT Version 4.0 was used to examine the year-to-year variation on this variable due to climate.

In the experiment, the development stages were similar for T1 and T2 while yield at maturity, leaf area index, and canopy height were similar for T2 and T3 (Table 1).

**Table 1 – Observed and predicted yield and phenology**

| Growth and Development Variables | Observed (2002 season) | | | Predicted (2002 season) | | |
|---|---|---|---|---|---|---|
| | N (kg $ha^{-1}$) | | | | | |
| | 0 | 60 | 120 | 0 | 60 | 120 |
| Anthesis (DAP) | 47 | 47 | 47 | 47 | 47 | 47 |
| 1st Pod (DAP) | 52 | 52 | 52 | 52 | 52 | 52 |
| Full Pod (DAP) | 73 | 73 | 77 | 73 | 73 | 73 |
| Physiological Maturity (DAP) | 95 | 95 | 101 | 95 | 95 | 95 |
| Yield at Maturity (kg $ha^{-1}$) | 1677 | 1996 | 1968 | 1872 | 1902 | 1902 |
| Grains at Maturity (grains $m^{-2}$) | 880 | 1140 | 934 | 1130 | 1060 | 1063 |
| Maximum LAI ($m^2$ $m^{-2}$) | 2.5 | 3.17 | 3.5 | 3.12 | 3.10 | 3.10 |
| Tops weight at Anthesis (kg $ha^{-1}$) | 839 | 1256 | 1243 | 892 | 863 | 863 |
| Leaf Number per stem at Maturity | 8 | 10 | 13 | 13 | 13 | 13 |
| Canopy Height (m) | 1.08 | 1.25 | 1.23 | 1.21 | 1.20 | 1.20 |

Simulated results showed that the model satisfactorily predicted growth and development and the only differences were observed between the control treatment (T1) and the treatments with N (Table 1).

The predicted seasonal yield was stable across years, and not much difference was found between the three fertilizer levels (Figure 1), except for 1982 and 1984, when 421mm and 118mm of rainfall was received during the growing season. Moreover, the two atypical years showed that the impact of water not only affected yield, but also the crop's response to N fertilizer. For the 16 years of this study, average yield was 1860, 2000 and 1950 kg $ha^{-1}$ for T1, T2 and T3, respectively.

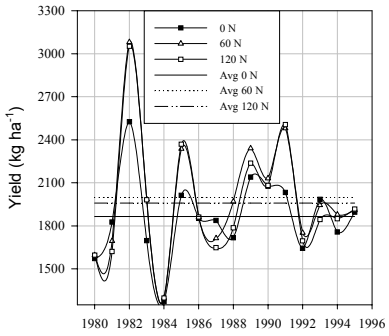


**Figure 2 – Predicted Annual Yield Variation for Carioca-Tybata.**

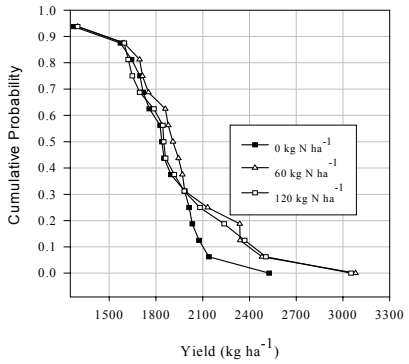


**Figure 3 – Cumulative Probability for Carioca-Tybata Yield.**

For the T2 and T3 treatments, yield levels higher than 2,100 kg $ha^{-1}$ were reached for 25% of the years while the control treatment had yield levels that were slightly less than 2,000 kg $ha^{-1}$ for the same probability. For the low productivity levels, yields less than 1,700 kg $ha^{-1}$ were observed in 20% of the years in treatment T2 and 25% of the years for the other two treatments. It was practically impossible to reach yield levels higher than 2,150 kg $ha^{-1}$ with no supplemental N fertilizer applied. However, this yield level was found in 20% of the years in treatment T2 and nearly of 14% of the years in T3 (Figure 2).

The results from this study showed that increases in the amount of N applied from 60 until 120 kg $ha^{-1}$ did not result in an increase in dry bean yield. Thus, an accurate knowledge of the local weather conditions and the impact on crop growth and development is critical for developing a sustainable technology while at the same time maintaining high productivity levels.

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# Ca, K, Fe, P and Na CONTENT IN DIFFERENT VARIETAL TYPES OF DRY BEAN USING TWO GROWING SYSTEMS: ORGANIC AND CONVENTIONAL

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## Introduction

People demand more and more foods that are both healthy and of high nutritional quality. Higher mineral content has been observed in foods cultivated under organic growing conditions (Bibak et al, 1998; Pither, 1990; Raigón et al., 2002). In dry beans, very few studies have been carried out in this respect so far. The objective of this work is to study the influence of organic cultivation on the quality of dry bean seeds and analyse whether or not changes occur in the relationship between traits as a result of this kind of cultivation.

## Methods and Materials

A total of 49 cultivars of different varietal types (including controls) were evaluated under both organic and conventional growing conditions in Valencia (Spain). The design was in random blocks with two repetitions and 20-25 plants/cultivars x repetition x growing condition. The evaluation was carried out at the level of individual plant. Twenty-one out of 49 were selected for general health reasons (including resistance to BCMV and BCMNV), external traits of the seed and yield; the chosen cultivars were grouped into 3 colours (white, red and cream) and 7 different seed shapes. In a mineralised sample (desiccated and calcinated) the content of Fe and P were measured using a visible UV light spectrophotometer; while Ca, K and Na content were measured using a flame photometer. The results are expressed as mg/100 g of dry matter. The results were measured using an ANOVA factorial with repetitions and the correlations between traits were estimated (for both cultivation systems).

## Results

In beans grown in organic agriculture, all the minerals, with the exception of Na, are found in greater quantities than in conventional agriculture (Table 1).

Table 1. Average values for Ca, K, Fe, P and Na content (in mg/100g dry matter) in the seeds of 21 cultivars of dry beans

| System of cultivation | Seed color | Ca | K | Fe | P | Na |
|---|---|---|---|---|---|---|
| Conventional | White | 21.1 | 2462.9 | 6.4 | 544.0 | 9.2 |
| | Red | 17.4 | 2050.8 | 5.4 | 507.8 | 16.6 |
| | Cream | 14.3 | 1600.8 | 4.2 | 274.6 | 11.2 |
| Organic | White | 21.6 | 2597.0 | 7.0 | 609.3 | 5.6 |
| | Red | 16.7 | 2272.5 | 5.8 | 758.7 | 9.5 |
| | Cream | 15.6 | 1699.0 | 6.8 | 424.5 | 7.1 |

In both cultivation systems, on average, the white cultivars displayed greater amounts of Ca, K and Fe, and lower amounts of Na; for P, the white and red varieties are more similar and contain higher amounts than the cream coloured ones; in almost all cases these cream coloured beans contain the lowest levels of Ca, K, Fe and P (Table 1). The white varieties with the highest mineral content were: for K, 99/311, USWK-6 and CELIA (conventional), and SIRIA and FRU4 (organic); for Fe, FRU4 (conventional and organic), and SIRIA and CLARA (conventional); for Ca, CLARA (conventional and organic), and SIRIA (conventional); for P, 99/335 (conventional and organic); those with lowest Na content were CELIA and USWK-6 (conventional) and CLARA and SIRIA (organic). IVT-7214-2 had the greatest Na content in conventional cultivation and MCM3031-10-4 in organic cultivation (data not shown). USWK-6 cultivar was kindly supplied by Dr. Miklas; IVT-7214-2 and MCM3031-10-4 are selections from known resistant IVT-7214 and MCM3031 materials. The others ones have been obtained

by our working group. The cultivation system (conventional or organic), the varietal type, the colour and the seed shape have a significant influence on the mineral content; however, the repetitions and the interactions are not significant, with the exception of what happens with Na. Table 2 indicates the most outstanding results of the ANOVA.

Table 2. Degrees of freedom and p-value for the ANOVA factorial with repetitions, considering the cultivation system with respect to the variety, colour and seed shape.

| Sources of variation | fd | Ca | K | Fe | P | Na |
|---|---|---|---|---|---|---|
| **System of cultivation** | 1 | 0.391 (ns) | 0.008 (**) | 0.014 (*) | 0.003 (**) | 0.000 (**) |
| **Cultivar** | 20 | 0.000 (**) | 0.000 (**) | 0.005 (**) | 0.000 (**) | 0.000 (**) |
| **System of cultivation** | 1 | 0.678 (ns) | 0.196 (ns) | 0.018 (*) | 0.004 (**) | 0.000 (**) |
| **Seed color** | 2 | 0.000 (**) | 0.000 (**) | 0.024 (*) | 0.001 (**) | 0.000 (**) |
| **System cultivation** | 1 | 0.864 (ns) | 0.015 (*) | 0.047 (*) | 0.002 (**) | 0.000 (**) |
| **Seed shape** | 6 | 0.000 (**) | 0.000 (**) | 0.142 (ns) | 0.001 (**) | 0.000 (**) |

Table 3. Phenotypic correlation coefficients (r) between traits. Values of r for conventional cultivation, top-right; for organic cultivation, bottom-left.

| r | Ca | K | Fe | P | Na |
|---|---|---|---|---|---|
| **Ca** | | 0.55 | 0.56 | -0.13 (ns) | -0.07 (ns) |
| | 0.59 | | 0.36 | 0.19 (ns) | -0.30 |
| **Fe** | 0.07 (ns) | 0.10 (ns) | | -0.04 (ns) | -0.10 (ns) |
| **P** | -0.04 (ns) | 0.29 | 0.10 (ns) | | 0.07 (ns) |
| **Na** | -0.25 | -0.23 | -0.28 | 0.01 (ns) | |

In conventional cultivation, the highest correlations are obtained between Ca and K and between Ca and Fe (both positive) and lower and negative between K and Na; the remaining ones are not significant. In ecological cultivation, a positive correlation is maintained between Ca and K; however, the correlation between Ca and Fe is not significant; by contrast, it increases between K and P; and negative correlations are found for K, Ca and Fe with respect to Na. The negative correlations with Na favour the selection of varieties with a high content of other minerals and low in sodium, with the corresponding repercussions on diet; this is more likely to happen with beans grown in organic agriculture.

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# THE INFLUENCE OF ORGANIC CULTIVATION ON PRODUCTIVE COMPONENTS IN DRY BEAN

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## • INTRODUCTION

There are scarce written references about the behaviour of different dry bean cultivars grown in pure organic cultivation systems. Our interest in this issue lies in wishing to offer the consumer a natural food and, at the same time, for its cultivation to be economically viable for the farmer, selecting varieties that fulfil the most suitable productive and nutritive traits. In this work we compare the productive traits in beans grown in conventional and organic cultivation systems, as well as the corresponding correlations, as a basis to consider organic cultivation of dry beans as a real and interesting alternative.

## METHODS AND MATERIALS

A total of 47 cultivars of different varietal types (including controls) were evaluated in organic cultivation and conventional cultivation in Valencia (Spain). The design was in random blocks with two repetitions and 20-25 plants/cultivar x repetition x cultivation type. The evaluation was carried out at individual plant level.

The two test plots had sandy loam soil. The climatic conditions were similar, given the proximity of the two plots. Fertilisation and phytosanitary treatments made the difference; thus, in the plot with conventional cultivation a granulated organic-mineral fertiliser was added to the soil and periodic phytosanitary treatments were carried out using mineral pesticides; in the organic plot, exclusively organic matter was added to the soil, originating from cattle, and no phytosanitary treatments were carried out, weeding was done solely by hand and aphids were fought against by introducing the predator *Coccinella septempunctata* in the plot.

The data were analysed using the ANOVA factorial with repetitions, and correlations between production components were estimated for each cultivation system, as well as the correlations between these and the mineral concentration of Ca, K, Fe, P and Na in the seed.

## RESULTS

There are significant differences between the growing systems and varieties for all the traits; the repetitions do not influence the most important productive characteristics; however, there is an interaction for all the traits between the cultivation system and variety (AB) (Table 1). Several cultivars showed high yield and large seed size together, in both conventional and organic cultivation. This demonstrates that, despite the interaction between cultivation system x cultivar, some cultivars would be suitable for both cultivation systems.

The highest average values for the productive characters have been reached in organic cultivation (Table 2). Average weight/seed was more similar in both.

Table 1. Degrees of freedom and p-value for the ANOVA factorial with repetitions, considering the cultivation system, cultivar and repetition

| Sources of variation | fd | Nº seed/pl | Yield/pl | Av.weight/ seed | Nº pods/pl | Nºseed/pod | Nº loc/pod |
|---|---|---|---|---|---|---|---|
| **System of cultivation (A)** | 1 | 0.000 (**) | 0.000 (**) | 0.009 (**) | 0.000 (**) | 0.000 (**) | 0.000 (**) |
| **Cultivar (B)** | 46 | 0.000 (**) | 0.000 (**) | 0.000 (**) | 0.000 (**) | 0.000 (**) | 0.000 (**) |
| **Repetition (C)** | 1 | 0.318 (ns) | 0.137 (ns) | 0.526 (ns) | 0.561 (ns) | 0.030 (*) | 0.000 (**) |
| **AB** | 46 | 0.000 (**) | 0.000 (**) | 0.000 (**) | 0.000 (**) | 0.000 (**) | 0.000 (**) |
| **AC** | 1 | 0.258 (ns) | 0.136 (ns) | 0.541 (ns) | 0.433 (ns) | 0.003 (**) | 0.426 (ns) |
| **BC** | 46 | 0.013 (*) | 0.018 (*) | 0.880 (ns) | 0.193 (ns) | 0.006 (**) | 0.000 (**) |

*, ** Significant differences at $\alpha$= 5%, and $\alpha$= 1%, respectively

Table 2. Average values for productive characteristics in organic and conventional cultivation

| System of cultivation | Nº seed/pl | Yield/pl | Av.weight/seed | Nº pods/pl | Nºseed/pod | Nº loc/pod |
|---|---|---|---|---|---|---|
| **Organic** | 320.2 | 267.2 | 0.474 | 84.2 | 3.854 | 4.713 |
| **Conventional** | 116.1 | 122.5 | 0.442 | 35.0 | 3.181 | 4.777 |

The greatest positive correlations were found between the total weight/plant and nº pods/plant, total weight/plant and nº seeds/plant, and between nº seeds/plant and nº pods/plant. The negative correlations were found between weight/seed and nº of seeds (per plant and per pod). Despite the different influence exerted by both cultivation systems on productive characteristics, the correlations were maintained within a similar range (Table 3).

Table 3. Phenotypic correlations between productive components, in conventional cultivation (top-right) and in organic cultivation (bottom-left)

| r | Nº seed/pl | Yield/pl | Av.weight/seed | Nº pods/pl | Nºseed/pod | Nº loc/pod |
|---|---|---|---|---|---|---|
| **Nº seed/pl** | | 0.79 | -0.17 | 0.88 | 0.56 | 0.22 |
| **Yield/pl** | 0.78 | | 0.40 | 0.75 | 0.35 | 0.07 (ns) |
| **Av.weight/seed** | -0.37 | 0.21 | | -0.09 (ns) | -0.25 | -0.07 (ns) |
| **Nº pod/pl** | 0.80 | 0.71 | -0.22 | | 0.21 | -0.03 (ns) |
| **Nºseed/pod** | 0.47 | 0.32 | -0.24 | -0.06 (ns) | | 0.40 |
| **Nº loc/pod** | 0.39 | 0.13 | -0.36 | 0.10 (ns) | 0.45 | |

In general, the correlations between the productive traits considered and mineral concentrations are not significant, except for those relating to weight/seed with Fe and K (conventional, -0.25 and –0.33, respectively) and only K (organic, -0.24), which are negative and low for the three cases.

**ACKNOWLEDGEMENTS**

We would like to acknowledge the *Universidad Politécnica de Valencia* for their economic support for the project "New high-protein/dietetic snacks, using vegetable products through organic agriculture (SPAE)".

# SPLIT APPLICATION OF BROADLEAF HERBICIDES IN DRY BEAN

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Broadleaf weeds have become a big problem in dry bean production at central valley of Chile. This crop is produced in irrigated areas, and new weed flushes after watering needs to improve weed control systems to avoid high density after bean flowering. Traditionally, herbicides combination is the most effective way to control high broadleaf weed. Thus, preemergent herbicides are applied before weed emergence, while bentazon or fomesafen are sprayed while weeds are actively growing in a single rate. These herbicides are able to maintain low weed density only a short time. The objective of this experiment was to evaluate the effect of successive sequential applications of bentazon and fomesafen on broadleaf weeds and dry bean yield at the central valley of Chile.

## Materials and methods

An experiment was carried out during the 2001-2002 season at the central valley of Chile, 36º 52´ S. L., 71º 55´ W. L. Beans were planted on November 8, and the single rate and the first rate of the split treatments were sprayed on December 13, with weeds no more than three leaves. The second spraying was 10 days later and the third spraying was 14 days later (Table 1). The experiment was conducted in a randomized complete block with five replications in 5.0 m x 2.0 m plots. The herbicides were sprayed with 200 L/ha of water using a $CO_2$ sprayer at 0.21 MPa. Grasses were controlled with clethodim.

Table 1.Herbicides treatments sprayed on black dry bean cv. Curi-INIA. Chillán 2001-2002

| ***Tratamientos*** | Dosis p. c. (kg o l/ha) |
|---|---|
| 1. Bentazon | 0.96 |
| 2. Bentazon and Bentazon | 0.528 and 0.528 |
| 3. Bentazon and Bentazon and Bentazon | 0.384 and 0.336 and 0.336 |
| 4. Fomesafen | 0.375 |
| 5. Fomesafen and Fomesafen | 0.1875 and 0.1875 |
| 6. Fomesafen and Fomesafen and Fomesafen | 0.125 and 0.125 and 0.125 |
| 7. Weedy check | - |

## Results

Weedy check had the greater broadleaf weed production when beans started pod production, and only bentazon at 0.96 kg/ha was similar, in spite of 35% of reduction. On the other side, split fomesafen 3 by 0.125 gr/ha decreased weed biomass about 80% respect to weedy check and 37% respect to the single rate of fomesafen. Split fomesafen 2 by 0.1875 kg/ha, and bentazon 3 by 0.384 or 0.336 kg/ha also decreased broadleaf weeds about 77% and 60% respect to weedy check, and 52% and 65% respect to 1 by 0.96 kg/ha of bentazon (Table 2). The main weeds were common lambsquarters, common ragweed, black nightshade, smallflower galinsoga, ladysthumb, wild radish, purslane, and jimsonweed. The main weeds affected by sequential respect to the single application were black nightshade and common lambsquarters.

Bean yield was affected by broadleaf weed competition, and all treatments increased yield respect to weedy check. The greater yield was by applying fomesafen and bentazon as split application as either 3 by rate or 2 by rate, although 2 by rate, of both herbicides, were not different respect to the traditional single application (Table 2). Split fomesafen and bentazon by 3 times increased yield respect to the single application about 23%.

Table 2. Herbicide treatments on broadleaf weed dry matter and dry bean yield. Chillán 2001-2002 [(1)]

| ***Treatments*** | Broadleaves Dry matter ($g/m^2$) | Beans Yield (qq/ha) |
|---|---|---|
| 1. Bentazon | 296.7 ab [(2)] | 24.6 b |
| 2. Bentazon y Bentazon | 164.8 b | 28.5 ab |
| 3. Bentazon y Bentazon y Bentazon | 141.7 bc | 30.2 a |
| 4. Fomesafen | 140.3 b | 25.2 b |
| 5. Fomesafen y Fomesafen | 103.3 bc | 27.2 ab |
| 6. Fomesafen y Fomesafen y Fomesafen | 88.3 c | 31.0 a |
| 7. Weedy check | 456.9 a | 10.7 c |
| C.V.(%) | 17.0 | 6.2 |

[1]Data were transformed to log (n) to stabilize variance but are reported as their original values.
[2] Means within a column followed by the same letter are not different, LSD (0.05).

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# YIELD RESPONSE OF 'OTHELLO' PINTO BEAN UNDER SIX IRRIGATION TREATMENTS

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## Introduction

Approximately 18,000 hectares of dry bean are planted every year in central Washington for seed or export to domestic or international markets. In Washington, bean is grown under irrigation and production of high yield and good quality bean is obtained. In any drought years, irrigation is very limited to the perennial crops and other cash crops. Breeders continue to work on high yielding and high quality new bean lines with resistance to viral and fungal diseases and very limited to the other environmental factors like soil fertility, micro nutrients and water requirement to produce the crop. In this experiment, Othello bean was grown 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 135 m long. Plant density is 10 x 56 cm. Research plots were pre-irrigated uniformly before land preparation. Soil nutrients levels were adjusted to 112 kg $ha^{-1}$ of N and 56 kg $ha^{-1}$ of $P_2O_5$. Eptam (4.7 l $ha^{-1}$ ) and Sonalan (2.3 l $ha^{-1}$) were applied pre-plant during land preparation. Othello seeds were treated with a mixture of 5% Apron XL and 10% Maxim 4FS and planted using a 4-row cone seeder on May 24, 2003. All plots were irrigated uniformly by furrow irrigation at 112 cm apart. There were 6 irrigation treatments of 5 replication. Irrigation treatments were:

Trt. 1: irrigated on July 8 only.
Trt. 2: irrigated on July 8, and 15.
Trt. 3: irrigated on July 8, 15, and 22.
Trt. 4: irrigated on July 8, 15, 22, and 29.
Trt. 5: irrigated on July 8, 15, 22, and 29 and August 6
Trt. 6: irrigated on July 8, 15, 22, and 29 and Aug 6 and 13 or control.

Each treatment consisted of 16 rows and only 4 middle rows were harvested. Each plot was 7.5 m in length and 2.23 m wide or 16.76 $m^2$ . All treatment plots received uniform irrigation until full canopy development. The first irrigation treatment started on July 8 and as scheduled every week thereafter for other treatments. Each irrigation is 24 hours set. PAM (polyacrylamide) was used to reduce the sediments in runoff water. Plots were cut and threshed using Hege small plot combine.

Seed harvested was at the seed moisture of less than 8%. Bean was cleaned and weighed for yield. Hundred seeds from each seed lot was weighed.

## Discussion

Normally under central Washington condition, Othello matured in 80 to 85 days after planting. Due to the drought stress, the canopy was smaller in treatments 1 and 2. With the soil moisture in the silt loam soil, the top leaves were still green. Flowering stage was short and number of pods per plant were reduced. Plants of the first and the 2nd treatment were smaller than those of the other treatments. With low soil moisture, plants produced less blooms and shortened reproductive stage and seed fill duration also reduced. There were significant difference in seed yield and seed weight at Pr>F = 0.0004 and 0.0001, respectively. As predicted the plots received water every week produced the highest yield and the plots received no water after full canopy development produced the lowest seed yield. Treatment 3 with 4 irrigations produced the 2nd highest yield but it was only 2000 kg $ha^{-1}$ less than the maximum amount of applied irrigation water.

Seed size did increase with the irrigation water except treatment 1. It may be due to the small pool of pods and seeds produced compared to treatments 2 and 3.

## Conclusion

The results of this study show that dependable amount of water available, Othello can produce yield with good quality bean. With low soil moisture, bean yield and seed size may reduce. High soil moisture at seed fill may not effect pod numbers but seed weight was increased. Low soil moisture at the blooming stage may reduce number of pods due to the abortion of flower. Less water input during pod fill will reduce seed size therefore lower 100-seed weight. Amount of soil moisture from precipitation or water applied will dictate bean yield and seed size of Othello.

Table 1. Effects of Irrigation Treatment on Yield and 100-Seed Weight of Othello Grown in Central Washington in 2002.

| Treatment | Seed Yield lb $a^{-1}$ | Seed Yield kg $ha^{-1}$ | 100 - Seed weight g |
|---|---|---|---|
| 1 | 2875 c | 3229 c | 34.2 bc |
| 2 | 3437 bc | 3853 bc | 32.2 d |
| 3 | 3950 b | 4428 b | 32.8 cd |
| 4 | 3714 bc | 4164 bc | 34.8 b |
| 5 | 3663 bc | 4106 bc | 35.3 b |
| 6 or control | 5716 a | 6407 a | 38.0 a |
| **Mean** | **3893** | **4364** | **34.5** |
| **CV (%)** | **15.6** | **15.6** | **2.9** |
| **Pr>F** | **0.0004** | **0.0004** | **0.0001** |

Each value is the average of 4 replications.

Means in a column with the same letter are not significant difference - LSD (0.05) = 2.13.

# DRY BEAN VARIETIES FOR NICHE MARKETS IN THE U.S.A.

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Dry beans are generally considered a large-scale bulk commodity crop yet they are also well suited to small-scale production for niche markets. Dry beans are harvested in the fall, are easily stored over the winter, and can be a good addition for direct market farmers at the beginning and end of the growing season. Little information is available regarding small-scale production of niche-market dry bean varieties in the United States. In our research we are investigating production potentials of niche-market dry bean varieties with a particular focus on early maturing varieties that are suitable for production in western Washington. Western Washington is characterized by cool summer temperatures and a low number of growing degree days during the growing season (1900 GDD in Vancouver), and most dry bean varieties are harvested 15 or more days later than in the mid-west. This report presents a summary of some of our preliminary findings. Based on our results, we have posted a web page that includes photographs of more than 50 bean varieties and their characteristics including plant height, seed color and shape, and days to maturity in our region, http://eastafricacrsp.wsu.edu/beans/beanVarieties.html.

## Materials and Methods

Replicated field trials were conducted in 2001 and 2002 at WSU Vancouver REU. In 2001, 33 dry bean varieties were planted late, on June 6, and in 2002, 70 dry bean varieties were planted on May 15. Plots measured 2 rows wide and 10 feet long, spacing between rows was 2 feet, and spacing in the row was 2 inches. The field was certified organic and was maintained accordingly. Plants were harvested from the center 5 feet of both rows for a total harvest area of 10 feet per plot. Whole plants were placed in burlap bags, and dried in field ovens for 16 hours at 68$^{o}$ C, until seed moisture was approximately 12%. Beans were threshed and cleaned by hand and total marketable bean weight (g) was measured. 100 beans were randomly selected and weighed from each plot.

## Results and Discussion

In 2002 we planted 3 weeks earlier than in 2001, and more varieties reached maturity due to the earlier planting date. Beans germinated well and grew vigorously in May and early June and thus mid-May appears to be a good planting time for dry beans in western Washington. Varieties differed significantly in days to harvest, total bean yield and weight of 100 beans (Table 1). The earliest varieties were harvested 105 days after planting (DAP) (August 28) and the latest was harvested 131 DAP (September 23). In our region, rains make it impossible to continue harvest beyond the end of September. Mean bean weight per 10 feet of row was 473 g in 2001 and 518 g in 2002. Varieties such as Maine Yellow Eye (286 g), Cannellini (320 g) and Speckled Bays (357 g) are considered low yielding however their marketability is high. Yield is not a primary criterion for selection of niche-market varieties. Yields in our plots increased slightly in 2002 as compared to 2001, likely due to our improved organic management practices, that is, we became better at supplying plant nutrients and controlling weeds organically. The weight of 100 beans did not differ significantly between the two years, therefore increased yield was due to increased number of beans and not due to increased bean size. Disease problems in this trial included halo blight, sclerotinia and several legume viruses. In 2003, we will focus our research efforts on organic management of halo blight and effects of halo blight on farm-stored seed quality.

**Table 1**. Days after planting (DAP) to harvest; bean yield (g) for 10-feet of row; and 100 bean weight (g) of niche market dry bean varieties at WSU VREU in 2001 and 2002.

| | Harv DAP | | BeanYield (g) | | Wt 100 Beans | |
|---|---|---|---|---|---|---|
| **Variety** | **2001** | **2002** | **2001** | **2002** | **2001** | **2002** |
| **Andrew Kent** | 120 | 127 | 572.6 | 685.3 | 58.3 | 74.4 |
| **Beka** | | 105 | | 617.6 | | 42.4 |
| **Black Coco** | 117 | 118 | 416.7 | 584.5 | 54.3 | 56.1 |
| **Candy** | | 119 | | 436.1 | | 139.0 |
| **Cannellini** | 115 | 120 | 320.4 | 528.0 | 59.3 | 52.2 |
| **Cardinal** | 119 | 115 | 452.8 | 506.6 | 63.8 | 62.0 |
| **Celina's Romano** | | 109 | | 161.3 | | 54.3 |
| **Childer's Golden** | | 116 | | 224.8 | | 21.6 |
| **Dwane Baptiste** | | 118 | | 708.1 | | 57.0 |
| **Etna** | 114 | 110 | 639.5 | 608.0 | 69.7 | 62.5 |
| **French Flageolet** | 115 | 129 | 518.1 | 417.1 | 25.4 | 31.5 |
| **French Shell Flambeau** | | 131 | | 575.6 | | 27.6 |
| **G18689** | 115 | 123 | 681.3 | 756.2 | 23.6 | 40.9 |
| **Hutterite** | | 122 | | 308.5 | | 47.2 |
| **Ireland Creek** | | 105 | | 554.1 | | 54.5 |
| **Lake Kivu** | | 111 | | 541.7 | | 52.1 |
| **LeBaron** | 110 | 105 | 344.4 | 401.5 | 34.7 | 61.3 |
| **Maefax** | | 107 | | 550.9 | | 41.8 |
| **Magpie** | | 119 | | 746.4 | | 35.2 |
| **Maine Yellow Eye** | 114 | 115 | 285.5 | 401.5 | 43.5 | 46.9 |
| **Major** | | 105 | | 649.1 | | 36.0 |
| **Mansel Magic** | | 108 | | 627.7 | | 100.2 |
| **Midnight Black Turtle** | 118 | 116 | 517.4 | 633.8 | 17.9 | 18.8 |
| **Molasses Face** | | 108 | | 562.8 | | 45.7 |
| **Montezuma Red** | 111 | 109 | 401.0 | 606.2 | 36.3 | 35.0 |
| **Mrocumiere** | | 123 | | 628.9 | | 54.7 |
| **Norwegian** | | 105 | | 664.2 | | 37.8 |
| **Nugget** | | 113 | | 256.2 | | 32.0 |
| **NW-63** | 116 | 110 | 655.0 | 513.2 | 34.6 | 33.6 |
| **Old Fashioned Soldier** | 114 | 112 | 575.9 | 634.6 | 66.2 | 55.0 |
| **Orca (USWA-27)** | 117 | 115 | 435.2 | 563.1 | 32.5 | 35.4 |
| **Red Soldier** | | 113 | | 510.1 | | 59.5 |
| **Serene** | | 112 | | 327.4 | | 66.2 |
| **Speckled Bays** | 116 | 111 | 357.1 | 400.5 | 49.5 | 49.4 |
| **Stevenson Blue Eye** | | 120 | | 487.4 | | 50.3 |
| **Thort** | 114 | 112 | 452.2 | 532.0 | 55.3 | 53.7 |
| **Tongue of Fire** | 116 | 110 | 502.9 | 294.9 | 58.8 | 52.0 |
| **Trout/Jacobs Cattle** | 114 | 110 | 394.3 | 357.7 | 66.5 | 66.7 |
| **UI-911** | 118 | 119 | 487.1 | 605.8 | 20.1 | 20.4 |
| **Vermont Cranberry** | 112 | 120 | 457.1 | 530.5 | 48.8 | 48.5 |
| **Zert** | | 112 | | 549.6 | | 44.8 |
| **P-value** | **0.000** | **0.0000** | **0.0000** | **0.0000** | **0.0000** | **0.0000** |

# SURVEY OF WASHINGTON DRY BEAN PRODUCTION

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Dry beans are grown in Washington as a seed crop, a bulk commodity grain crop and a direct market grain crop. In 2002, we surveyed dry bean farmers in Washington to better understand farmers' perceptions regarding dry bean production, storage and marketing in the region. This paper presents a few of our findings from this survey.

## Materials and Methods

We developed a simple questionnaire with 13 questions pertaining to dry bean production, storage and marketing. The questionnaire was distributed to 124 farmers by mail, and follow up was conducted by email (30) and telephone interviews (88). The distribution list for the questionnaire included names of dry bean farmers provided by extension agents, the Washington organic farmer list, Farmers Market managers and respondent farmers who referred us to other farmers who grew dry beans.

## Results and Discussion

Obtaining names of large-scale dry bean farmers was very difficult. Dry bean contracting companies did not give out names of their contract farmers and extension agents did not have lists for their counties. There are no dry bean meetings in the state where we could make contact with dry bean farmers. More work is needed to locate addresses of large-scale dry bean farmers in Washington. We were able, however, to locate a good number of commercial small-scale farmers through the organic farming list and farmers markets.

Of the 124 farmers we attempted to contact, only 46 farmers responded (37%). Factors that contributed to the low response rate included: farmers were no longer living at listed addresses; and farmers' listed telephone numbers had changed. Telephone interviews produced the greatest number of responses essentially because we made repeated calls to each farmer, and we conducted the interview at his or her convenience.

The 46 respondents were from 18 counties in Washington. In 2001, Washington Ag. Statistics reported 315 large-scale dry bean farmers located in 4 counties in eastern Washington. In our survey, 11 (24%) respondents were located in eastern Washington and 9 of these were large-scale farmers (Table 1). The remaining 37 (80%) respondents were small-scale farmers, and 29 (63%) were in western Washington. This survey did not adequately capture the large-scale dry bean producers in the state, however the survey did show that small-scale farmers are currently growing dry beans in western Washington.

Of the respondents, 21 (46%) were female. In some households, the husband and wife farmed together, and in these cases we based 'respondent farmer gender' on the gender of the primary person who responded to the questions. In our survey, all large-scale respondents were male whereas only half of the small-scale respondents were male. These results imply that females actively participate in small-scale farming and do not participate in large-scale farming.

**Table 1.** Gender and geographic distribution of farmers in Washington who responded to our dry bean production questionnaire in 2002.

| Respondent Farmers | Regions | | | | | Total |
|---|---|---|---|---|---|---|
| | Northwest | Southwest | North Central | South Central | East | |
| **Female** | 14 | 3 | 2 | 2 | 0 | 21 |
| **Male** | 11 | 1 | 1 | 1 | 11 | 25 |
| **Total** | 25 | 4 | 3 | 3 | 11 | 46 |
| **% Respondent Farmers** | 54 | 9 | 6.5 | 6.5 | 24 | 100 |
| **Large : Small*** | 0 : 25 | 0 : 4 | 0 : 3 | 0 : 3 | 9 : 2 | 9 : 37 |

* Number of large-scale farmers : Number of small-scale farmers

The 46 respondent farmers grew a total of 69 varieties of dry beans. Large-scale respondents grew on average 2 varieties of dry beans each year and pinto, red kidney and small red beans were the primary types of dry beans that they grew. Small-scale respondents grew 1-20 varieties of dry beans each year, 4 varieties on average per respondent, and there were no dominant types of dry beans grown by small-scale respondents. Total dry bean production area for each large-scale respondent was 18-450 acres, and 155 acres on average. Total dry bean production area for each small-scale respondent was a minimum of 10-row feet and a maximum of 1¼ acres, and 0.13 acres on average. All small-scale respondents operated commercial farms and sold or bartered their dry bean crop.

All 9 large-scale respondents produced dry beans on contract for seed-supplying companies and harvested beans were transported back to the company for storage and marketing. Of the small-scale respondents, 3 did not store beans and 34 stored their beans on-farm for 1 to 6 years for their own consumption or for direct marketing purposes. The most common small-scale on-farm storage techniques were sacks and glass jars.

Of the respondents, 24 purchased dry bean seed each year, 10 saved seed from their crop, 1 bartered seed, 7 purchased and saved seed, and 4 purchased, saved and bartered seed. Of the small-scale respondents, 8 did not save seed, 24 saved seed for 1-3 years and 5 saved seed for more than 3 years. Of the large-scale respondents, 8 did not save seed and 1 saved seed for up to 5 years.

Regardless of the scale of production, most of the respondents (31 or 67%) did not observe any disease problems in their dry bean crop and felt that they have had healthy crops in the past. The remaining 15 farmers observed disease symptoms such as mold, seedling wilt, brown leaf spot, pod rot and anthracnose. Symptoms of Beet Curly Top Virus and Bean Yellow Mosaic Virus were observed by 4 farmers. Most of the small-scale bean farmers were organic growers and did not use any chemical pest control measures. Respondents rated weeds as the number one problem in dry bean production (26%), followed by poor germination (22%), late maturity (20%), diseases (20%) and shriveled beans (13%). There was no correlation between saving seed and poor seed germination. Some respondents also reported that inadequate tools for small-scale dry bean threshing was a major constraint to increasing dry bean production.

# EVALUATIONS OF SNAP BEAN CULTIVARS WITH DETERMINATE AND INDETERMINATE GROWTH.

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## Introduction

Snap beans are largely produced in Brazil using climbing cultivars. However, the production of staked beans is labor intensive and requires a high initial investment.

Cultivars with determinate growth presents great potentiality in Brazil. That would bring advantage at planting time, in the development stage and in the harvest, mainly when it is growing in great extensions.

The objective of the experiment was to evaluate the agronomic and commercial traits of snap beans cultivars with determinate and indeterminate growth.

## Material and Methods

The experiment was conducted in a soil classified as Red Latosol (Eutrustox) (LVef), loamy texture, in the period of September to November of 2002 (sub tropical spring time) in the Londrina-Paraná State, Brazil.

It was used cultivars of determinate growth (Alessa, Macarrão Rasteiro, Mimoso Rasteiro and UEL - 1) and indeterminate growth (Macarrão Favorito AG480 and Feijão Teresópolis AG484). Fertilizers were applied, equivalent to 1 t/ha of the fertilizer NPK (04-14-08) in the sowing and 200 kg/ha of ammonium sulfate 15 days after the emergency (DAE).

The statistical design was randomized complete blocks, with four replications. The plots consisted of four two-meter row spaced at 0,5 m with 10 seeds sown per meter. The evaluations were made in eight plants removed at random from the two central rows of each plot. The appraised traits were: beginning, end and period of flowering, length (cm) and diameter (mm) of pod and fresh matter weight of commercial (equal or bigger than 10 cm) and non commercial pods commercial pods (smaller than 10 cm).

Results and Discussion

The beginning and the end of the flowering set over a smaller period in determinate growth cultivars than in climbing habit. The flowering duration in UEL-1 was as longer than indeterminate growth cultivars (Table 1). With a concentrated pod set and maturity that were well adapted to once-over destructive mechanical harvesting. In other hand, environmental problems like water or temperature stress should have more effect in production in cultivars with determinate growth habit.

The length and the diameter were larger in indeterminate growth cultivars (Table 2). That can be a commercial problem of snap beans pods produced by determinated growth cultivars. The Brazilian consumer, in a general way, prefers the fruits characteristics of indeterminate growth cultivars. In Alessa, the pods presents length and diameter closer to indeterminate growth cultivars. The genotypes with bigger productive potential of pods were the Feijão Teresópolis, UEL-1 and Alessa, that produced approximately two times more than the other three cultivars. The cultivars of habit indeterminate growth produced smaller amounts of green beans smaller than 10 cm length. The biggest sum of fresh matter was observed in UEL-1. To increase the

utilization of small pods like UEL-1, those should be applied, for example, in the production of soups or processed cut pods.

**Table 1** - Plant habit, beginning and end of flowering, period of flowering in six snap bean cultivars

| Cultivars | Plant habit | Beginning of flowering (DAE)* | End of flowering (DAE) | Period of flowering (days) |
|---|---|---|---|---|
| Macarrão Favorito AG480 | Indeterminate | 47 | 69 | 22 |
| Feijão Teresópolis AG484 | Indeterminate | 48 | 72 | 24 |
| Alessa | Determinate | 40 | 52 | 12 |
| Macarrão Rasteiro | Determinate | 42 | 57 | 15 |
| Mimoso Rasteiro | Determinate | 42 | 59 | 17 |
| UEL - 1 | Determinate | 36 | 58 | 22 |

*Days after emergence

.

**Table 2 –** Pod length average, pod diameter average, and fresh matter of commercial pods and fresh matter of non commercial pods, 32 plants production sum.

| Cultivars | Length (cm) | **Diameter** (mm) | Fresh Matter of commercial Pods* (g) | Fresh Matter of Non Commercial Pods** (g) |
|---|---|---|---|---|
| Macarrão Favorito AG480 | 14.8 | 9.6 | 2260.0 | - |
| Feijão Teresópolis AG484 | 19.4 | 12.0 | 4287.5 | 128.0 |
| Alessa | 14.5 | 8.9 | 3535.0 | 276.5 |
| Macarrão Rasteiro | 13.5 | 7.9 | 1835.0 | 183.1 |
| Mimoso Rasteiro | 11.7 | 7.9 | 1607.1 | 476.0 |
| UEL - 1 | 11.9 | 8.4 | 3955.8 | 834.,1 |

***Pods> 10,0 cm** Pods < 10,0 cm**


## Acknowledgments

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# PRODUCTION OF SNAP BEANS (cv UEL-2) IN RELATION TO DOSES AND SOURCES OF NITROGEN APPLIED IN COVERING

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## Introduction

Because of the development of new cultivators of snap beans it is necessary to set trial in order to calibrate dosages and to define forms of applications of nitrogen fertilizers. The nitrogen fertilization is very important for snap beans once there are no commercial inoculants with specific indication for this crop regarding to biological nitrogen fixation. The root nodulation normally is due to native strains of Rhizobium (Franco & Balieiro, 1999), not always efficient. The increase of available nitrogen in the soil by covering nitrogen fertilization, may improve the bean yield even when inoculated (Franco et al., 1979 , Vidor et al., 1989).On the other hand, when the nitrogen content is not enough to support the crop needs, 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 number and yield of commercial pods of snap beans.

## Materials and methods

The experiment was carried out in greenhouse. The substrate was collected from de surface layer of clay Oxissol from de Experimental Station of State University of Londrina. 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 wetter lost by evapotranspiration. Each vessel was fertilized with an equivalent rate of 400 kg ha-1 of the formulation 04-14-08. The reagent crop was the cultivar UEL-2 of snap bean, leaving two plants in each vessel. The experimental design was completely randomized with four replicates in 4x2 factorial arrangement, with 4 doses (0, 30, 60 and 90 kg ha-1 of N) and 2 sources (ammonium sulphate (**AS**) and urea (**U**). The nitrogen fertilization in covering was applied at the 23 days after emergence (**DAE**). The harvest was made 43 **DAE**, evaluating the number of commercial pods (**NCP**) and fresh mater weight of commercial pods (**FMWCP**). The data were submitted to variance analyses and polynomial regression.

## Results and discussion

The NCP and FMWCP increased linearly with the nitrogen doses when AS was used. Using **Ur**, the adjust was significant ($P<0,05$) for the second degree polynomium with maximum for **NCP** and **FMWCP** in 78,1 and 88,2 kg ha-1 of N, respectively (Fig 1 and 2).

**Figure 1. Number of commercial pods**

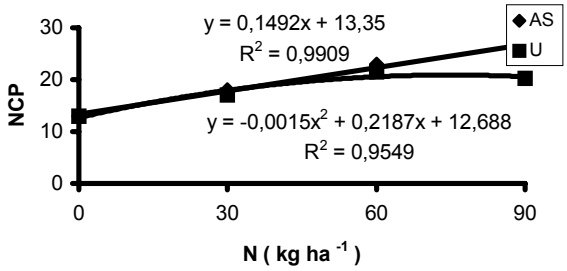
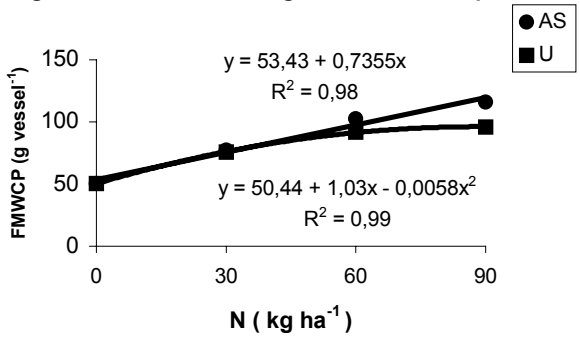



**Figure 2. Fresh matter weight of commercial pods**




In general it could be observed that: a) The **NCP** increased linearly with ammonium sulphate, with its maximum at 78,1 kg ha-1 of N for urea. b) The **FMWCP** increased linearly when ammonium sulfate was used, however for urea the maximum yield was got wit the dose of 88,8 kg ha-1 of N.

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# PRODUCTION AND PROTEIN CONTENT OF SNAP BEANS (CV UEL-2) COMMERCIAL PODS SUBMITTED TO COVERING NITROGEN FERTILIZATION

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## Introduction

Nitrogen is a nutrient demanded in great amount by most of the cultures, mainly for those cultivated in clay soils with low organic matter content. A strategy of handling of the application of the nitrogen will define the largest or smaller benefit of this nutrient for the production and quality of the products (Malavolta, 1980). Nitrogen fertilization is very important for the bean culture because there is not specific efficient commercial inoculating strain in the biological nitrogen fixation process. The increase of available nitrogen in the soil, by covering nitrogen fertilization, may increase the productivity even of the inoculated bean plant (Franco et al, 1979, Vidor et al., 1989). Pinto et al. (1999), observed that low nitrogen nutrition reduces soluble protein contents in the leaves, photosynthesis rate and dry matter accumulation in different cultivars of *Phaseolus vulgaris L.*. This experiment was carried out with the objective of evaluating the pod production and protein content in commercial pod of snap beans (cv UEL-2) submitted to covering nitrogen fertilization.

## Materials and methods

The experiment was carried out in greenhouse. The substrate was collected from de surface layer of clay Oxissol from de Experimental Station of State University of Londrina. 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 wetter lost by evapotranspiration. Each vessel was fertilized with an equivalent rate of 400 kg $ha^{-1}$ of the formulation 04-14-08. The reagent crop was the cultivar UEL-2 of snap bean, leaving two plants in each vessel. The experimental design was completely randomized with four replicates in 4x2 factorial arrangement, with 4 doses (0, 30, 60 and 90 kg $ha^{-1}$ of N) and 2 sources (ammonium sulphate (**AS**) and urea (**Ur**). The nitrogen fertilization in covering was applied at the 23 days after emergence (**DAE**). The harvest was made 43 **DAE**, evaluating the dry mater weight of commercial pods (**DMWCP**) and protein content in commercial pod (**PCCP**). The data were submited to the variance analyses and polynomial regression.

## Results and discussion

**DMWCP** increased lineally for the two N studied sources (Fig.1), indicating the possibility of obtaining of highest productivities of snap bean culture, by covering application, of nitrogen doses higher than 90 kg ha-1.

**Figure1. Dry matter production of commercial pods**

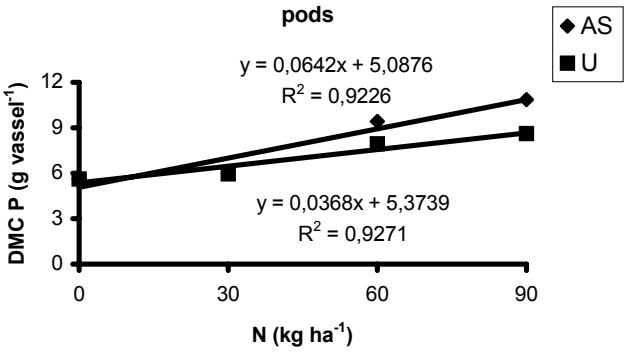
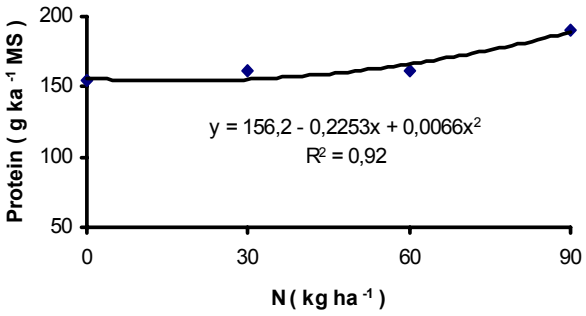



**Figure 2. Protein content in dry matter of commercial pods to source ammonium sulphate.**




It was not observed significant effect of N doses on **PCCP**, when the urea was used. However, the obtained data with the **AS** were adjusted to the polynomial function of second degree ($P < 0,05$), defining a minimum **PCCP** in 17 $kg^{-1}$ of N (Fig. 2). This result is in agreement with Pinto et al (1999), that observed low protein content in leaves in different bean species cultivated under low N nutritional level.

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# POSSIBLE CONTRIBUTION OF MESOAMERICAN PHENOTYPE IN SNAP BEANS CULTIVATED IN SECONDARY CENTERS

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## Introduction

Snap bean genotypes grown in Colombia are often affected by several biotic stresses. In order to carry out a pre-breeding process, we wanted to know the extent of genetic variability of the parental materials to be used (Kami and Gepts, 1991; Myers and Baggett, 1999). Phaseolin type is an important evolutionary genetic marker. In this paper, we wanted to compare, using morphological descriptors and biochemical markers (phaseolins), the snap bean varieties cultivated in secondary centers with the genetic pools distributed in the Americas, i.e. the Mesoamerican, Colombian or Andean domestication centers.

## Materials and methods

One hundred and sixteen snap beans genotypes from URG-CIAT bean germplasm bank coming from Europe, Asia, Africa and America, two commercial genotypes of snap beans [Blue lake Ferry and Milenio (G51158)]. As controls four common bean accessions, G4494(cultivated material proceeding from the Andes), G23725 (ecuatorian wild type), G21117 (Colombian wild type) and G5733 (cultivated genotype coming from Mesoamerica) were used, in order to make a comparison to the American genetic pools. Pod characteristics (fiber content 15% humidity, length, shape, seed number) and seed characteristics (color and weight) were used as morphological descriptors (Muñoz et al, 1993). Phaseolin patterns were analyzed by SDS-PAGE as in Gepts et al. (1986).

## Results and discussion

In previous reports (Gepts 1988; Ocampo et al. (2002) most of the European materials studied by them, are Andean in origin. However, data obtained in this study are not in agreement with those results. namely with respect to data from Europe , Africa and USA (Table 1). Seven phaseolin types were found. The "S" type was present at the highest frecuency (53% of the genotypes), followed by "T" type (17%), "C" type (19%), "CH" type (6.9 %), "Sb" type (2%), "$H_I$" type (0.86%) and heterozygotic "H(S+I)" type (0.86%). We found that twenty-two accessions (28%) were atypical, showing phenotypical characteristics of Andean materials (seed type) and Mesoamerican "S" phaseolin type. These genotypes with contrasting morphological and biochemical characteristics were found only in traditional varieties (n=78). These genotypes have creamy and spotted seeds, medium size, and rounded or kidney shape seeds (Table 2).

Fourty six genotypes out of sixty-two phaseolin S materials are traditional varieties. Nevertheless, in spite that the sample from USA is the most numerous and contains more S phaseolin genotypes, none of the accessions coming from USA showed morphotypes originated by hybridization between the American origin centers excepted the material with heterozygote phaseolin type [H(S+1)]. This type has been reported in common bean by Ocampo et al. (2000). These results suggest that the contribution of Mesoamerican types to the secondary centers of domestication and diversification of snap bean, is higher than the value accepted currently.

Table 1. Geographical distribution of phaseolin types found in snap bean.

| Continental region | Number of accessions | S | T | C | CH | $H_1$ | Sb | H(S+1) |
|---|---|---|---|---|---|---|---|---|
| **Mediterranean Europe** | 18 | 2 | 1 | 11 | 2 | 1 | 1 | |
| **Central-Eastern Europe** | 16 | 10 | 1 | 2 | 3 | | | |
| **Western Europe** | 6 | 5 | | 1 | | | | |
| **Asia** | 6 | 3 | 3 | | | | | |
| **China*** | 17 | 12 | 2 | | 2 | | 1 | |
| **Turkey*** | 16 | 5 | 6 | 4 | 1 | | | |
| **USA*** | 27 | 18 | 6 | 2 | | | | 1 |
| **Rest of America**[1] | 8 | 5 | 1 | 3 | | | | |
| **África** | 3 | 3 | | | | | | |
| **Total accessions** | 118 | 63 | 20 | 23 | 8 | 1 | 2 | 1 |

*Countries with highest contributions to the sample. [1]South America contribution was only two samples.

Table 2. Description of atypical "S" phaseolin type snap bean landraces found in this study.

| Accession | Country | Seed weight | Seed color | %Fiber | Seeds per pod | Pod length | Pod diameter ratio | Phaseolin type |
|---|---|---|---|---|---|---|---|---|
| G621 | TUR | 23.0 | Cream | 21.54 | 4,1 | 7,5 | 0,48 | S |
| G10134 | NLD | 42 | Color mix* | 13.59 | 5,5 | 12,63 | 0,48 | S |
| G10214 | PRT | 45 | Color mix* | 13.12 | 5,5 | 11.8 | 0,74 | S |
| G10220 | PRT | 48 | Cream | 17.94 | 6,1 | 14,64 | 0,62 | S |
| G10222 | PRT | 38.7 | Cream | 12.35 | 7,6 | 15,5 | 0,7 | S |
| G10233 | PRT | 38 | Cream | 8.04 | 7,9 | 11,71 | 0,52 | S |
| G13431 | CHN | 40 | Brown | 11.63 | 5,8 | 11,56 | 0,57 | Sb |
| G14722 | ITA | 23 | Cream | 18.36 | 6,3 | 11,15 | 0,94 | S |
| G15300 | ZMB | 26 | Color mix* | 6.98 | 7,5 | 11,49 | 0,76 | S |
| G15660 | MEX | 30 | Spotted | 22.77 | 4,3 | 10,33 | 0,53 | S |
| G15913 | NLD | 39.1 | White | 16.69 | 5,4 | 13,55 | 0,68 | S |
| G17861 | HUN | 32.5 | Cream | 17.54 | 6,5 | 12,5 | 0,97 | S |
| G18212 | ESP | 29 | Cream | 5.14 | 6,2 | 13,12 | 0,68 | Sb |
| G19268 | SUN | 41 | Spotted* | 13.25 | 4,9 | 11,4 | 0,74 | S |
| G19279 | CHN | 44 | Color mix* | 7.75 | 4,7 | 9,4 | 0,77 | S |
| G20401 | CHN | 29 | Cream | 15.54 | 5,8 | 11,03 | 0,54 | S |
| G20624 | IND | 31 | Cream | 14.87 | 7,6 | 15,4 | 0,87 | S |
| G23952 | CHN | 39 | Cream | 10.24 | 6,9 | 14,5 | 1,12 | S |
| G24544 | CHN | 41.4 | Spotted* | 8.29 | 6,4 | 14,66 | 0,69 | S |
| G50638 | CHN | 35.9 | Spotted* | 5.5 | 5,2 | 10,35 | 0,71 | S |
| G50639 | CHN | 30.5 | Spotted* | 20.14 | 6,1 | 12 | 0,64 | S |
| G50640 | CHN | 30 | Spotted* | 10.84 | 6,7 | 14,75 | 0,6 | S |

*Includes creamy seeds

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# GENETIC ANALYSIS ON AGRONOMIC TRAITS IN SNAP BEAN

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## Introduction

In snap bean, studies about inheritance of important agronomic traits for plant breeders are scarce. Besides, there are many controversial results considering the same characteristics (Leal et al. 1979; Rodrigues et al. 1998; Carvalho et al. 1999).

Nevertheless, planning and executing a breeding program require correct information on the genetic systems controlling interest traits, expecting gains can be maximized with use of the selection process (Vencovsky and Barriga 1992; Barelli et al. 1999).

In this case, diallel analysis is an adequate strategy, considering it offers large amount of genetic information. Among methodologies of diallel analysis, Hayman´s procedure (1954), has greatly contributed to breeding because it provides an efficient study of the genetic action involved in the control of traits and identifies the presence of epistatic interactions. It also estimates the genetic component of genotype determination and the limit of selection which may be obtained from assessed parents (Cruz and Regazzi 1995; Barelli et al. 1999; Bonett el al. 2001).

The present study was carried out using a diallel cross system based on Hayman´s methodology (1954) among five divergent snap bean accessions to obtain genetic information for agronomic traits to start a breeding program.

## Materials and Methods

The snap bean accessions UENF 1429, UENF 1432, UENF 1442, UENF 1445 and UENF 1448, from vegetables germplasm bank at UENF (Darcy Ribeiro North Fluminense State University), 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 Hayman`s diallel analysis (1954) was carried out based on Cruz and Regazzi (2001), considering that homozygous parents were different in only one locus (**T/t**), and presented desirable alleles in an $\mathbf{u_1}$ ratio and undesirable alleles in a $\mathbf{v_1}$ ratio. The GENES program (CRUZ, 2001) was used for this analysis.

## Results and Discussion

Additive genetic effects were predominant for pod weight per plant, number of seeds per pod, height of the insertion of the first pod and number of days to flowering while non-additive effects were more important for number of pods per plant. Using the accessions ´per se` in breeding programs would be possible to obtaining genetic gain for the first four traits, while breeding system with crossing are desirable for improve other traits.

The analysis also revealed that dominant alleles increased the number of pods per plant, pod weight per plant and number of seeds per pod. Moreover, although the recessive alleles were responsible for increase in the height of the insertion of the first pod and number of days to flowering, considering practical aspects from farmers point of view, major interesting is to reduce of these traits. Achieving this objective, dominant alleles are desirable to obtain pure lines with lower insertion of first pod and plants with short yield season.

Allelic interaction was overdominance to the number of pods per plant, while the partial dominance worked on the expression of other traits. Excepting number of pods per plant, there were high coefficients of genetic determination for the other traits, indicating that superior lines may be obtained using simple selection methods.

## Conclusions

1. Genes with additive action were responsible for the expression of the major traits evaluated.
2. There is great chance of developing superior genotypes in breeding programs.

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# POD CLASS DEFINITION BASED ON LENGTH AND WIDTH IN COMMON BEANS (*Phaseolus vulgaris* L.)

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Plant architecture improvement is one of the objectives of the bean breeding program. Among the various advantages presented by an erect bean plant type relates to the fact that pods do not touch the ground, which, in consequence, contributes to prevent rotting and harvest losses, assuring good grain quality. Pod size is related to this aspect and represents one of the plant traits that varied the most with species domestication. Wild *Phaseolus* relatives present much smaller pods than cultivated ones. The establishment of standards defining pod classes according to pod dimensions appears to be a valuable criterion to plant characterization besides being a practical and objective plant descriptor.

The objective of this work was to indicate acceptable limits to classify pod size utilizing pod measurements (length and width) analysis from 273 common bean genotypes with grains from several commercial Brazilian classes represented by cultivars and lines morphologically characterized in experiments conducted at Embrapa Arroz e Feijão, in Goiânia, GO, Brazil.

Evaluation was performed using 20 pods per genotype. Length was measured from the peduncle connection point to pod apex, excluding the pod beak, and width was taken on the middle portion of the pod, utilizing a Mitutoyo Digimatic Caliber. To examine the data distribution into classes from genotype length evaluation, two criteria were used: the average ± 1 standard deviation (A ± 1SD); and average ± 2 standard deviation (A ± 2SD).

Results obtained indicated an average pod size of 10.2 cm, with maximum and minimum values ranging from 15 to 7.5 cm. Utilizing the value (A ± 1SD), it was verified that 73.6% fit into the intermediate class size, 12.1% into short, and 14.3% into long. When values were established by the second criterion (A ± 2SD), classification was 94.5%, 0%, and 5.5%, respectively, into intermediate, short, and long pod classes. These results indicate that (A ± 1SD) criterion is better suited to pod classification, setting pod length as follows: < 8.79 cm - **short**; 8.80 to 11.55 cm - **intermediate**; and >11.55cm - **long** (Figure 1).

In terms of width, the average value obtained was 9.13mm, with maximum and minimum values ranging from 16 to 4.1mm. Even though not being related to plant architecture, pod width may be used to pod classification. However, the fact that this trait is less variable than pod length suggested a third criterion [(A±0,5 SD) + (A±1 SD)] to set class limits, in addition to the two ones previously used to classify pod length (A±1SD) and (A±2SD). Using (A±1SD), 7.3%, 83.9%, and 8.8% were classified, respectively, as slender, intermediate, and wide. When width limits were set using the second criterion (A±2SD), width distribution was 94.1% as intermediate, 1.1% as slender, and 4.7% as wide. Finally, when the third criterion was used it allowed pod width discrimination into five classes (Figure 2), as follows: 7.3% **very slender** (< 7.84 mm); 16.1% **slender** (7.85 - 8.49mm); 56.4% **intermediate** (8.50 - 9.77mm); 11.4% **wide** (9.78 - 10.42mm); and 8.8% **very wide** (> 10.42mm).

Such characteristics as erect plant type, resistance to lodging, uniformity of maturation, dehiscence resistance and desirable first pod insertion, associated to small sized pods without grain size reduction, are well suited to mechanical harvest and to achieve good product quality with high commercial value.

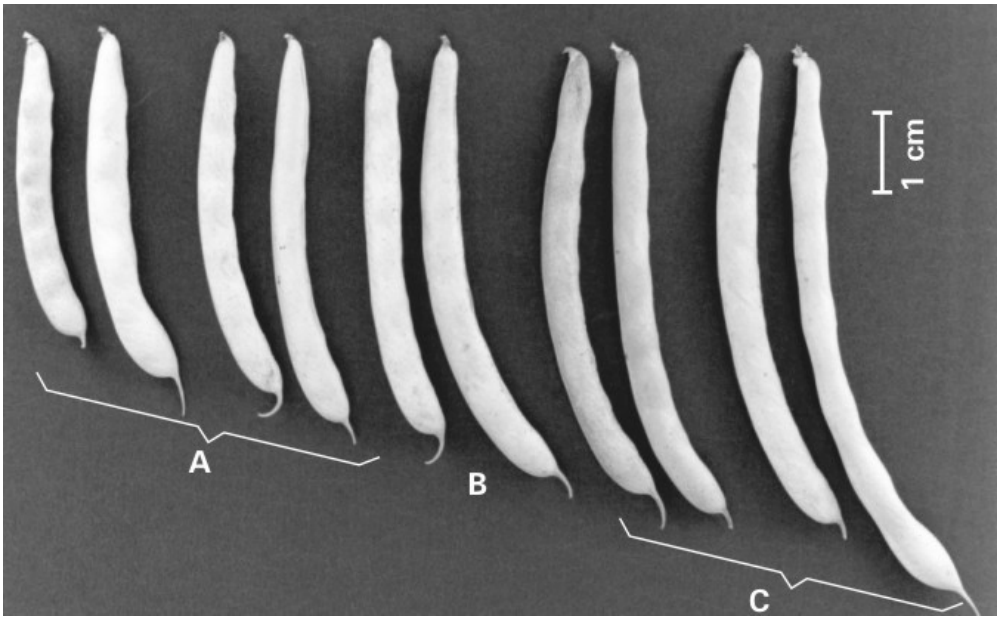


**Figure 1.** Common bean pod length classes: A - short; B - intermediate; C - long.

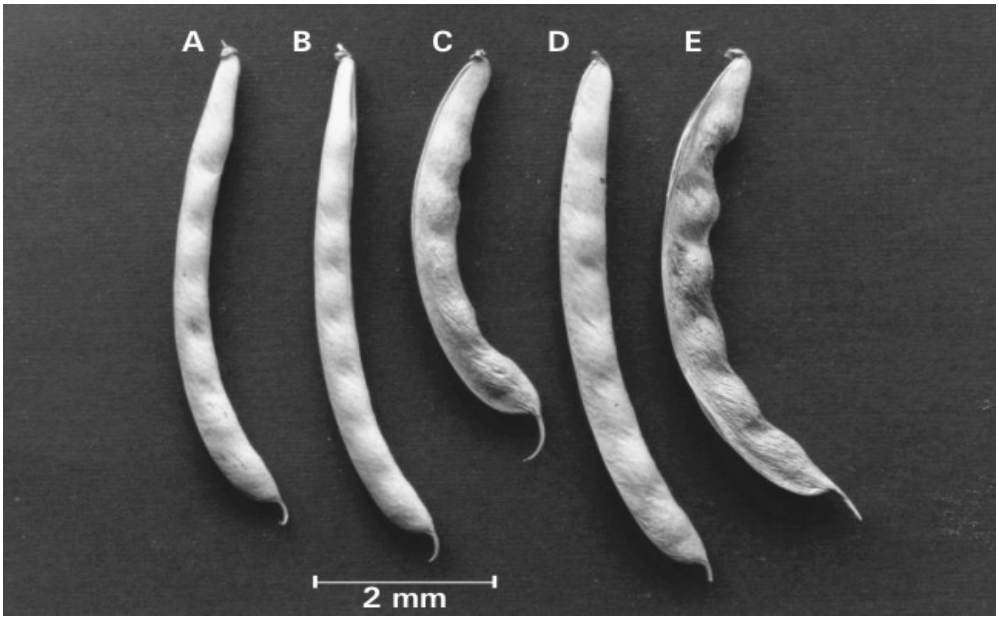


**Figure 2.** Common bean pod width classes: A - very slender; B - slender; C - intermediate; D - wide; E - very wide.

# SENSORY ANALYSIS OF BREEDING MATERIAL FROM GARDEN BEAN RESISTANT TO *ACANTOSCELIDES OBTECTUS*

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The purpose of the present investigation was to characterize new created garden bean lines resistant to *Acantoscelides obtectus* by using of sensory analysis and to estimate the possibility for their use as a raw material in canning. The study was carried out with 11 flat pod breeding lines and cultivar Starozagorski cher as a standard. The plants were harvested in industrial ripeness at dry matter content from 10.01% to 14.00%. The sterilization was performed according to Bulgarian Technological Instruction (1983) without adding of NaCl.

Sensory properties of bean were evaluated based on the following characters: *external appearance, colour, aroma, skin integrity, pod suture uniformity, pod flatness, crispness, succulence, tenderness, consistence, taste, free of string and parchment layer*. It was used a 0 to 5 scale of estimation, with step 0.25. The total sensory evaluation was not formed as average arithmetical quantity but as total panelist perception for organoleptic values of the samples. The obtain results were calculated by analysis of variance (Lakin, 1990) and Duncan's multiple range test (1955).

The breeding lines were harvested in optimal dry matter content. In this stage the seed beds in the pods were not well formed, the pods had shallow and narrow suture. That is why the assessment of the characters *skin integrity, pod suture uniformity and pod flatness* had close and high value of the organoleptic evaluation (Table 1). The same characters have been optimized in the first generations after hybridization. There was a great variation in the visual evaluation of pod colour – from intensively green and homogeneous in No 1 to uneven and non-intensive in No 6. The studied lines had a typical bean aroma – delicious and fresh, without external nuances. According to this character all lines obtained high sensory evaluation.

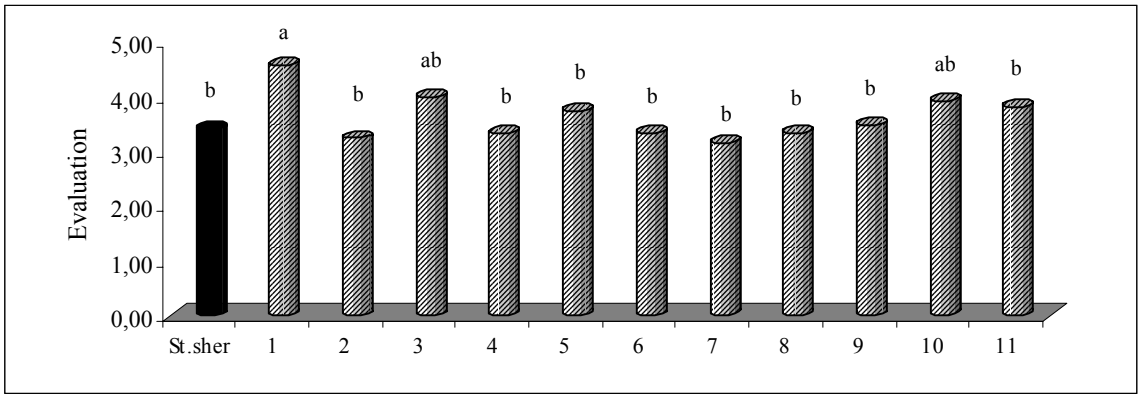
Concerning flavour properties most of the lines exceeded the standard cultivar Starozagorski cher. Regarding to the character *free of string* the mean evaluation was the lowest one (3.6), as well as the coefficient of variability was highest one (10.85%). The estimation of lines 1, 10 and 11 was comparatively high because the string was practically absent and almost it didn't feel during consumption. In the lines 2 and 7 the string had hard structure. It was located along the pod length and during consumption caused a nasty taste. The same breeding material obtained poor evaluation on the remaining taste characters - the pods were tougher, hard cracking, dry, with thick and rough parchment layer. This fact could be explained only with the quality of the parents, involved in the hybridization and different gene recombinations in the breeding lines. In some of them unfavourable characters had transferred together with the resistance to *Acantoscelides obtectus*. In others, such as line 1, the recombinogenesis had not only eliminated great part of the negative properties of the resistance carriers but also had improved the organoleptic properties of the pods to a considerable extend.

Line 1 had the highest total sensory evaluation (Fig.1). This fact proves the possibility for creation of cultivars, combining resistance to *Acantoscelides obtectus* with good sensory characters. Breeding and introduction in practice of garden bean cultivars resistant to *Acantoscelides obtectus* is the only way to obtain high quality and healthy products and in the same time to protect the environment from using pesticides.

Table 1. Sensory evaluation of the studied garden bean lines

| Breeding lines | ***Sensory characters*** | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | External appearance | Colour | Aroma | Skin integrity | Pod suture uniformity | Pod flatness | Crispness | Succulence | Tenderness | Consistence | Free of | | Taste |
| | | | | | | | | | | | String | Parchment layer | |
| 1 | 4.2 | 4.4 | 4.8 | 4.0 | 4.3 | 4.2 | 4.4 | 4.3 | 4.3 | 4.4 | 4.4 | 4.5 | 4.5 |
| 2 | 3.8 | 4.0 | 4.4 | 4.0 | 3.7 | 3.7 | 3.5 | 3.5 | 3.4 | 3.3 | 3.0 | 3.3 | 3.3 |
| 3 | 3.9 | 3.9 | 4.3 | 3.5 | 3.7 | 3.5 | 4.1 | 4.1 | 4.1 | 4.1 | 3.6 | 4.0 | 3.9 |
| 4 | 4.4 | 4.2 | 4.3 | 4.0 | 4.2 | 4.2 | 3.5 | 3.6 | 3.5 | 3.7 | 3.4 | 3.5 | 3.3 |
| 5 | 3.8 | 4.1 | 4.3 | 4.0 | 3.7 | 3.5 | 4.0 | 3.9 | 3.8 | 3.8 | 3.7 | 3.8 | 3.6 |
| 6 | 3.4 | 2.9 | 4.5 | 3.9 | 3.7 | 3.8 | 3.8 | 3.8 | 3.8 | 3.8 | 3.4 | 3.7 | 3.4 |
| 7 | 3.6 | 3.6 | 4.3 | 3.9 | 3.3 | 3.6 | 3.2 | 3.3 | 3.2 | 3.3 | 3.2 | 3.2 | 3.3 |
| 8 | 3.8 | 3.8 | 4.5 | 4.1 | 3.9 | 3.6 | 3.5 | 3.5 | 3.5 | 3.4 | 3.4 | 3.4 | 3.6 |
| 9 | 3.3 | 3.5 | 4.5 | 3.8 | 3.8 | 3.4 | 3.8 | 3.8 | 3.8 | 3.8 | 3.4 | 3.8 | 3.8 |
| 10 | 3.5 | 3.8 | 4.6 | 3.9 | 3.7 | 3.7 | 4.2 | 4.1 | 4.1 | 4.0 | 3.9 | 3.8 | 3.9 |
| 11 | 4.3 | 4.1 | 4.3 | 4.0 | 4.1 | 3.9 | 3.8 | 3.8 | 3.8 | 3.8 | 3.9 | 3.9 | 3.7 |
| $\overline{x}$ ± sd | 3.8 ± 0.4 | 3.8 ± 0.4 | 4.4 ± 0.2 | 3.9 ± 0.2 | 3.8 ± 0.3 | 3.7 ± 0.3 | 3.8 ± 0.4 | 3.8 ± 0.3 | 3.8 ± 0.3 | 3.8 ± 0.3 | 3.6 ± 0.4 | 3.7 ± 0.4 | 3.7 ± 0.4 |
| CV (%) | 9.50 | 10.62 | 3.67 | 4.09 | 7.37 | 7.21 | 9.42 | 7.95 | 8.86 | 8.99 | 10.85 | 9.83 | 9.86 |
| St.cher (standard) | 4.1 | 3.8 | 4.4 | 4.0 | 3.9 | 3.7 | 3.7 | 3.8 | 3.7 | 3.8 | 3.3 | 3.6 | 3.4 |

$\overline{x}$ - mean, sd - standard deviation, CV - coefficient of variability




a,b - Duncan's multiple range test ($p<0.05$)

Figure 1. Total sensory evaluation of the studied garden bean lines resistant to *Acantoscelides obtectus*

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# AN INVESTIGATION OF VARIETAL PREFERENCES EXHIBITED BY THE POTATO LEAFHOPPER, *EMPOASCA FABAE* (HARRIS) IN EDIBLE BEANS

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## Introduction

The potato leafhopper (PLH), *Empoasca fabae* (Harris) is an economically significant pest of edible beans in Ontario, causing potential yield losses of $12 million per annum. PLH exhibits distinct preferences for 'Berna Dutch Brown' bean over all other cultivars of edible beans grown in Ontario. The underlying factors responsible for this preference were investigated during the summer of 2001 and 2002 at Ridgetown College.

**Y-tube olfactometre:** A y-tube olfactometre, consisting of two glass branches was used to determine the effect of olfactory cues on PLH selection behaviour. Edible bean leaves were presented as binary comparison tests. Potato leafhopper adults collected from apples and soybeans were starved for a 30-minute period prior to placement in the Y-tube chamber. A movement of 5 cm down either branch of the apparatus was recorded as a choice. Berna was compared to White Bean and the experimental line EMP 419, with 100 trials for each pair. 70% ethanol was used to sterilize the apparatus following each replication. The right-left orientation of each pair was switched after 50 repetitions to accommodate for any directional preferences. A control comparison was made with Berna in one side of the chamber, and no leaf in the other side of the chamber. This assessment confirmed that there was a significant response to the leaf stimulus (Table 1.1). All binary comparisons demonstrated that there were no significant preferences for any particular variety over another when choice was entirely based on scent (Table 1.1). These results are realistic, when considering that migrating potato leafhoppers settle into an area from an altitude at which olfactory cues are not apparent.

**Table 1.1.** Y-tube olfactometre binary comparison results for PLH adults selecting varieties of edible beans based on olfactory cues, after a starvation period of 30 minutes.

| Varieties Compared | | #PLH | | $X^2$ df=1 Value 3.84 | P value $\propto$ 0.05 |
|---|---|---|---|---|---|
| A | B | A | B | | |
| Berna | EMP | 46 | 54 | 0.64 | 0.572 |
| Berna | WB | 49 | 51 | 0.04 | 0.258 |
| Berna | Berna | 27 | 23 | 0.32 | 0.572 |
| Berna | No leaf | 42 | 8 | 36.56* | <0.0001* |

* - significantly different at 5%

**Visual cues:** The effects of visual cues were investigated using a video-monitoring device mounted above a Plexiglas chamber. Intact bean leaves were presented through uniform circular windows cut into black Bristol board. The uniformity of each exposed leaf eliminated possible contributions of leaf size and shape. For each of the 20 trials, the order of Berna, White bean, EMP 419 leaves and a black control were randomized. 50 potato leafhopper adults,

collected from soybean fields at Ridgetown College during the summer of 2002, were placed in an opening located at the top of the chamber. All trials consisted of an observation period of two hours, in which the number of leafhoppers landing and remaining on each variety was recorded. When analyzed as a single-factor analysis of variance, with 3 degrees of freedom, results supported a significant preference for Berna leaves over EMP 419 and White Bean when chosen on the basis of colour (Table 1.2.). The influence of non-volatile surface compounds appeared to be minimal. Once the leafhoppers chose a leaf, they remained there for the duration of the two-hour period, with no movement between leaves observed.

**Table 1.2. T**he affects of leaf colour on varietal preferences exhibited by PLH adults when presented with a choice between White Bean, Berna, EMP 419 and a black standard.

| Potato leafhoppers per variety | | | | |
|---|---|---|---|---|
| | Berna | White Bean | EMP 419 | Control |
| Mean per trial | 9.45* | 4.25 | 3.70 | 0.05 |
| Sum | 189 | 85 | 74 | 1 |

*- significantly different at 5%.

**Trap cropping simulation:** This experiment was conducted during the summer of 2001 and 2002 at Huron Research Station and Ridgetown College, with two blocks per location. Different arrangements of Berna were interplanted amongst rows of Navigator White bean. Arrangements of Berna consisted of 1, 2 and 4 rows to examine the differences in pest populations when preferred plants were more abundant. Transect sampling was performed to observe gradient effects between varieties. Sampling commenced the first week in July and continued for five weeks, with adult and nymph counts and damage scores assigned on a weekly basis. On all sampling dates, there were significantly more leafhopper adults and nymphs and higher damage scores in the rows of Berna, with a gradual linear decrease in potato leafhopper numbers in rows of white bean with increasing distance from rows of Berna. In 2002, a Cruiser (thiamethoxam) seed treatment was applied to half the Berna, which effectively controlled potato leafhopper populations for the first three weeks of sampling. These results are favourable given that the initial residual activity of insecticidal seed treatments can dramatically reduce the application of foliar insecticides during emergence times when PLH control is most crucial.

# QUANTITATIVE TRAIT LOCI FOR LEAFHOPPER (*EMPOASCA FABAE* AND *EMPOASCA KRAEMERI*) RESISTANCE IN THE COMMON BEAN

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**Abstract:** A population of 108 recombinant inbred lines (RILs) ($F_{5:6-8}$), derived from a leafhopper (*Empoasca fabae* and *E. kraemeri*)- susceptible cultivar ('Berna') and a leafhopper-resistant line (EMP 419) was used to identify molecular markers genetically linked to leafhopper resistance. Bulked segregant analysis was used to screen 203 RAPD markers to identify markers linked to genes for leafhopper resistance. Composite interval mapping identified QTL for resistance to both *E. fabae* and *E. kraemeri* on core-map linkage groups B1, and B7

**Introduction**: Leafhoppers of the genus Empoasca (Homoptera: Cicadellidae) are important insect pests of field beans (*Phaseolus vulgaris*) in South and Central America, and in North America, including Southern Ontario, Canada. A recurrent selection breeding program at CIAT in Colombia has developed a series of *E. kraemeri* resistant lines (designated EMP). One such line (EMP 419) was crossed to 'Berna', a highly susceptible cultivar, and the resultant $F_2$ plants were self-pollinated to create a population of recombinant inbred lines (RILs) segregating for leafhopper resistance (Murray et al., 2001).

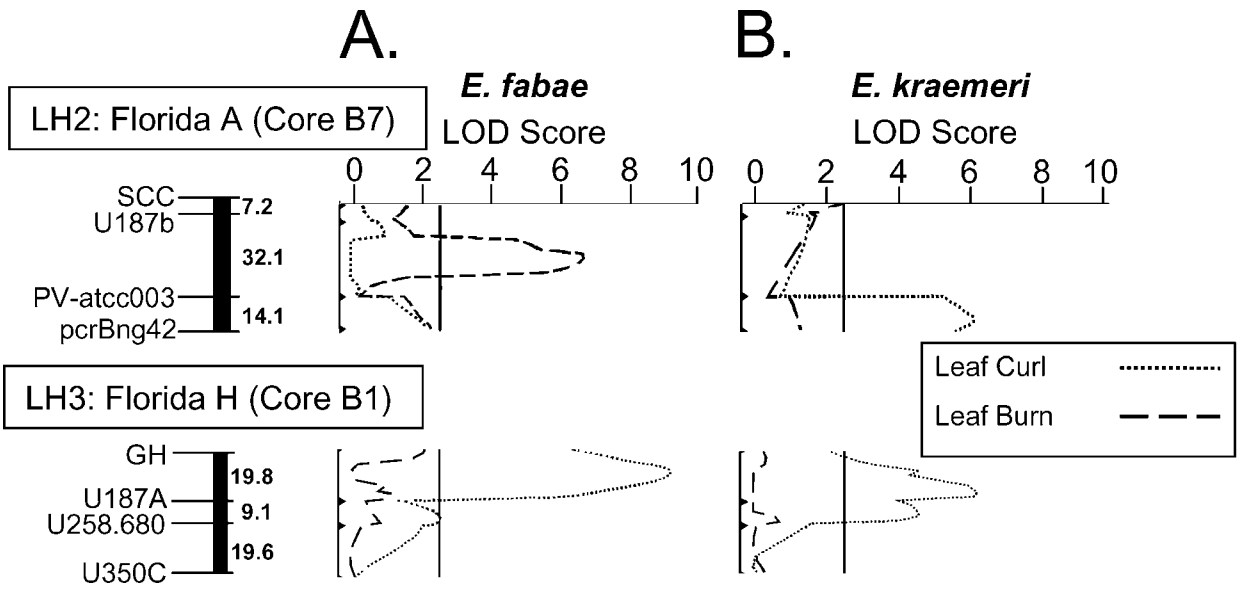
**Methodology.** From the Berna x EMP 419 cross, a population of $F_{5:6-9}$ RILs was developed. Leaf burn (necrosis) and leaf curl (cupping) were scored from 0-5 with a score of zero indicating no damage and a score of 5 indicating a severely damaged plant: *E. fabae* damage was evaluated during two seasons at 2 locations in Ontario. *E. kraemeri* damage was evaluated during a single season in Colombia. Bulked segregant analysis was used to identify markers associated with *E. fabae* resistance. Genomic DNA was pooled from the 10 most leafhopper resistant RILs and 11 most leafhopper susceptible RILs respectively. A total of 203 RAPD primers obtained from the University of British Colombia were used to amplify DNA from the bulk samples. RAPD markers polymorphic between the resistant and susceptible bulks, in addition to several CAPs and SSR markers were scored in the RIL population. A genetic map was constructed using Mapmaker/EXPv3.0. Estimations of QTL map positions using a composite interval mapping (CIM) procedure, and simulations to estimate LOD score thresholds, were conducted using Windows QTL Cartographer v1.21.

**Results and Discussion:**

*Genetic map:* Of the 203 RAPD primers tested in the DNA bulks, 23 produced a total of 43 bands that could be scored in the study population. A map consisting of 8 linkage groups, designated LH1-LH8 (leafhopper), was formed from 32 markers genotyped in the RIL population. LH1, LH2 and LH3 are shown in Fig. 1. Two previously mapped loci (bng42: Vallejos et al. 1992; PV-atcc003:Yu et al. 2000) were used to anchor the LH2 to core map linkage group B7 (Fig. 1). To anchor LH3 on the core map, RAPD marker U258.680 was scored on a subset of the Bat93 X JaloEEP558 population (Freyre et al. 1998). Two-point linkage analysis for this marker revealed that it was linked to marker D1662 (LOD=3.07, 19 cM) on core map linkage group B1.

*Leafhopper resistance* QTL: For E. fabae, a QTL for resistance to leaf burn was positioned by CIM in linkage group LH2 between U187b, and PV-atcc003 and two QTL for resistance to leaf curl were detected on LH3, a major QTL between growth habit (GH) and U187A and a minor one flanked by U187A and U258.680 (Fig. 1A). For E. kraemeri, QTL conditioning leaf curl resistance were positioned on LH2 (PV-atcc003 and pcrBng42 interval), and on LH3 (GH - U258.680 interval). A QTL for E. kraemeri resistance to leaf burn was centered on the locus controlling seed coat color (Fig. 1B).

**Fig. 1.** A partial genetic linkage map of common bean enriched for markers associated with leafhopper resistance. Linkage group numbers and the corresponding, Florida linkage groups (Vallejos et al. 1992) core linkage groups (Freyre et al. 1998) are indicated in the boxes above each linkage group (LH1, LH2, and LH3). Distances are in cM (Haldane). QTL conditioning resistance for A) *E. fabae* in Ontario and B) *E. kraemeri* in Colombia are shown.

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# IDENTIFICATION OF GERMPLASM WITH RESISTANCE TO THE SOYBEAN APHID TRANSMITTED VIRUS COMPLEX

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## Introduction

A virus disease complex causing plant stunting, pod necrosis and malformation as well as yield loss in snap beans (*Phaseolus vulgaris* L.) was observed in the 2000-2002 growing seasons. The disease was widespread, and has been reported in the snap bean production areas of Wisconsin, Illinois, Minnesota, Michigan, Iowa, Kentucky, New York and Ontario, Canada (Larsen, et al., 2002). In snap beans, the disease is thought to be caused by a virus complex consisting predominantly of CMV (Cucumber Mosaic Virus), AMV (Alfalfa Mosaic Virus) and TSV (Tobacco Streak Virus) (Grau, et al., 2002). Viruses CMV and AMV are transmitted in a non-persistent and stylet-borne manner by the soybean aphid (*Aphis glycines*) which was first discovered in the Midwest in 2000 and is thought to have been introduced from Asia. Although not aphid transmissible, TSV has also been implicated in the virus complex.

Cultural practices; e.g. carefully timed foliar applied insecticides (Orthene, Capture and Dimethoate) and nicotinic-based insecticidal seed treatments (Cruiser and Gaucho) may provide some protection from the aphid on snap bean (Wyman, 2002). Virus symptoms were most severe in late season plantings, which may be related to weather conditions and the buildup of the soybean aphid populations. Foliar sprays and seed treatments may offer some protection; nevertheless, a genetic solution allowing the transfer of favorable genes to adapted cultivars is the best long-term solution to the future security of the snap bean industry.

## Materials and Methods

A replicated field trial of 240 accessions was planted at Arlington, WI Research Station in mid-July. Two weeks prior to planting the trial, mixed spreader rows consisting of a soybean and virus susceptible snap bean cultivar, Hystyle were planted. Germplasm accessions included 170 accessions from the USDA Regional Plant Introduction Station in Pullman, WA, 10 commercial cultivars and 60 recombinant inbred lines from a cross of Eagle x Puebla 152.

One month after the spreader rows were planted, the snap beans in the spreader rows were inoculated using infected CMV, AMV and TSV tissue (Larsen, et al., 2002). Carborundum was included as an abrasive agent. Soybean aphid (winged adults and nymphs) counts were taken at 4, 5 and 6 weeks after the trial was planted.

At 55 days after planting, a 10 leaf sample from each of the 480 plots were taken for ELISA (Enzyme-Linked Immunosorbent Assay). Agdia (Elkhart, IN) CMV, AMV and TSV antibody specific ELISA kits were used

## Results

Initial composite sample results indicated that although the spreader rows were inoculated with TSV, only 12 plots tested positive for the virus. In contrast, approximately 60% of the plots tested positive for AMV and 100% tested positive for CMV.

Visual ratings of virus symptoms were also taken. Thirty-one accessions were phenotypically symptomless. These plots were resampled for AMV and CMV using ELISA. One leaf per plant in each plot was taken for individual plant ELISA. Each leaf was numbered according to it's corresponding position within the plot so if it tested negative for both CMV and AMV, seed could be harvested from the correct individual. Of the 592 individual plants

harvested, 147 plants tested negative for both AMV and CMV (Tables 1 and 2). Mature seed was harvested from approximately 50% of the 147 plants. This seed will be evaluated in the greenhouse in collaboration with Dr. Craig Grau, Univ. of Wisconsin, Dept. of Plant Pathology in the Spring 2003.

These results suggest that genetic variability exists within *Phaseolus vulgaris* and could serve as a source of resistance to this soybean aphid transmitted virus complex.

Table 1. ELISA results for accessions scored as visually symptomless in the field

| Accessions | Total No. Evaluated | Visually Symptomless | ELISA (-) AMV and CMV |
|---|---|---|---|
| PIs | 170 | 21 | 2 |
| Cultivars | 10 | 2 | 0 |
| E x P RILs | 60 | 8 | 0 |

Table 2 ELISA results of individual plant screening for CMV and AMV

| Entry | %AMV(-) | %CMV(-) | %AMV& CMV(-) |
|---|---|---|---|
| 151 | 100.0 | 100.0 | 100.0 |
| 13 | 100.0 | 80.0 | 80.0 |
| 21 | 100.0 | 0.0 | 0.0 |
| 51 | 61.3 | 12.5 | 6.3 |
| 186 | 88.9 | 55.0 | 43.9 |

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# DEFENSE RESPONSE IN COMMON BEAN GENOTYPES THAT ARE RESISTANT TO *Apion godmani* Wagner.

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## INTRODUCTION

Plants are exposed to numerous organisms that threat to infect the tissues, especially in wounds after mechanical damage. Receiving a stimulus from the surface or after the invasion threat from the microorganisms, plant result in synthesis of defense compounds. The defense response, involves accumulation of phytoalexins, of enzymes that degrade microbial cell walls and the deposition of phenolic compounds and proteins that act as chemical and physical barriers to the invading agents.

Common bean defense response against phytopathogenic fungi includes phytoalexin accumulation, with phaseollin as the main phytoalexin synthezised (Soriano and Medina, 1989). When bean pods are infested with the insect pest *Apion godmani* W., the amount of phaseollin accumulated in elicited pods was lower in bean genotypes susceptible to the insect ovoposition than those resistant to it; it was suggested that the presence of the insect inhibit the bean pod defense response, rendering the plant more susceptible to microbial disease (Jacinto et al., 2001). To determine if there is a genetic correlation between susceptibilty to insect attack and ability to response to defense elicitation, in this work, bean plants of different genotypes were elicited to compare phytoalexin accumulation as related to behaviour resistance or susceptibility to insect ovoposition of bean pods in the field.

## MATERIAL AND METHODS

Seeds of common bean (*Phaseolus vulgaris* L.) cvs. Mex-332, J-117, Amarillo-155 (insect-resistant) and Canario-155, Bayomex and Desarrural (insect-susceptible) were surface-esterilized and allowed to germinate in aseptic conditions, then the plantelets were grown in vermiculite in a incubating chamber and watered daily with a modified Hoaglands solution. Seven days old plants were inoculated in the hypocotyls with an elicitor solution of decagalacturonide (0.1μg/μL, glucuronic acid eq.) and after 24 hours the hypocotyls (2 cm segments) were extracted to measure phaseollin levels *P. vulgaris* cv. Flor de mayo was used as reference of defense response to the elicitor. Control plants received distilled water instead of elicitor solution.

## RESULTS AND DISCUSSION

It was found that there were not correlations between the response of the plants to insect attack and phytoalexin response in hypocotyls of the bean genotypes. The three bean genotypes that in the field behaved as susceptible to insect ovoposition, when treated with the defense elicitor hypocotyls were capable of accumulating phaseollin (Table 1.), with the genotype Desarrural accumulating twice as much phaseollin as the control. On the other hand, the insect-resistant bean genotypes in one case (J-117) there was not phaseollin accumulation after elicitor treatment. The genotype Mex-332 accumulated ten times as much as genotype Amarillo-155.

Table 1. Accumulation of phaseollin in bean genotypes resistant and susceptible to and *A. godmani* W.

| Resistant | Phaseollin µg / gfw | Susceptible | Phaseollin µg / gfw |
|---|---|---|---|
| Mex – 332 | 31.6 | Canario – 107 | 8.9 |
| J – 117 | 0.9 | Bayomex | 4.9 |
| Amarillo - 155 | 3.9 | Desarrural | 27.36 |

It was concluded that was no correlation between the ability of bean genotypes to accumulate phytoalexins as a defense response in the stage of hypocotyls and susceptibility of genotypes to the bean pod weevil attack (*Apion godmani* W.), however it would be necessary to confirm the results in the pod of genotypes affected in natural conditions.

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# RESISTANCE OF MEXICAN BEAN LANDRACES TO BEAN POD WEEVIL *APION GODMANI* WAGNER, IN HIGHLANDS OF MÉXICO

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**INTRODUCTION**. Biological factors reduce bean productivity in the highlands of México. The most important insect pests are the Mexican bean beetle *Epilachna varivestis* Mulsant, the bean pod weevil *Apion godmani* Wagner, and the bruchid *Acanthoscelides obtectus Say,* and the diseases common blight *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye., halo blight *Pseudomonas syringidae* pv. p*haseolicola* (Burkholder), the rust *Uromyces appendiculatus* var. *appendiculatus* (Per.) Unger, and anthracnose *Colletotrichum lindemuthianum* (Sacc & Magn.) Briosi & Cav. Development of varieties with multiple resistance to pests (insects and diseases), is necessary for bean production in a sustainable agriculture. The first step is to identify sources of resistance to bean pests and pathogens in the highlands of Mexico. A project is carried on to select bean Mexican landraces with high levels of resistance to bean pod weevil *Apion godmani*, anthracnose *Colletotrichum lindemuthianum* and common blight *Xanthomonas campestris* pv *phaseoli.* Parts of the results are presented.

**MATERIALS AND METHODS**. We evaluated 189 Mexican bean landraces, which during the years 1994, 1995 and 1996 showed levels of resistance to bean pod weevil, anthracnose, and common blight. This genotypes were sown on may 28 of 1997 at Santa Lucía de Prías, Estado de México. Genotypes APN-83, México-332 and Amarillo-153 with well-known resistance to bean pod weevil as well as susceptible genotypes: Canario-107, Bayomex, Jamapa, Zacatecas-45 and another three introduced genotypes were included as controls. The experimental plot was one 5 meters row with two replications, in one plot pesticides were used to control all insect pests (mainly Mexican bean beetle and bean pod weevil) and some diseases like anthracnose, common blight, and rust. Second replication was not applied with pesticides to allow natural populations of bean pod weevil. Mechanical control was used, through handpicking part of adults and larvae population of Mexican bean beetle to avoid total destruction of foliage and pods in bean plants. A comparison of yield in both plots was performed. Before harvest, 100 pods were collected in the plot with no application of pesticides, and 30 pod in the replication with pesticide applications. The resistance or susceptibility to bean pod weevil, was score according to the percentage of seed damage in the 100 pods sample. We use as a reference the damage in the most susceptible control treatment, according to Garza *et al*. (1996). All plants of each plot were harvested and grain production estimated.

## RESULTS AND DISCUSSION

EVALUATION OF RESISTANCE/SUSCEPTIBILITY TO BEAN POD WEEVIL. The local susceptible check, Canario-107 and Bayomex, exhibited the highest percentage seed damage, with 94.9 and 90.7%, respectively, only surpassed by two introduced genotypes Japón-13412 and Japón-13398 with 99.2 y 96.7 % seed damage; Jamapa showed 66.8 % of damage percentage. APN-83, resistant variety of Central America, was classified as susceptible with 67.4 % seed damage. 40 genotypes showed susceptibility to pod weevil. 82 genotypes showed high resistance to *Apion godmani* Wagner, with percentage seed damage among 5.1 and 23.5%, including the resistant check Amarillo-153 y México-332. Some Mexican bean landraces (20) had better

response of resistance than the check; the five outstanding bean landraces were Pue-33-a-3, Méx-38-a, Oax-98, Oax-71-c y Méx-328 with less than 7% seed damage (Table 1).

**Table 1**.- Percentage seed damage by bean pod weevil in bean genotypes.

| | Genotype | % seed damage plot without pesticides | % seed damage plot with pesticides |
|---|---|---|---|
| 37 | Ph. vulg. 7236 Japón-13412 | 99.2 S | 18.8 |
| 73 | Ph. vulg. 7234 Japón-13398 | 96.7 S | 16.8 |
| 201 | Canario-107 | 94.9 S | 32.8 |
| 200 | Bayomex | 90.7 S | 20.5 |
| 206 | APN-83 | 67.4 S | . |
| 203 | Jamapa | 66.8 S | 11.2 |
| 202 | Amarillo-153 | 12.3 R | 3.4 |
| 205 | México-332 | 11.5 R | 3.0 |
| 188 | Ph. vulg. 2096 Pue-33a-3 | 6.8 R | 2.5 |
| 179 | Ph. vulg. 393 Méx-38-a | 6.4 R | 0.8 |
| 34 | Ph. vulg. 1954 OAX-98 | 5.9 R | 0.0 |
| 35 | Ph. vulg. 1942 OAX-71-C | 5.9 R | 0.0 |
| 146 | Ph. vulg. 1706 Méx-328 | 5.1 R | 4.2 |

**YIELDS**. The bean landraces Ver-140, Pue-473, Pue-2, Ver-188, Pue-241, Chis-202-A, Mich.102-B, Pue-220, Méx-110, Pue-513, Qro-36-D, Pue-217, Ver-161 and Ph. vulg. 4823 showed highest yields, in the plot without pesticides, grain production was between 850 and 1010 gr/row (Table 2). This landraces showed intermediate and high resistance to bean pod weevil. Interesting results exhibited genotypes Oax-39 y Chih-11-G, because they had high grain production, 825 and 785 gr/row, respectively, in spite of they had 60.1 and 50.1 % seed damage for *Apion godmani*. The landraces Qro-36-D, Sin-4-C, *Ph. vulg*. 3836, Oax-118, Méx-45-A, Gro-42 and Méx-930 had the same behavior, and showed higher yields in the plot without pesticides, in comparison to the plot with pesticides, in spite of having over the 31% seed damage. These results suggest the possibility of a tolerance mechanism.

**Table 2.-** Outstanding yielding Mexican bean landraces, sown at Santa Lucia de Prías, Edo Méx. Mexico, in 1997.

| | Genotype | Yields (kg/5 m row) in plot without pesticides | Yields (kg/5 m row) in plot with pesticides |
|---|---|---|---|
| 10 | Ph. vulg. 2703 Ver-140 | 1010 | 1095 |
| 118 | Ph. vulg. 2466 Pue-473 | 985 | 165 |
| 169 | Ph. vulg. 2001 Pue-2 | 975 | 495 |
| 14 | Ph. vulg. 2741 Ver-188 | 940 | 920 |
| 202 | Amarillo-153 | 913 | 1109 |
| 116 | Ph. vulg. 2264 Pue-241 | 905 | 715 |
| 20 | Ph. vulg. 1115 Chis-202-A | 890 | 1520 |
| 61 | Ph. vulg. 7324 Mich-102-B | 885 | 900 |
| 108 | Ph. vulg. 2862 Pue-220 | 885 | 400 |
| 82 | Ph. vulg. 3052 Méx-110 | 870 | 515 |
| 126 | Ph. vulg. 2505 Pue-513 | 870 | 960 |

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# EVALUATION OF RWANDAN VARIETIES FOR DISEASE RESISTANCE

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In Rwanda, common beans are grown by 95% of farmers and are central to the farm economy and people's diet. Per capita consumption is over 60 kg per year, one of the highest in the world (ISAR bean program, 2001). The annual production of 300,000 tons (ISAR bean program, 2001) does not meet the national demand and Rwanda is obligated to import beans from neighboring countries. The main factors that limit bean production include the lack of essential soil nutrients and the presence of seed borne bean diseases. Bean anthracnose caused by *Colletotrichum lindemuthianum,* bean common mosaic necrosis virus (BCMNV), and common bacterial blight (CBB; *Xanthomonas axonopodis* pv *phaseoli*), are among the important diseases constraining bean production in Eastern and Central Africa. BCMNV is particularly a serious problem in these areas because of the prevalence of subsistence agriculture with farmers saving their own seed for the next growing season. In Rwanda, anthracnose is the second biotic cause of yield loss after angular leaf spot (ISAR bean program, 2001)**.** The objective of this study was to evaluate ten Rwandan bean varieties for reaction to races 7, 73, and 256 of anthracnose (*C. lindemuthium*), NL3 strain of BCMNV and screen the same varieties for the presence of markers linked to loci conferring resistance to anthracnose, CBB and BCMNV.

**Materials and Methods:**

All analyses were conducted on 10 Rwandan varieties RWR1802, RWV524, RWR13121, RWK10, NG224-4, CAB19, SCAM80CM/15, RWV167, RAB487, and G2331 released in 2001 by Rwanda's Institute of Agronomic Sciences (ISAR) and shown on Table 1.

**a) Genotypic evaluation:** Leaf samples were collected from seedlings of the 10 Rwandan genotypes grown in the greenhouse and DNA was extracted using the miniprep procedure outlined by Afanador et al. (1993). Amplification reactions were carried out according to SCAR marker protocols (Melotto et al.1996; Melotto et al.1998; Miklas et al.2000; Awale and Kelly, 2001)

**b) Green house evaluation:** For anthracnose screening, the Rwandan varieties were inoculated with races 7, 73, and 256 of anthracnose (Balardin and Kelly, 1998) nine days after planting. Disease reaction was recorded one week after inoculation. For viral inoculations, unifoliate leaves were rub-inoculated with viral homogenate of the NL 3 strain of BCMNV nine days after planting. Disease reaction was recorded at 7 days and 21 days after inoculation and were rated as susceptible – mosaic, top necrosis due to the presence of the unprotected *I* gene or mild mosaic due to the presence of *bc-1*$^2$ gene.

**Results and Discussion:**

Based on the marker data, RWV167 appears to carry *Co-5* and *Co-4*$^2$ genes conferring resistance to anthracnose. The same variety also showed resistance to races 7, 73, and 256 of anthracnose suggesting that this variety would be a good source for anthracnose resistance. The variety G2331 was resistant to three races of anthracnose and it appeared to possess the SAB 3 SCAR marker linked to *Co-5* gene. In addition, G2331 appeared to possess SCAR markers SAP6 and BC 409, linked to two major QTLs conferring resistance to CBB (Miklas et al., 2000). RAB 487, RWV524, and RWK 10 varieties appeared to have SAS13 marker but did not possess the SH18

or SBB14 markers indicating that they should possess another resistance allele at the *Co-4* locus. RWK10 and RAB487 possessed the *I* gene marker and this was confirmed as a top necrosis reaction after inoculation with NL 3. These *I* gene varieties will need to be protected by *bc-3* gene to be successfully grown in Rwandan conditions where strains of BCMNV are prevalent. These data will be useful in designing a breeding program to enhance disease resistance of local bean varieties for production in Rwanda.

**Table 1.** Molecular markers linked to different disease resistance genes in Rwandan varieties and their reaction to races 7, 73, 256 of anthracnose, and NL3 strain of BCMNV.

| ***Markers *and diseases*** | Cultivars | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **RWR 13121** | **NG224-4** | **RAB 487** | **RWV167** | **G 2331** | **RWV524** | **RWK10** | **SCAM80 CM/15** | **RWR 1802** | **CAB19** |
| **SW13** (*I gene)* | - | - | + | - | - | - | + | - | - | - |
| **SAB3** (*Co-5)* | - | - | - | + | + | - | - | - | - | - |
| **SAP6** (CBB) | + | - | - | - | + | - | - | - | - | + |
| **BC409** (CBB) | - | - | + | + | + | - | - | - | - | - |
| **SAS13** (Co-$4^2$) | - | - | + | + | - | + | + | - | - | - |
| **SH18** (Co-$4^2$) | - | - | - | + | - | - | - | - | - | - |
| **SBB14** (Co-$4^2$) | - | - | - | + | - | - | - | - | - | - |
| **Anthracnose races # 73** | R | R | R* | R | R | R | R | R | R | S |
| **7** | S | S | R* | R | R | R | S | S | R | R |
| **256** | S | R | R | R | R | S | R | R* | S | S |
| **BCMNV§** | MM | M | TN | M | M | M | TN | MM | M | MM |

∗+: Presence of marker linked to the disease resistance gene -: absence

**¶§** BCMV: Bean Common Mosaic Virus, MM: Mild Mosaic, M: Mosaic, TN: Top Necrosis, NR: Non-Reaction

# R: Resistant, R*: Some plants were resistant, some susceptible. S: anthracnose susceptible.

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# TWO GENES FROM *Phaseolus coccineus* L. CONFER RESISTANCE TO BEAN GOLDEN YELLOW MOSAIC VIRUS

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In Central America and Caribbean, Bean Golden Yellow Mosaic (BGYM) can cause significant reductions in the yield and quality of beans. Symptoms caused by this whitefly-transmitted geminivirus include mosaic with intense foliar yellowing (chlorosis), leaf curling, pod deformation and severe plant stunting. Plant breeders have developed and released bean cultivars with resistance to BGYM (Beaver and Miklas, 1999; Beebe, 1994; Rosas et al., 1997) and researchers have identified specific resistance genes such as *bgm-1* and *bgm-2*, that confer resistance to leaf chlorosis (Velez et al., 1998), and *Bgp* that prevents pod deformation (Molina-Castaneda and Beaver,1998). The identification of additional genes for BGYM resistance resistance would permit bean breeders to broaden the base of resistance to this important disease. The scarlet runner bean (*Phaseolus coccineus* L.) accession G35172 was identified at CIAT as a novel source of resistance to BGYM in Central America and the Caribbean and Bean Golden Mosaic (BGM) in Brazil (Singh et al., 2000). Ferwerda (2001) reported that G35172 had a recessive gene for resistance to BGYM, but segregation ratios varied when different isolates of BGYMV from Puerto Rico and Florida were used for screening. Muñoz (2002) reported that two recessive genes from G35172 confer resistance to BGYM. The purpose of this research was to study the inheritance and nature of BGYM resistance derived from G35172. The advanced line USPR-VCI-6 ($BC_1F_{2:5:8:9}$), derived from the cross 'HP8437-95*2/G35172', was obtained by combined pedigree and bulk selection methods for field resistance to BGYMV for six generations. HP8437-95 is a BGYM susceptible small red line from the cross 15R-148/3M-81. However, the $F_9$ lines with resistance derived from G35172 had the susceptible marker band (530 bp) diagnostic for absence of the *bgm-1* gene for resistance to BGYM (Urrea et al., 1996). Muñoz (2002) identified BGYM susceptible $F_3$ plants from the cross 'Morales/ USPR-VCI-6' which suggested that the resistance derived from G35172 and the *bgm-1* and *Bgp* resistance genes found in Morales are different. In order to study the inheritance of resistance from G35172, an $F_2$ population was developed from the cross 'Arroyo Loro//USPR-VCI-6. Arroyo Loro is susceptible to BGYMV whereas the other parent USPR-VCI-6, derived from the cross HP8437-95*2/G35172', is resistant to BGYM. A portion of the $F_2$ population (241 plants) was planted at Isabela, Puerto Rico in June, 2002. Spreader rows of susceptible plants were mechanically inoculated (Morales and Niessen, 1988) six days after planting. Whitefly populations were used in the field and the greenhouse to promote the spread of the virus. Each $F_2$ plant was evaluated for the absence or presence of leaf mosaic at 30 days and for pod deformation at 55 days after planting. Another portion of the $F_2$ population (51 plants) was planted in the greenhouse at the University of Puerto Rico, Mayaguez Campus in December 2002. Each plant was mechanically inoculated six days after planting and evaluated for leaf mosaic at 30 days and for pod deformation at 55 days after planting. Phenotypic ratios from the field and greenhouse experiments suggest that one recessive gene confers resistance to leaf mosaic, and a dominant gene controls resistance to pod deformation caused by BGYM. Chi square tests found both greenhouse and field data to fit a 9:3:3:1 segregation ratio (Table 1). Both parents and individual $F_2$ plants were screened with the SCAR SR2, which is linked to the

*bgm-1* gene. Neither the parents nor the $F_2$ plants showed the resistant band to this gene, thus providing further evidence that the recessive resistance to leaf mosaic derived from G35172 and *bgm-1* are different. In Brazil, Bianchini (1999) screened a bean population for reaction to BGM that was derived from a cross with *P. coccineus*. He also identified plants with resistance to leaf mottling and pod deformation.

Table 1. Chi-square tests of $F_2$ plants screened in the field and GH for BGYM reaction.

| | Mosaic[1] (+) Deformed pods ( - ) | Mosaic (+) Deformed pods (+) | Mosaic ( - ) Deformed pods ( - ) | Mosaic ( - ) Deformed Pod (+) | $X^2$ | P |
|---|---|---|---|---|---|---|
| **FIELD:** | | | | | | |
| Observed | 144 | 41 | 49 | 7 | 5.18 | 0.1591 |
| Expected | 135.0 | 45.8 | 45.8 | 14.5 | | |
| GREENHOUSE: | | | | | | |
| Observed | 22 | 9 | 14 | 6 | 6.298 | 0.098 |
| Expected | 28.6 | 9.7 | 9.7 | 3.1 | | |

[1] Mosaic (+) = Number of plants with leaf mosaic symptoms; Mosaic ( - ) = Number of plants without leaf mosaic symptoms.

[2]. Pod (+) = Number of plants with deformed pods; Pod ( - ) = Number of plants with normal pod development.

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# MAPPING OF GENOMIC REGIONS ASSOCIATED WITH RESISTANCE TO ANGULAR LEAF SPOT IN COMMON BEANS

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Common bean angular leaf spot (ALS) caused by the fungus *Phaeoisariopsis griseola* is a common disease in tropical and sub-tropical regions. In fact, this disease affects bean crops grown in more than sixty diffentent countries causing yield losses that may reach 80% of the expected production. Incorporation of genes determining partial resistance is a promissing breeding strategy for developing resistant bean cultivars to ALS. This strategy has been shown to be effective in other cultures leading a more durable resistance (Johnson, 1984). The lack of information on the inheritance mode of the ALS resistance genes in beans is one of the main reasons this strategy is not being used to develop cultivars resistant to this disease.

The present work aimed at evaluating and mapping the loci controlling partial resistance to ALS in common beans. A set of recombinant inbred lines (RILs) at the $F_{8-9}$ generation was used. The RILs were produced from a cross between BAT 93 (Mesoamerican) and Jalo EEP558 (andean), performed by Gepts et al. (1993).

## Materials and Methods

The seeds of the 50 RILs used in this work were kindly provided by Dra. Siu Mui Tsai from the Centro de Energia Nuclear na Agricultura (CENA/USP, São Paulo, Brazil). First, the parents BAT 93 and Jalo EEP558 were inoculated with spores from 10 *P. griseola* pathotypes to select the one that would be used to inoculate the RILs. The pathotypes correspond to monosporic cultures isolated in different bean growing regions in Brazil.

The RILs were planted in May 2002, in a greenhouse from BIOAGRO/UFV. The plants were inoculated 25 days after germination with a suspension of $2 \times 10^4$ conidia/ml. Evaluation of the disease symptoms was done 17 and 21 days after inoculation, based on a 1-to-9 scale in which 1 was assigned to plants with no symptoms and 9 to plants severely affected by the disease.

The molecular data (RFLP) was obtained at http://agronomy.ucdavis.edu/gepts/gepts.htm. All statistical analyses were done with the aid of the GQMOL program developed by prof. Cosme Damião Cruz from UFV (www.ufv.br/dbg/gqmol/gqmol.htm).

## Results and Discussion

In the initial evaluation of the parents for resistance/susceptibility to ALS it became clear that Jalo EEP558 was resistant and BAT 93 was susceptible to pathotype 63.55. So this pathotype was used to inoculate the RILs. One hundred-and-three RFLP markers segregating 1:1 (http://agronomy.ucdavis.edu/gepts/gepts.htm) were analyzed together with the phenotypic data. Five RFLP markers (D1367, D1287, D1492, D1157 and D1390) co-segregated with regions associated with partial resistance to ALS at $P<0.05$. An additional marker (D1512) was included at $P<0.08$. These same regions are also linked to partial resistance to other types of diseases in beans. Marker D1512 is associated with resistance to anthracnose (Geffroy et al., 2000).

Markers D1390 and D1492 are associated with resistance to common bacterial blight (Nodari et al., 1993; Tsai et al., 1998). Markers D1157, D1367 D1287 and D1390 are linked to the partial control of common bacterial blight and wildfire (Boscariol, 1997). Markers D1157 and D1492 were mapped in linkage group 5 and markers D1287 and D1367 were mapped in linkage group 2 (Gepts et al., 1993).

Disease resistance genes in plants are often organized in clusters. This type of organization favors unequal crossing over, which may lead to different haplotypes associated with partial resistance. The fine mapping and characterization of these clusters may help the breeder to design appropriate breeding strategies to develop disease resistant cultivars.

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# INHERITANCE OF ANGULAR LEAF SPOT RESISTANCE IN SELECTED COMMON BEAN GENOTYPES

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## Introduction

Evidence of co-evolution of *P. griseola* and common bean implies that combining resistance from the Andean and Mesoamerican gene pools is a good strategy for disease control. To effectively combine and pyramid useful diverse genes, sufficient characterization of the genetics of resistance is necessary. Very little is known about the nature and inheritance of ALS resistance in common bean although this information is important to facilitate breeding for ALS resistance, to deploy ALS resistance genes, and for gene tagging and the development of molecular markers for marker assisted selection (MAS). The objective of this study was to elucidate the inheritance of ALS resistance in selected ALS differential genotypes and promising resistant germplasm.

## Materials and Methods

Segregating populations (F1, F2, and F1 backcrosses to resistant and susceptible parents) were developed by crossing the snap bean variety, Sprite (susceptible*)* with 5 ALS differential varieties (Don Timoteo, Amendoin, PAN 72, Mexico 54, Cornell 49242) and two potential sources of resistance (G 10474 and G 10909) (Table 1). Andean genotypes (Don Timoteo, Amendoin) were screened under green house conditions using Andean races while Mesoamerican genotypes (PAN 72, , Mexico 54, Cornell 49242, G 10474 and G 10909) were screened using Mesoamerican races (Table 1). Evaluations for disease severity were assessed using a CIAT 1 – 9 scale, where 1 represents no visible symptoms and 9 = severe symptoms and disease expression. Ratings of 1 to 3 were considered resistant and ratings > 4 as susceptible. Area under disease progress curves was calculated to assign genotypes to resistance and susceptibility classes and the Chi-squared test in the SAS program was used to test different genetic inheritance models.

## Results and Discussion

The observed segregation ratios revealed that a single dominant gene conditions ALS resistance in the genotypes, Don Timoteo, PAN 72 and G 10474 (Table 1). Two dominant genes control resistance in Cornell 49242, G 5686 and G 10909, while G 2858 has two duplicate genes, Mexico 54 a single recessive gene, and Montcalm and Amendoim have two recessive genes each (Table 1). However, results with Amendoim were somewhat ambiguous. Sartorato et al, (1999) reported that ALS resistance in Mexico 54 was conditioned by a single dominant gene, *Phg*-2, while Caixeta et al. (2002) reported that Mexico 54 has three ALS resistance genes, designated *Phg*-2, *Phg*-5 and *Phg*-6. The ALS races that we used (31-55), as well as the background in which we elucidated the nature of ALS resistance in Mexico 54 (the snap bean variety, Sprite), differs from the ones used by the Brazilian group (races 63-39, 63-23, variety Ruda-Mesoamerican). It is possible that the background in which we are detecting the single resistance gene in Mexico 54 has an influence on the outcome of the tests. Nevertheless, the results reported show the complex nature of inheritance of resistance to *P. griseola*. Major genes (whether recessive or dominant) are involved in conferring resistance to ALS.

Table 1. Nature and inheritance of angular leaf spot resistance in some differential varieties and selected resistant sources.

| Source | PG race | Generation | Observed | Expected | $X^2$ | Interpretation |
|---|---|---|---|---|---|---|
| Cornell 49242 | 63-31 | F1 | 18:0 | | | 2 dominant Genes |
| | | F2 | 49:22 | 9:7 | 0.09 | |
| | | BC-P1 (res) | 64:9 | | | |
| | | BC-P2 (sus) | 13:25 | 1:3 | 0.19 | |
| Mex 54 | 31-55 | F1 | 20:69 | | | |
| | | F2 | 39:120 | 1:3 | 0.89 | 1 rec. gene |
| | | BC-P1 (res) | 32:24 | 1:1 | 0.28 | |
| | | BC-P2 (sus) | 28:86 | 1:3 | 0.91 | |
| PAN 72 | 15-0 | F1 | 137:0 | | | 1 dominant gene |
| | | F2 | 47:15 | 3:1 | 0.88 | |
| | | BC-P1 (res) | | | | |
| | | BC-P2 (sus) | 17:16 | 1:1 | 0.86 | |
| G10474 | 63-63 | F1 | 40:0 | 1:0 | | 1 dominant gene |
| | | F2 | 57:25 | 3:1 | 0.25 | |
| | | BC-P2 (sus) | 9:8 | 1:1 | 0.80 | |
| G10909 | 63-63 | F1 | - | | | 2 dominant genes |
| | | F2 | 92:73 | 9:7 | 0.89 | |
| | | BC-P2 (sus) | 7:35 | 1:3 | 0.21 | |
| Amendoim | 15-0 | F1 | 0:136 | | | Recessive |
| | | F2 | 7:107 | 1:15 | 0.96 | 2 rec. genes |
| | | BC-P1 (res) | 5:87 | 1:15 | 0.74 | 3 rec. genes |
| | | BC-P2 (sus) | 0:58 | | | |
| Timoteo | 62-0 | F1 | 59:3 | | | 1 dominant gene |
| | | F2 | 47:24 | 3:1 | 0.11 | |
| | | BC-P1 (res) | 28:3 | | | |
| | | BC-P2 (sus) | 17:17 | 1:1 | 1.0 | |

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# AN ANGULAR LEAF SPOT DISEASE RESISTANT LANDRACE COMPONENT FROM IRINGA, TANZANIA

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In Tanzania like many regions of Africa, bean production systems are characterized by the extensive use of varietal mixtures or landraces by resource poor farmers. These landraces represent a potential source of germplasm for improvement of the bean crop (Martin and Adams, 1987a). In her work, Gondwe 1998 reported that resistance to the halo blight disease could be found in local landraces. The present work was aimed at identifying desirable traits associated with landraces with an objective to make use of and preserve the genetic diversity present in indigenous germplasm.

## Materials and Methods.

Two farmer's mixtures were collected from Iringa and Njombe districts in 1998. Forty components were manually separated basing on seed size and seed colour. About 19 components were evaluated together with other bean genotypes at ARI Uyole(1700m), Tanzania, between 1999 and 2002 growing seasons. Each bean landrace component/ improved cultivar was grown in a single row one meter (in 1999) and later (2001 – 2002) three meters long. In 2001-2002, the experimental design was RCBD with three replications. The beans were planted at a spacing of 10cm within and 50cm between rows. Agronomic practices were carried out as recommended. Disease severity scores were made using the 1 – 9 scale where 1 = no to little disease symptoms and 9 = severe infection and or death of plant due to disease.

## Result and Discussion

It is evident from the results presented in Table 1 that the landrace component Iramix 10a has resistance to the angular leaf spot disease. Although Iramix 10a was susceptible to other diseases, the identified trait can be used in breeding for resistance to this important fungal disease. These results also show that landraces, the product of selection by farmers, are rich storehouses of genetic material. Therefore, selection of superior traits such as disease resistance in our landraces is important because it provides sources of germplasm that are adapted to local conditions. Multilocational screening of this component could be important in order to make these results more useful. It would also be useful to evaluate this component against described races of *P.griseola* prevalent in region to assess the potential usefulness of the resistance observed

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**Table 1. Angular Leaf Spot mean disease severities for bean genotypes evaluated at Uyole, in 1999, and 2001 – 2002 seasons.**

| Bean cultivar Code No. | Mean ALS disease severity scores ( 1 – 9) | | |
|---|---|---|---|
| | 1999 | 2001 | 2002 |
| Iramix 1a | 3.5 | 6.3abc | 6.0abcd |
| Iramix 1b | 5.5 | 6.3abc | 6.7abc |
| Iramix 2 | 4.5 | 7.3ab | 7.0ab |
| Iramix 3 | 4.5 | 4.7de | 5.0cde |
| Iramix 4a | 2.0 | 5.7bcd | 6.0abcd |
| Iramix 5 | 2.0 | 4.3de | 5.0cde |
| Iramix 6 | 5.5 | 6.3abc | 6.3abcd |
| Iramix 8a | 6.5 | 5.0cd | 5.7bcde |
| Iramix 9b | 4.5 | 7.0ab | 6.7abc |
| Iramix 9d | 2.5 | 5.7bcd | 5.0cde |
| Iramix 10b | 2.0 | 5.0cd | 4.3e |
| Iramix 10a | 1.5 | 2.0f | 1.0f |
| Iramix 12 | 5.5 | 7.7a | 7.3ab |
| Exc. 52 | 2.0 | 2.3f | 1.3f |
| Sam-1 | 5.5 | 7.0ab | 6.3abcd |
| Sam-4 | 3.5 | 3.3ef | 1.3f |
| Edmund | 4.5 | 7.7a | 6.3abcd |
| A 52 | 7.0 | 7.3ab | 6.0abcd |
| A 43 | 4.0 | 8.0a | 6.7abc |
| RM U13 | 2.5 | 7.0ab | 6.7abc |
| Canadian Wonder | 4.0 | 7.7a | 6.7abc |
| Tendergreen | 3.5 | 7.4a | 7.7a |
| Iramix 1a-i | 3.5 | - | - |
| Iramix 1a-ii | 4.5 | - | - |
| Iramix 1c | 4.5 | - | - |
| Iramix | 5.5 | - | - |
| Iramix 2a | 3.5 | - | - |
| Iramix 4b | 2.0 | - | - |
| Iramix 5a | 4.5 | - | - |
| Iramix 7a(r) | 6.5 | - | - |
| Iramix 7a(p) | 6.5 | - | - |
| Iramix 7b-i | 4.0 | - | - |
| Iramix 7b | 7.0 | - | - |
| Iramix 8b | 2.0 | - | - |
| Iramix 9a | 2.0 | - | - |
| Iramix 10d | 2.0 | - | - |
| Iramix 9c | 6.0 | | - |
| Iramix 10a | 2.5 | - | - |
| Sam-2 | 4.0 | - | - |
| OT 1b | 2.5 | - | - |
| Nyamuhanga | - | 7.0ab | 7.0ab |
| Kombati | - | 7.7a | 7.0ab |
| Uyole 94 | - | 7.0ab | 6.0abcd |
| Kablanketi | - | 7.7a | 7.0ab |
| Kigoma | - | 7.0ab | 7.7a |
| Imp. Kabl. | - | 5.7bcd | 2.0f |
| Ex. Bukoba | - | 6.9ab | - |
| MTB | - | NG | - |
| RB 296 | - | - | 1.0f |
| Uyole 98 | - | - | 4.3e |

NG = seeds did not germinate. - not tested.
Iramix = component of a mixture from Iringa. Sam =component of a mixture from Njombe.

**Tagging resistance allele of the common bean to angular leaf spot by SSR and RAPD markers.**

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The consumption of common bean in Brazil has reduced more than 22% in the past 25 years, mainly due to irregular supplies, and high prices. Increasing grain yield may reverse this picture. Nevertheless, the bean crop faces several threats such as diseases, among which angular leaf spot caused by the fungus *Phaeisariopsis griseola*, is one of the most important.

The most efficient method of disease control is the use of resistant cultivars. Although, some information on resistance sources and genetic control are already available, the procedures for artificial inoculation are not yet accurate resulting in low selection gain. An alternative is to select under natural incidence of the disease, which generally occurs only in the drought season, when the inoculum pressure is enough to assure selection efficiency. The use of molecular markers for identifying vertical resistance alleles stands for an important contribution for bean breeding, mainly because they make possible to select genotypes with one or more resistance alleles at any time.

Most cultivars used in Brazil and consequently the disease-causing races are Mesoamerican in origin. Thus, resistance alleles from Andean origin, like one from Jalo cultivar, should contribute to improve resistant cultivars in a more efficient and durable way. The objective of this study was to identify RAPD or SSR markers linked to the resistant allele from cv. Jalo as an aid in selecting for angular leaf spot.

The cross ESAL 550 x Carioca MG was performed and the $F_1$ and $F_2$ generations were obtained. Line ESAL 550 was selected within the cultivar Jalo, which possesses large, yellow seeds (50 g per 100 seeds); resistance to angular leaf spot and is incompatible to most small seeded cultivars of Mesoamerican origin. 'Carioca MG' possesses seeds similar to those of the Carioca cultivar, slightly smaller and darker (20 g per 100 seeds), is highly susceptible to angular leaf spot, but is compatible to cross with ESAL 550. One hundred and twenty $F_{2:3}$ families whose phenotypes were identified under conditions of natural disease incidence were used.

Total DNA extraction from all families was done using the usual CTAB procedure. About 2 g of young leaves was taken from 12 plants of each family. The DNA of the 10 most resistant families and of the 10 most susceptible was bulked to make up the two contrasting bulks, one resistant and the other susceptible (Michelmore et al, 1991).

The bulks were evaluated with 1,080 10-mer primers (Operon Technologies Inc., Alameda, Ca, USA), and 32 pairs of SSR primers designed for *Phaseolus vulgaris* (Yu et al. 2000). The reactions were performed in Eppendorf MasterCycler thermocycler according to manufacturer recommendations. The polymorphic DNA fragments in the bulks and in the $F_{2:3}$ families were resolved in 1% agarose gel for RAPD, and 2,5% for SSR, stained with ethidium bromide and photographed under UV light.

$F_{2:3}$ families segregation based on the disease reaction, RAPD and SSR markers were analyzed by $\chi^2$ test. Recombination frequencies between the resistance gene and one marker or between two markers were estimated according to Allard (1956), and also using the GQMOL software (Cruz & Shuster 2001).

The 120 $F_{2:3}$ families segregated 90 resistant (R) and 30 susceptible (S), in an exactly 3R : 1S ratio ($\chi^2$= 0; P= 0.0000) showing monogenic inheritance, with resistance being due to the dominant allele. Similar results have been observed from other Mesoamerican resistance sources. However, Jalo cultivar is one of the few Andean bean used in Brazil and has shown almost

complete resistance for more than 30 years. Therefore its resistance should be more durable because in Brazil most cultivars utilized is of Mesoamerican origin and the predominant races of the pathogen surely were adapted to overcome the resistance alleles of that origin.

The two RAPD markers and one SSR marker presented segregations in $F_2$ which confirm the dominance monogenic inheritance that qualify them as genetic markers. Interesting that most SSR markers have shown codominance inheritance, although the one found showed dominance as have sometimes happened.

In the co-segregation analysis one RAPD marker is in coupling phase, amplified by the primer OPBB04, and the other is in repulsion, and was amplified by the primer OPP07. The third marker (282bp) is also in coupling phase, and was amplified by the following SSR pair of primers (PV-atct001): 5'CAATTAAAACTCAACCAACCCAAATA3' and 5'TTTCCCGCCTA GAATATGTGAGA3'. The genetic distances and respective LOD scores, their standard errors and confidence intervals are in table 1. Note that the marker OPBB04 on one side and the other two on the other side flanks the reaction gene. Among the three markers, the SSR is the most useful to be used in breeding programs, because it is closer to the resistance allele. The relatively low recombination frequency among them characterizes it as a useful marker for indirect selection, considering the small percentage of plants with a marker, erroneously selected in a segregating population. For example, the expected frequency of susceptible plants selected as resistant in a $F_2$ population is 4.5%.

Table 1. Recombination frequencies (r) between the reaction gene and the markers, considering the co-segregation of two of them at a time, and the respective Haldane distance (cM), LOD score, standard error (SE), and inferior ($CI_I$) and superior ($CI_S$) confidence intervals.

| Loci | Distance (cM) | r (%) | LOD ***Score*** | SE | $CI_I$ | $CI_S$ |
|---|---|---|---|---|---|---|
| OPP07/R allele | 24,4 | 19,20 | 6,33 | 0,04 | 0,11 | 0,27 |
| OPBB04/R allele | 53 | 32,70 | 1,26 | 0,08 | 0,16 | 0,48 |
| PV-atct 001/R allele | 7.6 | 7,08 | 16,21 | 0,0005 | 0,03 | 0,11 |
| OPP07/OPBB04 | 149,6 | 47,49 | 0,02 | 0,07 | 0,33 | 0,61 |
| OPP07/PV-atct 001 | 21,9 | 17,71 | 6,82 | 0,03 | 0,10 | 0,25 |
| OPBB04/ PV-atct 0013 | 73,9 | 38,65 | 0,54 | 0,07 | 0,23 | 0,53 |

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# MICROSATELLITE MARKERS FOR COMMON BEAN

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## Introduction

Marker-assisted selection (MAS) has been proposed as a useful method in plant breeding as it can help speed up the selection process and reduce the selection costs during the early generations of a breeding program. Cregan et al. (1999) emphasized the importance of the DNA markers not only for assisting selection, but also for genomic analysis, evolutionary studies and gene cloning. Therefore, molecular markers are important to plant breeders as a source of genetic information, as well as for use in the indirect selection of economic traits linked to the marker (Kelly, 1995).

Most of the markers identified in common bean are RAPD and AFLP types, however, new markers such as the microsatellites (SSRs) have gained popularity among breeders and plants geneticists. This class of codominant marker that detects a high level of allelic variation associated with the economical and simple PCR procedure, has become an efficient tool for studies of eukaryotic genes (Panaud et al., 1996). In addition to these traits, they are the preferred markers to assist in breeding several crops because they are randomly distributed in the genome and they are easily reproducible (Rallo et al., 2000). The use of these efficient markers in common bean has been limited by the lack of available primers developed for the species. In this work, microsatellite markers were developed for common bean, using bacteria artificial chromosome (BAC) clones.

## Materials and Methods

Four BAC clones chosen from the genomic library of the bean cultivar Sprite (Van Houten and McKenzie, 19??; Melotto and Kelly, 2002) and made available through the bean breeding program at Michigan State University were subcloned, using three different combinations of restriction enzymes: *Alu*I/*Hpa*I, *Rsa*I/*Nae*I, and *Hinc*II/*Xmn*I. The small insert library was hybridized with three SSR probes. After two hybridization cycles, the positive clones were sequenced. The sequences were analyzed for the redundancy and presence of SSR sequences. Specific primers, complementary to the sequences that flank the SSRs, were designed and tested in the original BAC clones and in ten bean cultivars. Six cultivars selected; Rudá, BAT 332, Cornell 49-242, Mexico 54, MAR-2, and AND 277 came from the common bean breeding program for resistance to angular leaf spot, developed by BIOAGRO-UFV, Viçosa, Brazil; BAT 93 and JaloEEP558, the parents of the consensus bean map (Freyre et al., 1998) were chosen; and Black Magic, and SEL1308 the parents of mapping population developed at MSU to study anthracnose resistance in common bean (Melotto and Kelly, 2001) were chosen.

## Results and Discussion

The subcloned fragments were hybridized with the microsatellite probes, $(AT)_{15}$, $(CT)_{15}$ and $(ATT)_{10}$. These oligonucleotides were chosen because they are the most frequent SSRs in higher plant genomes (Morgante and Olivieri, 1993; McCouch et al., 1997); and the same AT/TA and CT/AG, repetitions were reported as the most common in the bean species (Yu et al. 1998, 1999). From 890 colonies analyzed, 8% or 72 hybridized positively. When the clones were

sequenced, 27 showed microsatellite sequences (Table 1), and some exhibited more than one microsatellite. Primers were designed and tested, but three did not generate any product, while five amplified several bands. Twenty-one primers pairs amplified a clearly defined and unique band, of which 15 were polymorphic and six were monomorphic. The allele numbers per locus ranged from one to six, showing a high degree of polymorphism for these markers. According to Weber (1990), the number of alleles in a microsatellite is usually correlated with the number of repetitions they possess; in general, a higher number of repetitions leads to higher polymorphism. However, some primers observed in this research had SSRs with only three repetitions and amplified two alleles, whereas others with 5, 6 and 11 repetitions produced a monomorphic band. These observations suggest the lack of any correlation between the number of repetitive units and the number of detected alleles in common bean. A similar lack of correlation was also reported by Panaud et al. (1996), Yu et al. (1999), and Rallo et al. (2000).

The described procedure was shown to be efficient for the development of microsatellite markers in common bean. The procedure was relatively fast, with lower associated costs and labor, compared to other methods that involve the construction of a genomic library. In this study, however, the developed primers can only be used to analyze the small area of the genome that contained the BAC clones. These SSR markers will be highly useful to saturate the specific area of the genome where major disease resistance traits are known to reside (Melotto and Kelly, 2001). This group of new microsatellite markers, combined with the other available markers, should also provide an important tool in the breeding and genetic of common bean. The future identification of other markers utilizing other libraries should be performed, so that the entire bean genome can be saturated with SSR markers.

**Table 1. Sequenced clones results**

| Sequenced clones | 62 |
|---|---|
| Clones without insert | 6 |
| Clones with *Escherichia coli* DNA | 3 |
| Redundant sequences | 4 |
| Problems with the sequencing | 6 |
| Sequences with no microsatellites | 16 |
| **Sequences with microsatellites** | **27** |

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# SIMULTANEOUS TRANSFER OF RESISTANCE GENES FOR RUST, ANTHRACNOSE AND ANGULAR LEAF SPOT TO CULTIVAR PEROLA ASSISTED BY MOLECULAR MARKERS

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The common bean (*Phaseolus vulgaris*) is susceptible to several diseases under field conditions. The best strategy to overcome this situation is the use of resistant cultivars. The development of cultivars which are simultaneously resistant to several types of pathogens depends on a multidisciplinary effort. One of the strategies to reach this goal is the pyramiding of resistance genes. Usually in this process resistance genes are transferred through backcrosses from cultivars or lines not adapted for commercial use to elite cultivars. Molecular markers can be a useful tool in this process to facilitate selection of specific alleles and to accelerate the recovery of the recurrent progenitor´s genome. In this sense, there is a great expectation on the success of the use of molecular markers in combination with conventional breeding methods to transfer resistance genes to commercial bean varieties. This work aimed at selecting bean progenies containing resistance genes to rust (caused by *Uromyces appendiculatus*), anthracnose (caused by *Colletotrichum lindemuthianum*) and angular leaf spot (caused by *Phaeoisariopsis griseola*), with the aid of molecular markers.

## Materials and Methods

Cultivar Pérola ("carioca type") was first crossed to an advanced line ("carioca type") from the Common Bean Breeding Program from BIOAGRO/UFV containing genes for resistance to rust (from cv. Ouro Negro), anthracnose (genes *Co-6*, *Co-4* and *Co-10*) and angular leaf spot (gene *Phg-1*). The $F_1$ plants were then selfed until $F_5$. The resistance genes were selected in generations $F_1$ to $F_4$ based on molecular markers and inoculations with the pathogens causing the three diseases of interest. The inoculations were made sequentially, that is, after inoculation and evaluation of the symptoms of one disease, the other pathogen was inoculated, and so on. Evaluation of the disease symptoms were according to Stavely et al. (1983) for rust; Pastor-Corrales (1992) for anthracnose; and Pastor-Corrales and Jara (1995) for angular leaf spot.

The markers previously identified in our laboratory, were tested to determine if they were polymorphic between the resistant donor parent and cultivar Pérola. DNA was extracted from leaves according to Doyle and Doyle (1990). RAPD amplification reactions were according to Williams et al. (1990); microsatellite analysis was according to Routman and Cheverud (1994) and the SCAR protocol was described by Corrêa et al. (2000).

## Results and Discussion

DNA amplification revealed that most resistance loci analyzed were polymorphic between the recurrent parent (Pérola) and the donor parents except for marker SSR Satt174 (Figure 1). Therefore, it was not possible to use this marker in the selection process.

In the beginning of the pyramidation process, we had nine $F_1$ plants resistant to the three pathogens and harboring the corresponding molecular markers. Generations $F_2$ to $F_4$ were conducted by the genealogical method, with inoculation of the pathogens for the three diseases and verification of the presence of the markers associated with the resistance genes. At the moment, we have approximately 60 resistant $F_5$ families and in a next step they will undergo field tests for productivity.

The selection of these families with "carioca-type" grains, resistant to rust, anthracnose and angular leaf spot demonstrates the viability of the use of molecular markers to select resistance genes in a pyramiding program. The larger the number of genes involved the more useful the molecular markers become. However, inoculation is still important along the pyramiding process to confirm the data obtained through molecular markers.

**Table 1.** Molecular markers linked to resistance genes of the common bean to rust, anthracnose and angular leaf spot identified at BIOAGRO/UFV

| Markers | Distance | Phase | Gene of Resistance | Disease | Cultivar |
|---|---|---|---|---|---|
| $OPB03_{1800r}$ | 3.7 cM | repulsion | *Co-4* | Anthracnose | TO |
| $OPY20_{830a}$ | 0.0 cM | coupling | *Co-4* | Anthracnose | TO |
| $OPAZ20_{940a}$ | 7.1 cM | coupling | *Co-6* | Anthracnose | AB 136 |
| $OPH13_{490a}$ | 5.5 cM | coupling | *Phg-1* | Angular leaf spot | AND 277 |
| $OPBA8_{560a}$ | 6.0 cM | coupling | *Ur-Ouro Negro* | Rust | Ouro Negro |
| $OPF10_{1050a}$ | 6.9 cM | coupling | *Ur-Ouro Negro*, *Co-10* | Rust and Anthracnose | Ouro Negro |
| $OPX11_{550a}$ | 5.8 cM | coupling | *Ur-Ouro Negro* | Rust | Ouro Negro |
| $Satt174_{300}$ | 4.1 cM | coupling | *Ur-Ouro Negro* | Rust | Ouro Negro |
| $SCARF10_{1050a}$ | 6.9 cM | coupling | *Ur-Ouro Negro*, *Co-10* | Rust and Anthracnose | Ouro Negro |

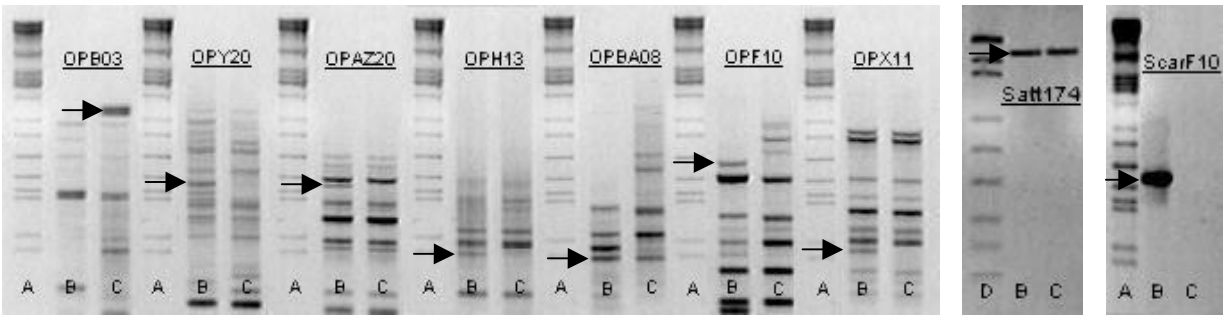


**Figure 1**. Molecular markers for different common bean resistance genes amplified in the resistant line and in cultivar Pérola. (**A**) - Size markers (lambda phage DNA digested with *Eco*RI, *Bam*HI and *Hin*dIII); (**B**) - resistant Line; (**C**) - cultivar Pérola; (**D**) - size markers (pUC18 digested with *Msp*I). The arrows indicate the marker bands for the resistance genes listed in Table 1.

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# RELATIONSHIPS BETWEEN YIELD LOSSES CAUSED BY ANGULAR LEAF SPOT ON BEANS AND DISEASE SEVERITY

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**Introduction.** Angular leaf spot (ALS) caused by *Phaeoisariopsis griseola* is one of the most important bean diseases in Brazil, especially in irrigated areas under center pivot (Paula Jr. & Zambolim, 1998). Leaf area of diseased plants is greatly reduced, which may have implications in the photossynthetic process. Leaf area reduction due to the attack of foliar pathogens has been reported as an important cause of yield losses on several crops (Waggoner & Berger, 1987). The objective of this research was to study the reduction of leaf area on bean plants infected with different disease levels of ALS and the effects on yield.

**Material and Methods.** The experiments were carried out in a field of the Plant Pathology Department of the Federal University of Viçosa, State of Minas Gerais (Brazil), where beans have been planted three times a year for at least six years. Because of that, inoculum potential of ALS has been high in this area. Seeds of bean cultivar Carioca were sown on April 28, 1998, with 14 seeds/m. Rows were spaced 0.5 m apart. Plot size was eight rows, each row was 5 m long. The epidemic of ALS was natural and the symptoms and signs were homogeneous in the field. Plants from one meter of the central row were weekly sampled to evaluate disease severity and leaf area. The trial was carried out in a randomized complete block design with four replications. Severity was evaluated with the aid of a diagrammatic scale described by Godoy et al. (1997). Leaf area was measured using a leaf area meter. Chlorothalonil + thiophanate methyl (Cerconil) at 1.5 L/ha was used to control the disease and to achieve different disease levels. The following treatments were tested: T0 = fungicide application weekly; T1, T2 and T3 = fungicide application when the severity reached 3, 6 and 9%, respectively; T4 = without fungicide application. The frequency of fungicide application varied from 0 to 7 times. Applications were done during the following plant developmental stages: V3 (first trifoliate leaves), R5 (preflowering), R6 (flowering), R7 (pod growth), R8 (pod filling), and R9 (maturation). Yield was determined considering two central rows in each plot.

**Results and Discussion.** Table 1 and Figures 1 and 2 summarize the results. Disease severity was negatively correlated with number of fungicide applications. High severity levels were always related to high reduction of the leaf area. Leaf area was reduced 71, 53, 18 and 63% in the treatments T1, T2, T3 and T4, respectively, compared to T0. Leaf area obtained in the treatment T4 was six times lower than that obtained in T0. Leaf area reduction correlated positively with yield reduction. On the other hand, disease control allowed more retention of leaves and consequently higher yield. Losses caused by ALS varied from 14 (T1) to 42% (T4).

**Table 1. Relationships among ALS severity, leaf area, yield and yield losses**

| Treat | Number of Applications | Plant Developmental Stages at Fungicide Application | Severity (%) | Leaf area ($cm^2$) | Yield (Kg/ha) | Yield Losses (%) |
|---|---|---|---|---|---|---|
| T0 | 7 | V3, R5, R6, R7, R8, R9, R9 | 5.53 | 909.9 | 1503 | 0 |
| T1 | 5 | R6, R7, R8, R9, R9 | 6.05 | 739.9 | 1292 | 14 |
| T2 | 4 | R7, R8, R9, R9 | 7.92 | 426.6 | 1151 | 23 |
| T3 | 1 | R9 | 13.70 | 264.0 | 976 | 35 |
| T4 | 0 | - | 18.30 | 136.8 | 883 | 42 |

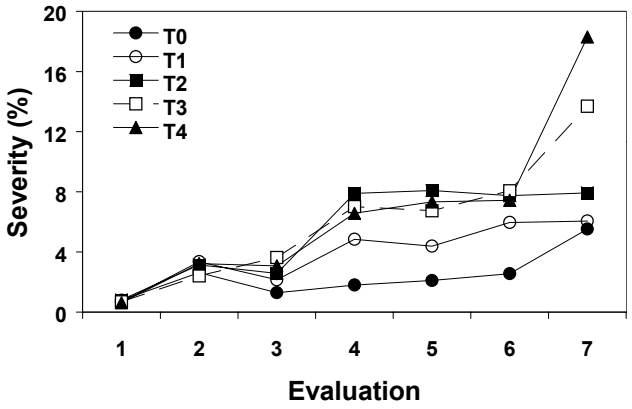


**Figure 1. Disease severity evaluated at weekly interval from the stage V3.**

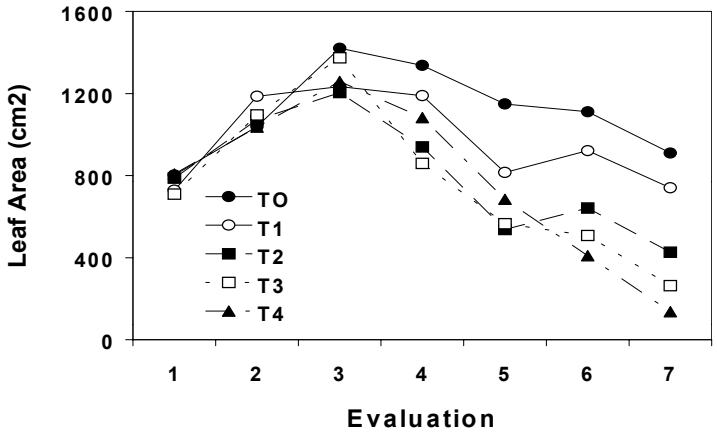


**Figure 2. Leaf area evaluated at weekly interval from the stage V3.**

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# RELATIONSHIPS BETWEEN DISEASE SEVERITY (ANGULAR LEAF SPOT, RUST AND ANTHRACNOSE), HEALTH LEAF AREA, HEALTH LEAF AREA ABSORTION AND YIELD ON COMMON BEANS

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**Introduction.** Angular leaf spot (*Phaeoisariopsis griseola*), rust (*Uromyces appendiculatus*) and anthracnose (*Colletotrichum lindemuthianum*) are important bean diseases in Brazil. Resulting yield losses depend on many factors including environmental conditions and resistance of cultivars. Several authors have tried to develop and standardize methods to estimate yield losses caused by plant diseases (Madden, 1983; Campbell & Madden, 1990). Generally, mathematical relationships can not be applied at different pathossystems or localities (Campbell & Madden, 1990). Leaf area reduction due to the attack of foliar pathogens has been reported as an important cause of yield losses on several crops (Waggoner & Berger, 1987). The objective of this work was to study the correlation of some characteristics of bean pathossystems involving angular leaf spot, rust and anthracnose with yield data.

**Material and Methods.** The experiments were carried out during two years in a field of the Plant Pathology Department of the Federal University of Viçosa, State of Minas Gerais, Brazil. Ten seeds/m of the cultivars Carioca and Vermelho were sown. An area of 256 $m^2$ consisting of 16 plots was planted with each cultivar. Plot size was 16 $m^2$. Each plot consisted of eight rows, which were 4 m long spaced 0.5 m apart. The pathogens at different inoculum levels (0, $10^2$, $10^4$, and $10^6$ spores/ml) were separately inoculated to obtain different disease intensities. In each plot, six plants were target and weekly evaluated for disease severity and leaf area. The trials were carried out in a randomized complete block design with four replications. Disease severity was evaluated with the aid of a diagrammatic scale described by Godoy et al. (1997). Leaf area was measured using a leaf area meter. Irradiation data was collected in a meteorological station near by the experimental area. Relationships between health leaf area duration (HAD), area under disease progress curve (AUDPC), health area absorption (HAA) and the yield were determined.

**Results and Discussion.** Table 1 summarizes the results. High $R^2$ values were obtained with HAD, independent of the cultivar, disease and planting time. The $R^2$ values varied from 0.48 to 0.78. The AUDPC did not adjust in six of the ten adjusted regressions. In these six cases, the $R^2$ values varied from 0.10 to 0.37. HAD was the best characteristic to estimate yield.

Table 1. Relationships between HAD, HAA, AUDPC and yield of beans infected by *Phaeoisariopsis griseola* (PG), *Uromyces appendiculatus* (UA) and *Colletotrichum lindemuthianum* (CL)

| Models | $b_0$ | $b_1$ | $R^2$ | Pathossystem |
|---|---|---|---|---|
| $Y = b_0 + b_1HAD$ | -1.226 | 0.114 | 0.52 | PG-Y2-C |
| $Y = b_0 * exp(b_1HAA)$ | 0.197 | 0.026 | 0.46 | PG-Y2-C |
| $Y = b_0 * exp(b_1AUDPC)$ | 23.171 | -1.066 | 0.37 | PG-Y2-C |
| $Y = b_0 + b_1HAD$ | -2.777 | 1.339 | 0.78 | PG-Y2-V |
| $Y = b_0 * exp(b_1HAA)$ | 0.820 | 0.015 | 0.67 | PG-Y2-V |
| $Y = b_0 * exp(b_1AUDPC)$ | 26.656 | -0.40 | 0.19 | PG-Y2-V |
| $Y = b_0 + b_1HAD$ | -3.195 | 0.202 | 0.70 | UA-Y2-C |
| $Y = b_0 * exp(b_1HAA)$ | 0.487 | 0.016 | 0.52 | UA-Y2-C |
| $Y = b_0 * exp(b_1AUDPC)$ | - | - | ns | UA-Y2-C |
| $Y = b_0 + b_1HAD$ | 6.117 | 0.119 | 0.55 | UA-Y2-V |
| $Y = b_0 * exp(b_1HAA)$ | 1.148 | 0.014 | 0.43 | UA-Y2-V |
| AUDPC | - | - | ns | UA-Y2-V |
| $Y = b_0 + b_1HAD$ | -2.969 | 0.190 | 0.70 | CL-Y2-C |
| $Y = b_0 + b_1HAA$ | 123.28 | 6.87 | 0.57 | CL-Y2-C |
| AUDPC | - | - | ns | CL-Y2-C |
| $Y = b_0 + b_1HAD$ | 0.075 | 0.518 | 0.73 | CL-Y1-C |
| $Y = b_0 * exp(b_1HAA)$ | 0.578 | 0.061 | 0.71 | CL-Y1-C |
| AUDPC | - | - | ns | CL-Y1-C |
| $Y = b_0 + b_1HAD$ | -2.865 | 0.157 | 0.48 | UA-Y1-C |
| $Y = b_0 * exp(b_1HAA)$ | 0.736 | 0.019 | 0.30 | UA-Y1-C |
| $Y = b_0 * exp(b_1AUDPC)$ | - | - | ns | UA-Y1-C |
| $Y = b_0 + b_1HAD$ | -0,316 | 1.653 | 0.70 | UA-Y1-V |
| $Y = b_0 * exp(b_1HAA)$ | 0.674 | 0.036 | 0.81 | UA-Y1-V |
| $Y = b_0 * exp(b_1AUDPC)$ | 17.836 | -0.774 | 0.10 | UA-Y1-V |
| $Y = b_0 + b_1HAD$ | -2.147 | 0.095 | 0.59 | CL-Y1-C |
| $Y = b_0 * exp(b_1HAA)$ | 0.323 | 0.035 | 0.38 | CL-Y1-C |
| $Y = b_0 * exp(b_1AUDPC)$ | 6.123 | -0.012 | 0.16 | CL-Y1-C |
| $Y = b_0 + b_1HAD$ | -6.112 | 0.142 | 0.54 | CL-Y1-V |
| $Y = b_0 * exp(b_1HAA)$ | 0.544 | 0.029 | 0.37 | CL-Y1-V |
| $Y = b_0 * exp(b_1AUDPC)$ | - | - | ns | CL-Y1-V |

Y1 and Y2 = years 1 and 2, respectively; C and V = cultivars Carioca and Vermelho, respectively; ns = not significant.

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# GENETIC DIVERSITY OF *Phaeoisariopsis griseola* BY THE RAPD METHOD

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Angular leaf spot, caused by the fungus *Phaeoisariopsis griseola* (Sacc.) Ferr., has been recognized as one of the most important air borne disease of common beans (*Phaseolus vulgaris* L.) in Brazil. Control methods for this disease includes cultural practices, chemical control and genetic resistance.

The development of new bean cultivars resistant to angular leaf spot needs the previous acknowledgment of the pathogen variability. It has been demonstrated that *P. griseola* presents great variability. The method universally accepted to determine such variability is through the inoculation of a bean differential set. The advent of the DNA recombinant technique offers new methodologies for a more complete and secure investigation of the genetic of plants and phytopathogenic fungi. Diversity and characterization studies of several plant species and fungi have been realized by the Random Amplified Polymorphic DNA (RAPD). The major advantages of this approach are its simplicity, the universality of used primers, tolerance to a wider range of DNA concentration and the lack of environment influences in the results.

Erlenmeyer flasks of 250 mL of liquid medium (200 g Potato and 10 g glucose per liter of water) were inoculated with 5-6 agar disks of 0,7 cm in diameter from each isolate. The cultures were placed in a rotary shaker (110-120 rpm) and incubated at room temperature for 12-15days. Mycelia were harvested by filtration through filter paper and placed in liquid nitrogen and then transferred to -80 $^{o}$C refrigerator until DNA extraction. DNA extraction was performed according to the SDS procedure using TE buffer for final dilution of the samples. RAPD reactions were carried out with the primers OP K 07, K 09, L 12, L 14, L 17, R 03, R 04 e R 17. Amplification reactions were performed in thermocycler model PTC-100™. Each reaction of 25 µL contained 25 ng of DNA, 0.1 mM of each dNTP, 2.0 mM $MgCl_2$, 10 mM Tris-HCl, pH 8.0, 50 mM KCl, 0,4 uM of one primer decamer, and one unit of Taq DNA polymerase. Each amplification cycle consisted of one initial DNA denaturation step of 3 minutes at 94 $^{o}$C followed by 45 cycles of 1 minute at 94 $^{o}$C, 1 minute at 35 $^{o}$C and 2 minutes at 72 $^{o}$C and a final extension step of 10 minutes at 72 $^{o}$C. Amplification products were separated by electrophoresis in a 1.5% agarose gel and visualized under UV light and photographed with the Eagle Eye II photosystem (Stratagene Inc, La Jolla, CA). The DNA bands obtained for each individual were scored based on their presence (1) or absence (0). Only the most intense bands were considered. Cluster analysis was done by the Unweighted pair-group average and Euclidean distances. All calculations were done with the program Statistica, version 5.0.

The analyze of 98 *P. griseola* isolates collected at Damolandia and Inhumas county, State o Goiás, Brazil, revealed great genetic diversity. According to the method used and at a distance level of 62,5%, isolates were clustered in 4 groups (Figure 1.). Groups 1 and 4 were formed by isolates originated only from Damolandia (4 isolates) and Inhumas (2 isolates) counties, respectively, while groups 2 and 3 were formed by isolates from both counties. Although the obtained dendrogram did not show any clustering in these groups according to the isolate origin, it was possible to observe a tendency of the isolates in these two groups be from Inhumas and Damolandia counties, respectively. A non linkage among RAPD markers and the pathotypes used in this study was observed. No pathotype specific band were observed in the present study. By the distance matrix it was possible to observe that the distance among isolates varied from

0,00 to 5,39, indicating great variability among the isolates of the fungus *P. griseola* what is in accordance with the variability found for this pathogen determined by inoculating the differential set. Although the RAPD allows a more complete study of the *P. griseola* genome, when compared to the virulence test, unhappily this technique does not permit the identification of which amplified locus is linked to virulence in the fungus. This fact makes this technique not appropriated for the identification of *P. griseola* pathogenic variability.

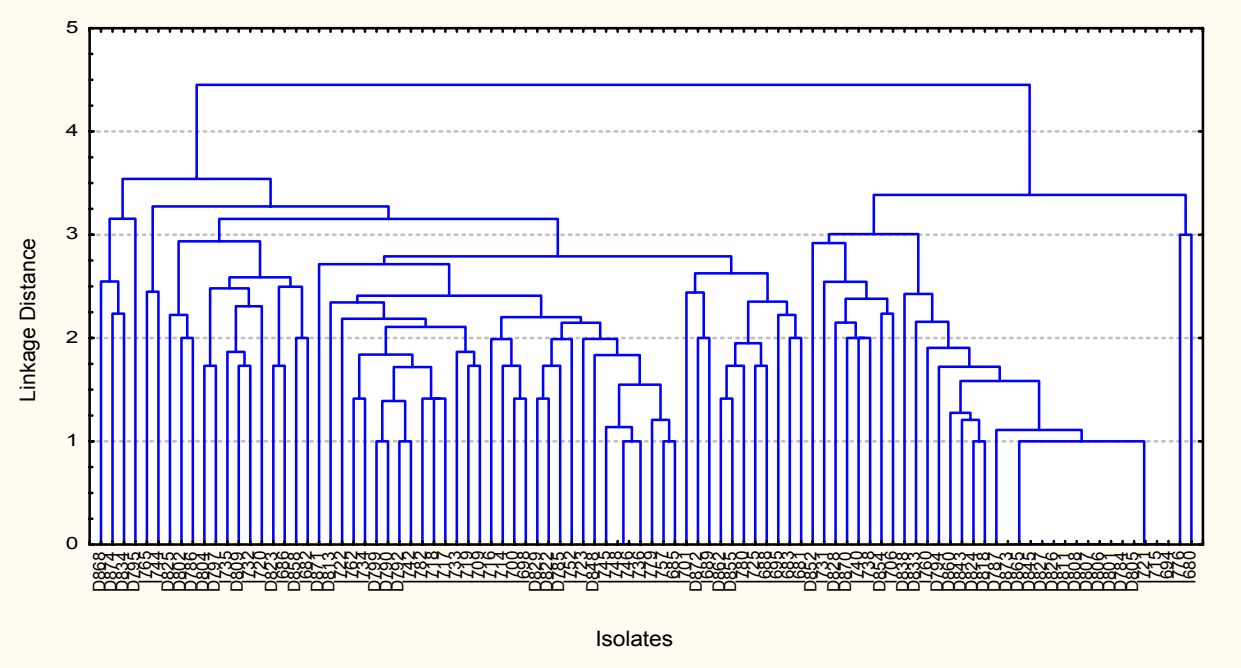


**Figure 1.** Dendogram of 96 isolates of *Phaeoisariopsis griseola* based on the RAPD method using 8 primers (OP K 07, K 09, L 12, L 14, L 17, R 03, R 04 e R 17). Embrapa Rice & Beans, 2002. (D = Damolandia; I = Inhumas).

# VIRULENCE PATTERN OF COLLETOTRICHUM LINDEMUTHIANUM IN COMMON BEAN IN ECUADOR

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**Introduction:** Anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. & Magn.) Scrib. is one of the most important pathogens of common bean in Ecuador. The disease is confined to cool or moderate temperatures and high humidity. Methods of control as sanitation and chemical control are available but are not implemented by most bean growers and in addition are expensive especially for the small farmers in Ecuador. Breeding for resistance offer a good approach in order to reduce the environmental pollution and avoid the boom and bust cycle of introduced with non-durable resistance. Incorporating sources of resistance to the existing bean cultivars requires adequate knowledge of the virulence pattern of the pathogen populations. The objective of this study is to determine the virulence pattern of *Colletotrichum lindemuthianum* by using standard world differential set together with local bean cultivars from Ecuador as supplemental set.

**Materials and Methods:** In 2002, a total of 31 infected leaf samples with *C. lindemuthianum* were collected from the major bean growing area in Ecuador. Race determination work was carried out in greenhouse at the Santa Catalina Experimental Research Station. Quito, Ecuador. A set consisting of 12 international differentials (Balardin et al., 1997) supplemented with nine local cultivars from Ecuador was used in this study. Seedlings were grown in the greenhouse at average temperature of 16ºC. Inoculation of primary leaves was made 14 days after planting by using a spore suspension containing 1.2 x $10^6$ conidias/ml of *C. lindemuthianum.* Incubation was carried out by replacing the plants in a growth chamber at 16ºC and 100% of relative humidity for 48 hours. Seven days after incubation, the reaction type to anthracnose was recorded by using 1-9 scale as described by Pastor-Corrales, 1994. When the reaction type was between 4-9, an isolate was usually considered to be virulent on the differential. (Balardin, et al. 1997). Race identification on the differential set was made through the use of the binary notation proposed by Pastor-Corrales, 1994.

**Results and Discussion:** From 31 samples examined, a total of 12 group of isolates (A-L) showing different virulence pattern were identified on both international and the supplemental local cultivars (Table 1). On the international differentials, the races 0, 3, 4, 256, 260 and 1346 were detected, indicating that using the international supplemental is not adequate to differentiate between the existing virulence in Ecuador. In addition, the lack of stability of the host pathogen interaction, smallness of the race specific effects should also not be ignored. The results obtained demonstrate that the known/unkown resistance genes/factors in the differentials Cornell 49242, Widusa, Kaboon, PI 207262, Tu and G2333 are still effective in the country. The cultivar Paragachi proved to be susceptible to all isolates evaluated and probably does not carry any resistance factor (Table 1). The local cultivars were very useful in examining the virulence of *C. lindemuthianum* in Ecuador. Most of the local cultivars are land races and extensively cultivated in the country (Table 1). Therefore, a host/pathogen co-evolution appears to have been taking place among the pathogen populations in Ecuador. Therefore is highly recommended to include

the local cultivars from Ecuador to the international differential set in order to differentiate adequately between the virulence factors that exist among the populations of *C. lindemuthianum* in Ecuador. It is also worth noting that some isolates of *C. lindemuthianum* in Ecuador carry virulence to the Mesoamerican source of resistance *Co-3*, *Co-4*, *Co-6* and *co-8*, which is not expected in the Andean region (Pastor-Corrales, M. 1994). Presence of virulence to these genes might be explained by pathogen adaptation to these sources of resistance during evaluation of Mesoamerican germplasm in trials in Ecuador.

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**Table 1.** Compatible (+)/incompatible (-) reaction of 12 group of isolates of *Colletotrichum lindemuthianum* on 12 standard differentials and nine local cultivars in Ecuador.

| Differentials | Co genes | Binary Value | Group of isolates | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | A | B | C | D | E | F | G | H | I | J | K | L |
| Michelite | -- | 1 | - | - | - | - | - | - | + | - | - | - | - | - |
| M.D.R.K. | *Co-1* | 2 | - | - | - | - | - | - | + | - | - | - | - | + |
| P. Marrow | *Co-1*[3] | 4 | - | - | - | - | - | - | - | + | - | - | + | - |
| Cornell 49242 | *Co-2* | 8 | - | - | - | - | - | - | - | - | - | - | - | - |
| Widusa | -- | 16 | - | - | - | - | - | - | - | - | - | - | - | - |
| Kaboon | *Co-1*[2] | 32 | - | - | - | - | - | - | - | - | - | - | - | - |
| Mexico 222 | *Co-3* | 64 | - | - | - | - | - | - | - | - | - | - | - | + |
| PI 207262 | -- | 128 | - | - | - | - | - | - | - | - | - | - | - | - |
| To | *Co-4* | 256 | - | - | - | - | - | - | - | - | + | + | + | + |
| Tu | *Co-5* | 512 | - | - | - | - | - | - | - | - | - | - | - | - |
| AB136 | *Co-6, co-8* | 1024 | - | - | - | - | - | - | - | - | - | - | - | + |
| G2333 | *Co-4*[2]*, Co-5, Co-7* | 2048 | - | - | - | - | - | - | - | - | - | - | - | - |
| | | **Race**[1] | **0** | **0** | **0** | **0** | **0** | **0** | **3** | **4** | **256** | **256** | **260** | **1346** |
| **Local cultivars** | | | | | | | | | | | | | | |
| Paragachi[3] | | | + | + | + | + | + | + | + | + | + | + | + | + |
| C. Imbabura[2] | | | + | + | + | + | - | + | + | - | + | + | + | + |
| Cocacho[2] | | | - | - | + | + | + | + | + | + | + | + | + | + |
| Magola[2] | | | - | - | - | + | - | + | + | - | + | + | + | - |
| Je.Ma[3] | | | - | - | - | - | + | + | - | + | - | + | + | - |
| Mil Uno[4] | | | - | + | - | - | - | - | - | - | - | + | + | + |
| San Antonio[2] | | | - | - | - | + | + | - | - | - | + | - | - | - |
| G916[4] | | | - | - | - | - | - | + | - | - | - | + | + | - |
| CAP9[4] | | | - | + | - | - | - | - | - | - | - | + | - | - |

**Isolates within each group** A: SCCl20, SCCl33; B: SCCl18, SCCl22, SCCl23, SCCl28; C: SCCl11; D: SCCl46; E: SCCl47, SCCl48; F: SCCl6, SCCl9, SCCl17; G: SCCl1, SCCl2, SCCl3, SCCl4, SCCl5, SCCl7, SCCl8, SCCl10, SCCl15, SCCl50, SCCl54; H: SCCl13, SCCl21; I: SCCl31, SCCl37; J: SCCl16, SCCl19; K: SCCl38

[1] Binary nomenclature identification (Pastor Corrales, M. 1994). [2] Local cultivars. [3] Bred cultivars. [4] Bred advanced lines.

# ALLELISM TEST FOR RESISTANCE TO RACE 38 OF ANTHRACNOSE IN COMMON BEAN DIFFERENTIAL CULTIVAR, 'WIDUSA'.

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Widusa is one of the twelve differential cultivars proposed by Pastor Corrales (1991) for the characterization of the pathogenic diversity of *Colletotrichum lindemuthianum* (Sacc.& Magnus) Lambs.-Scrib, causing anthracnose. Although the genetic characterization for anthracnose resistance of these differential cultivars is of major importance, not all have been extensively studied.

In this report we present the results of an allelism test addressed to identify the gene(s) conferring resistance to race 38 present in Widusa. The results obtained strongly support that Widusa has two resistance genes against this race, one of them dominant and the other, recessive.

Race 38 of *C. lindemuthianum* is among the most common races found in Northern Spain (Ferreira et al, 1998). The inoculations were carried out in a climate chamber on 8 to 10-days-old seedlings. The seedlings were sprayed with an aqueous conidial suspension derived from monosporic cultures containing $10^6$ spores per ml and maintained at 20-22ºC, 95-100% humidity and 12 h photoperiod. The responses of the plants were evaluated after 7-9 days.

Apart from 'Widusa', the plant materials used in this study were the differential cultivars 'Michelite', 'Mexico 222', 'TO', 'TU', 'AB 136' and 'PI 207262', all of them resistant to race 38, 'Andecha', a susceptible to race 38 white seeded cultivar, proceeding from a selection of Asturian (Spain) landraces, 'Xana', a susceptible to race 38 cultivar derived from 'Andecha', and 'A1183', a resistant to race 38 line obtained through a backcross breeding program in which Andecha was the recurrent parental and 'Sanilac' the resistance donor. 'A1183' has the dominant resistance gene, Co-2, proceeding from 'Cornell 49-242' via 'Sanilac' (Mendez de Vigo, 2001).

Table 1 shows the segregations for resistance to race 38 in F2 populations derived from crosses between different materials. The segregation in the F2 population derived from the cross 'Widusa' x 'Xana' (R x S) shows a good fit only to a 13R : 3S ratio. This suggests that two independent genes, one of them dominant and the other recessive, are involved in the resistance to race 38 present in 'Widusa'. The segregations of F2 populations derived from 'PI 207262' (R) x 'Andecha' (S), 'Mexico 222' (R) x 'Andecha' (S), and 'PI 207262' (R) x 'A1183' (R) indicate that the resistant parentals involved in these crosses have a single dominant gene conferring resistance to race 38. Therefore, the fit to a 61R : 3S ratio observed in the F2 populations derived from 'Widusa' x 'PI 207262', 'Widusa' x 'A1183', 'Widusa' x 'Mex 222' and 'Widusa' x 'TU', agrees with the hypothesis of two resistance genes (dominant + recessive) being present in 'Widusa'.

On the other hand, the lack of segregation in the F2 populations derived from crosses between 'Widusa' and 'Michelite', 'TO' and 'AB136' indicates that these three differential cultivars share at least one resistance gene with 'Widusa'. The segregation observed in the F2 population derived from the cross 'AB 136' (R) x 'Xana' (S) fit either a 15R : 1S ratio or a 61R : 3S ratio. This indicates that 'AB 136' has at least two independent dominant genes conferring resistance to race 38 and opens the possibility of a third recessive gene being also present in this material. Unfortunately, we have not yet F2 populations derived from crosses between materials susceptible to race 38 and differential cultivars 'TO' or 'Michelite'.

**Table 1.- Allelism test for genetic characterization of the resistance to race 38 of *C. lindemuthianum* in Widusa.**

| F2 population | Reaction | Observed values | | Expected values | | | $\chi^2$ | prob. |
|---|---|---|---|---|---|---|---|---|
| | | Res. | Sus. | Ratio | Res. | Sus. | | |
| Widusa x Xana | R x S | 277 | 64 | 13:3 | 277.1 | 63.9 | 0.0001 | 0.99 |
| PI 207262 x Andecha | R x S | 197 | 54 | 3:1 | 188.3 | 62.8 | 1.627 | 0.20 |
| Mexico 222 x Andecha | R x S | 76 | 20 | 3:1 | 72.0 | 24.0 | 0.889 | 0.35 |
| PI 207262 x A1183 | R x R | 252 | 23 | 15:1 | 257.8 | 17.2 | 2.09 | 0.15 |
| Widusa x PI 207262 | R x R | 303 | 12 | 61:3 | 300.2 | 14.8 | 0.544 | 0.46 |
| Widusa x A1183 | R x R | 142 | 8 | 61:3 | 143.0 | 7.0 | 0.140 | 0.71 |
| Widusa x Mex 222 | R x R | 153 | 6 | 61:3 | 151.5 | 7.5 | 0.297 | 0.59 |
| Widusa x TU | R x R | 145 | 12 | 61:3 | 149.6 | 7.4 | 3.070 | 0.08 |
| Widusa x TO | R x R | 114 | 0 | - | - | - | - | - |
| Widusa x Michelite | R x R | 116 | 0 | - | - | - | - | - |
| Widusa x AB136 | R x R | 286 | 0 | - | - | - | - | - |
| AB136 x Xana | R x S | 170 | 9 | 15:1 | 167.8 | 11.2 | 0.456 | 0.50 |
| AB136 x Xana | R x S | 170 | 9 | 61:3 | 170.6 | 8.4 | 0.046 | 0.83 |

In order to confirm the 13 : 3 (resistant : susceptible) segregation in the F2 population derived from Widusa x Xana, 78 F2:3 families proceeding from this F2 progeny (a minimum of 16 individuals per F2:3 family) were evaluated for resistance to race 38. The results obtained (Table 2) agree with the expected segregation under this hypothesis.

**Table 2.- Characterization of F2:3 families of Widusa x Xana for their resistance to race 38 of *C. lindemuthianum.***

| | Observed values[a] | | | | Expected values[a] (7:6:2:1) | | | | $\chi^2$ | prob. |
|---|---|---|---|---|---|---|---|---|---|---|
| | R | DS | RS | S | R | DS | RS | S | | |
| F2:3 families | 34 | 33 | 5 | 6 | 34.1 | 29.3 | 9.8 | 4.9 | 3.055 | 0.38 |

a: R= F2:3 families with all plants resistant; DS= F2:3 families showing "dominant" segregation for resistance (13R:3S or 3R:1S) ; RS= F2:3 families showing recessive segregation for resistance (1R:3S); S= F2:3 families with all plants susceptible.

Using anthracnose race 65, Alzate Marin et al. (2001, 2002) concluded that 'Widusa' has a single dominant resistance gene, also present in 'PI 207262' and different to the gene(s) conferring resistance in 'TO' and 'SEL 1308'. The results obtained in the present study would indicate the presence of two additional independent genes conferring resistance to race 38 in this differential cultivar.

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# Evaluation of *Phaseolus vulgaris* Germplasm For Resistance To Five Anthracnose Races Isolated in Northern Spain

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Bean anthracnose, caused by the fungus *Colletotrichum lindemuthianum* (Sacc.& Magnus) Lambs.-Scrib., is a serious problem in Northern Spain. At the moment, five different races of this fungus have been identified in this area (Ferreira et al. 1998), and some resistance sources as A321, A493, A252 and Sanilac (*Co-2*) have been successfully used in our breeding programs. However, due to the likely emergence of new pathogenic strains, the identification of other sources of resistance would be of major interest. In this report we present the results of an evaluation for resistance to five races of anthracnose of 292 *Phaseolus vulgaris* accessions conserved in the SERIDA collection (Villaviciosa, Asturias, Spain).

*C. lindemuthianum* races 3, 6, 38, 102, and 787 (binary nomenclature; Pastor Corrales, 1991) were used in this work. These races were collected between 1992 and 1997 from common bean cultivars grown in Northern Spain. The inoculations were carried out on 10-days-old seedlings. The seedlings were sprayed with an aqueous conidial suspension derived from monosporic cultures containing $10^6$ spores per ml and maintained at 20-22ºC, and 90-100% humidity. At least ten plants form each accession were evaluated after 7-9 days according to the criterions described by Schoonhoven and Pastor Corrales (1987).

A total of 246 landraces, 35 international lines and 8 breeding lines obtained in SERIDA (Xana, A1183, A1220, A1231, A1239, A1258, X1358 and X1319) were evaluated for resistance against the five races. In each evaluation the set of differential cultivars proposed by Pastor Corrales (1991) was incorporated as control. The results are summarized in Table 1.

According to the severity and homogeneity of anthracnose symptoms, four types of accessions were considered: resistant accessions (R), in which all plants were clearly resistant; susceptible accessions (S), in which all plants showed severe disease symptoms; accessions with intermediate reaction (I), in which all plants were moderately susceptible; and accessions containing both resistant and susceptible plants (R/S), probably proceeding from mixed populations. The accessions belonging to these two last types are included in the collection as local germplasm accessions.

In regard to the virulence of individual races to local materials, it ranged from 84% of race 6 to 57% of race 3. These values differ of those found in international materials, ranging from 55% of race 3 to 28% of race 102.

Table 2 shows the resistance spectra of the materials in which the reactions to all five pathogenic races were analyzed. Twenty-five out of 32 possible resistance spectra (combinations) were found, being the susceptibility to all races the most frequent one.

In addition to the differential cultivars Cornell 49 242, PI 207262, AB136 and G2333, and the breeding lines A1220, A1183, A1258, A1231, A1239, X1319 and X1358 (Table 1), eight international lines (Catrachita, A252, A321, A493, Sanilac, SEL1360, SEL1308 and BAT93) were resistant to all five races. None of the landraces presented a good resistance to all five races. The local germplasm accessions V369, V225 and V309, showed a good resistance to four races and an intermediate or mixed reaction for the fifth one.

The variation found suggests the presence of an important polymorphism in the genetic control for the resistance to *C. lindemuthianum* and offers the opportunity to identify new genes or alleles implicated in this resistance.

Table 1.- A summary of the reaction of 246 landraces, 35 international lines and 8 breeding lines to anthracnose races 3, 6, 38, 102 and 787 (R= resistant, S= susceptible, I = intermediate; R/S= mixed populations).

| Race | *Type of accession* | Type of reaction R | S | I | R/S | Total | % Susceptible |
|---|---|---|---|---|---|---|---|
| 3 | Landraces | 77 | 131 | 12 | 9 | 238 | 57.2 |
| | International lines | 14 | 19 | 1 | 0 | 34 | 55.9 |
| | Differentials | 10 | 2 | 0 | 0 | 12 | 16.7 |
| | Breeding lines | 8 | 0 | 0 | 0 | 8 | 0.0 |
| 6 | Landraces | 24 | 192 | 8 | 3 | 236 | 84.6 |
| | International lines | 16 | 17 | 2 | 0 | 35 | 48.6 |
| | Differentials | 10 | 2 | 0 | 0 | 12 | 16.7 |
| | Breeding lines | 7 | 1 | 0 | 0 | 8 | 12.5 |
| 38 | Landraces | 40 | 190 | 3 | 4 | 246 | 80.2 |
| | International lines | 19 | 13 | 3 | 0 | 35 | 37.1 |
| | Differentials | 9 | 3 | 0 | 0 | 12 | 25.0 |
| | Breeding lines | 7 | 1 | 0 | 0 | 8 | 12.5 |
| 102 | Landraces | 50 | 150 | 20 | 8 | 237 | 65.8 |
| | International lines | 24 | 10 | 1 | 0 | 35 | 28.6 |
| | Differentials | 8 | 4 | 0 | 0 | 12 | 33.3 |
| | Breeding lines | 8 | 0 | 0 | 0 | 8 | 0.0 |
| 787 | Landrace | 75 | 136 | 7 | 9 | 236 | 59.9 |
| | International lines | 16 | 18 | 0 | 1 | 35 | 51.4 |
| | Differentials | 7 | 5 | 0 | 0 | 12 | 41.7 |
| | Breeding lines | 8 | 0 | 0 | 0 | 8 | 0.0 |

Table 2.- Frequency of the resistance spectra for races 3, 6, 38, 102 and 787, in the landraces and international lines of the SERIDA collection. Intermediate reactions were considered as susceptible; Mixed populations were considered as resistant.

| Resistance spectrum 3 | 6 | 38 | 102 | 787 | Landraces | Int. lines |
|---|---|---|---|---|---|---|
| S | S | S | S | S | 87 | 5 |
| R | S | S | S | S | 7 | 0 |
| *S* | **R** | S | S | S | 4 | 0 |
| S | S | R | S | S | 15 | 1 |
| S | S | S | R | *S* | 18 | 6 |
| S | S | S | S | R | 12 | 1 |
| R | R | S | S | S | 0 | 0 |
| R | *S* | R | S | S | 2 | 0 |
| R | S | S | R | *S* | 2 | 0 |
| R | S | S | *S* | R | 32 | 0 |
| *S* | R | R | S | S | 0 | 0 |

| Resistance spectrum 3 | 6 | 38 | 102 | 787 | Landraces | Int. lines |
|---|---|---|---|---|---|---|
| **S** | R | *S* | R | *S* | 6 | 0 |
| *S* | R | S | S | R | 0 | 0 |
| S | S | R | R | *S* | 0 | 3 |
| S | S | R | *S* | R | 1 | 0 |
| S | S | S | R | R | 0 | 0 |
| R | R | R | S | S | 0 | 0 |
| R | R | *S* | R | *S* | 0 | 0 |
| R | R | S | S | R | 2 | 0 |
| R | *S* | R | R | *S* | 2 | 0 |
| R | *S* | R | *S* | R | 3 | 0 |
| R | S | S | R | R | 13 | 2 |

| Resistance spectrum 3 | 6 | 38 | 102 | 787 | Landraces | Int. lines |
|---|---|---|---|---|---|---|
| *S* | R | R | R | *S* | 0 | 2 |
| *S* | R | R | *S* | R | 1 | 0 |
| *S* | R | *S* | R | R | 0 | 1 |
| S | S | R | R | R | 0 | 0 |
| R | R | R | R | *S* | 2 | 0 |
| R | R | **R** | *S* | R | 5 | 4 |
| R | R | *S* | R | R | 5 | 0 |
| R | *S* | R | R | R | 8 | 0 |
| *S* | R | R | R | R | 0 | 1 |
| R | R | R | R | R | 3 | 8 |

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# ALLELISM STUDIES FOR ANTHRACNOSE RESISTANCE GENES OF COMMON BEAN CULTIVAR AND 277

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AND 277, a common bean cultivar of Andean origin, is an important source for resistance to bean angular leaf spot. In Brazil it has been observed that this cultivar is also resistant to several pathotypes of *Colletotrichum lindemuthianum*, the causative agent of anthracnose. However, the anthracnose resistance gene(s) present in AND 277 have not been characterized so far. The purposes of this work were (1) to characterize this cultivar by inoculation with 10 pathotypes of *C. lindemuthianum*, (2) to determine the inheritance of anthracnose resistance gene(s) present in AND 277 by analysing segregating populations derived from crosses with susceptible cultivar Perry Marrow, and (3) to characterize the anthracnose resistance locus present in AND 277 by studying its allelic relationship with *Co-1* (MDRK), *Co-1*$^2$ (Kaboon), and *Co-10* (Ouro Negro).

To study the anthracnose resistance spectrum of AND 277, 12 plants of this cultivar and 12 plants of cultivar Rudá (susceptible control) were inoculated with spores of *C. lindemuthianum* pathotypes 7, 55, 64, 65, 73, 81, 87, 89, 119 and 453. Spores (1.2 x $10^6$ conidia/ml) from *C. lindemuthianum* pathotypes were applied to one primary leaf of 10-day-old plants with the aid of a De Vilbiss no. 15 atomizer powered by an electric compressor. The pathotypes inoculated are listed in Table 1. The plants were incubated in a mist chamber (20-22$^o$ C, 100% relative humidity) for seven days and then the disease symptoms were scored visually using a 1 to 9 scale. Resistant (R) phenotype was assigned to plants with no or limited symptoms (grades 1 to 3) whereas plants graded 4 or greater were considered to be susceptible (S) (Pastor-Corrales et al., 1992). To avoid cross-contamination, each pathotype was inoculated in a separate chamber. For the allelism tests, $F_2$ seeds (AND 277 *vs.* Perry Marrow, MDRK, Kaboon and Ouro Negro) and those of their respective progenitors and from one susceptible control were sown in the greenhouse. The inoculation conditions and symptom evaluation were conducted as described before. *C. lindemuthianum* pathotypes 65, 81 or 453 were used depending on the cross.

Inoculation of AND 277 with 10 *C. lindemuthianum* pathotypes demonstrated that AND 277 is resistant to pathotypes 64, 65, 73, 81, 87, 89, 119 and 453 of *C. lindemuthianum* and susceptible to pathotypes 7 and 55 (Table 1). The susceptible control (cv. Rudá) was susceptible to all races tested (not shown). Races 65, 73, 81 and 89 are the most frequently found races in Brazil.

The inheritance studies showed a 3:1 ratio in the $F_2$ generation (Perry Marrow *vs.* AND 277), indicating that anthracnose resistance in AND 277 is determined by one dominant gene. The allelism studies showed a segregation ratio of 15:1 for crosses involving AND 277 and cultivar Ouro Negro, indicating that two independent dominant genes govern anthracnose resistance in these segregating populations. Indeed Ouro Negro is known to possess the anthracnose resistance gene *Co-10*. However, the allelism studies involving the crosses AND 277 *vs.* Kaboon and MDRK did not show any segregation, indicating that AND 277 carries an allele of the *Co-1* gene (Table 2).

The comparison between the resistance spectrum of AND 277 (this work) and those of cultivars MDRK (*Co-1*), Kaboon (*Co-1*$^2$) and Perry Marrow (*Co-1*$^3$) (Rava et al., 1994) indicates that AND 277

might have an alternative allele of the *Co-1* gene (Table 1). As *Co-1*$^2$ and *Co-1*$^3$ have already been characterized (Melotto & Kelly, 2000), we propose this new allele to be tentatively designated *Co-1*$^4$. Due to the need of Andean sources for resistance to anthracnose in Brazil and in other parts of the world, new information on the inheritance of resistance to *C. lindemuthianum*, and the identification of a new *Co-1* allele are extremely relevant.

**Table 1.** Resistance (R)/susceptibility (S) reactions of cultivar AND 277 to different *Colletotrichum lindemuthianum* pathotypes and comparison to the resistance spectra of cultivars MDRK, Kaboon, and Perry Marrow.

| Pathotype (Binary nomenclature) | Group/pathotype (Classical nomenclature) | AND 277[a] | MDRK[b] | KABOON[b] | PERRY MARROW[b] |
|---|---|---|---|---|---|
| 7 | DELTA/Delta | S | S | R | S |
| 55 | MEXICAN I /Mexican I | S | S | S | S |
| 64 | MEXICAN I /Mexican I | R | R | R | R |
| 65 | ALFA/Epsilon | R | R | R | R |
| 73 | ALPHA/Alpha BR | R | R | R | R |
| 81 | ALPHA /Eta | R | R | R | R |
| 87 | DELTA/Mu | R | S | R | S |
| 89 | ALPHA/Alpha BR | R | R | R | R |
| 119 | DELTA/Lambda | R | S | S | S |
| 453 | BRASILEIRO I /Zeta | R | R | R | S |

[a]Data obtained in this work; [b] data obtained by Rava et al., 1994.

**Table 2.** Characterization of the anthracnose resistance gene present in cultivar AND 277 in crosses with cultivars Perry Marrow, MDRK, Kaboon and Ouro Negro.

| Population | Race | Reaction | Observed ratio[1] | | Expected ratio | $\chi^2$ | P value |
|---|---|---|---|---|---|---|---|
| | | | R | S | | | |
| Perry Marrow x AND 277 | ***453*** | ***SXR*** | 48 | 19 | 3:1 | 0.4029 | 52.55 |
| AND 277 x MDRK | 65 | RxR | 128 | 0 | 1:0 | - | - |
| AND 277 x Kaboon | 65 | RxR | 169 | 0 | 1:0 | - | - |
| AND 277 x Ouro Negro | 81 | RxR | 206 | 15 | 15:1 | 0.1088 | 74.14 |

[1]R = resistance reaction and S = susceptibility reaction.

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# Characterization of the Anthracnose Resistance in the Differential Cultivar Widusa

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## Introduction

Anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. and Magn.) Scrib. is one of the most widespread and economically important fungal diseases of common bean (*Phaseolus vulgaris* L.). Genetic resistance is the most effective method of control of anthracnose in common bean where ten major resistance genes have been characterized. Inheritance of anthracnose resistance in the differential cultivar Widusa has not been fully characterized. To facilitate the use of Widusa as a source of resistance to anthracnose, we investigated its mode of inheritance in a series of allelism tests with previously characterized resistance genes.

## Material and Methods

Parental, $F_1$ and $F_2$ generations of seven crosses with Widusa shown on Table 1, and susceptible check varieties were grown in the greenhouse. 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 ($1.2 \times 10^6$ spores $mL^{-1}$) of each race of *C. lindemuthianum* (Table 1). After seven days of incubation in a mist chamber, seedlings were evaluated for their disease reaction using a scale of 1 to 9 (Balardin et al. 1990; Pastor-Corrales, 1991). Plants with a disease reaction score of 1-3 were considered resistant, whereas plants that rated 4-9 were considered susceptible.

## Results and Discussion

The inheritance studies supported an expected 3:1 ratio of resistant to susceptible individuals in the $F_2$ population from the R x S cross of Widusa x Michigan Dark Red Kidney (MDRK) inoculated with race 7. These data indicate that Widusa carries a single dominant gene for resistance to race 7. Results of allelism tests in $F_2$ populations derived from crosses involving Widusa with Cornell 49242 (*Co-2*) (race 7), TO (*Co-4*) (races 7 and 73), TU (*Co-5*) (race 73), and BAT 93 (*Co-9*) (race 7), showed segregation ratios of 15R:1S when populations were inoculated with races that produced a resistant reaction in both parents. This indicates that these $F_2$ populations showed independent segregation at two loci, where either of the dominant resistance genes in the parents is capable of conferring resistance. In the cross Widusa x PI 207262 (*Co-4*$^3$, *Co-9*), the $F_2$ segregation ratio was 63R:1S, showing that three independent dominant genes were involved for resistance to race 73. Therefore, according to these results, the anthracnose resistance gene in Widusa is independent of *Co-2*, *Co-4*, *Co-4*$^3$, *Co-5,* and *Co-9* genes. In the cross of Widusa with G 2333 (*Co-4*$^2$, *Co-5*, *Co-7*) (race 73), the $F_2$ segregation ratio was 255R:1S which indicated that four independent dominant genes were segregating for resistance, one from Widusa and the other three from G 2333 (Young et al., 1998). The combined results of these allelism tests support the hypothesis that the gene that confers resistance to anthracnose in Widusa is independent of *Co-2, Co-4*, *Co-4*$^2$, *Co-4*$^3$*, Co-5, Co-7,* and *Co-9* genes. The $F_2$ population derived from the cross Widusa and MDRK, when inoculated with race 65, showed no segregation among 200 individuals, suggesting that Widusa carries an allele at the *Co-1* locus. Our data are not in agreement with those of Alzate-Marin et al. (2001) who showed a lack of segregation of Widusa in crosses with PI 207262 whereas we demonstrate clear independence in crosses with PI 207262 and complementary with the resistance allele at the *Co-1* locus in MDRK.

Since Widusa is an Andean differential cultivar (Drijfhout and Davis, 1989), it is not surprising that it carries an allele at the *Co-1* locus. The *Co-1* locus is the only Andean anthracnose resistance locus identified in common bean to date. Previous studies have revealed that *Co-1* is a complex locus with a multi-allelic series where three alleles have been previously identified (Melotto and Kelly, 2000). Since Widusa has a different resistance spectrum from all 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 propose that the anthracnose resistance allele in Widusa be designated *Co-1*$^5$ as the *Co-1*$^4$ allele was reported in AND 277 (Alzate-Marin et al., 2003).

Table 1. Allelism tests for genetic characterization of anthracnose resistance in Widusa

| Population | Race | Reaction* | Resistance Gene | Observed Ratio | | Expected ratio | $\chi^2$ | *P* |
|---|---|---|---|---|---|---|---|---|
| | | | | R | S | R:S | | *value* |
| Widusa x MDRK | 7 | R x S | **Co-1** | 164 | 57 | 3 : 1 | 0.074 | 0.79 |
| Widusa x Cornell 49242 | 7 | R x R | *Co-2* | 137 | 11 | 15 : 1 | 0.353 | 0.55 |
| Widusa x TO | 7 | R x R | *Co-4* | 174 | 12 | 15 : 1 | 0.013 | 0.92 |
| Widusa x BAT 93 | 7 | R x R | *Co-9* | 229 | 17 | 15 : 1 | 0.183 | 0.67 |
| Widusa x MDRK | 65 | R x R | ***Co-1*** | 200 | 0 | --- | --- | --- |
| Widusa x TO | 73 | R x R | *Co-4* | 200 | 11 | 15 : 1 | 0.387 | 0.53 |
| Widusa x TU | 73 | R x R | *Co-5* | 92 | 5 | 15 : 1 | 0.199 | 0.66 |
| Widusa x PI 207262 | 73 | R x R | *Co-4*$^3$, *Co-9* | 352 | 6 | 63:1 | 0.029 | 0.86 |
| Widusa x G 2333 | 73 | R x R | *Co-4*$^2$, *Co-5*, *Co-7* | 294 | 1 | 255 : 1 | 0.020 | 0.89 |

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

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# IDENTIFICATION OF THE SECOND ANTHRACNOSE RESISTANT GENE PRESENT IN THE COMMON BEAN CULTIVAR PI 207.262

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The common bean breeding program being conducted at BIOAGRO/UFV, Vicosa, MG, Brazil, uses the cultivar PI 207.262 as a source for anthracnose resistance. A previous work reported the presence of two independent dominant anthracnose resistance genes in PI 207.262, one of them being an allele of the *Co-4* gene tentatively designated *Co-4*$^3$ (Alzate-Marin et al., 2001). In the same way, it was shown that no segregation was observed in an $F_2$ population derived from the cross PI 207.262 *vs.* Widusa, indicating that this cultivar carries at least one of the resistance genes present in PI 207.262. Later, allelism studies involving cultivars TO and Selection 1308 showed that the resistance gene present in Widusa is not an allele of *Co-4*. Thus, the gene shared by Widusa and PI 207.262 seemed to be distinct from all anthracnose resistance genes characterized by allelism studies so far (Alzate-Marin et al., 2002). On the other hand, PI 207.262 (also known as Tlanepantla 64 or G 1320 by CIAT identification) is one of the ancestors of BAT 93 (Voysest, 1983), and the most probable source of the *Co-9* gene present in this cultivar (Geffroy et al., 1999). This study aimed at identifying the second anthracnose resistance gene present in PI 207.262 by analyzing its possible allelic relationships with anthracnose resistance genes present in BAT 93, Widusa and TO.

$F_2$ seeds (BAT 93 *vs.* PI 207.262, Widusa and TO) and those of their respective progenitors and one susceptible control were sown in the greenhouse. Spores ($1.2 \times 10^6$ conidia/ml) from *Colletotrichum lindemuthianum* pathotype 65 were applied with the aid of a De Vilbiss no. 15 atomizer powered by an electric compressor to one primary leaf of 10-day-old plants. The plants were incubated in a mist chamber (20-22$^o$ C, 100% relative humidity) for seven days and then the disease symptoms were scored visually using a 1 to 9 scale. Resistant (R) phenotype was assigned to plants with no or limited symptoms (grades 1 to 3) whereas plants graded 4 or greater were considered to be susceptible (S) (Pastor-Corrales et al., 1992).

The allelism studies showed a segregation ratio of 15:1 for the cross BAT 93 *vs*. TO confirming the independence of dominant genes *Co-9* and *Co-4*. In the crosses BAT 93 *vs.* PI 207262 and BAT 93 *vs*. Widusa no segregation was observed indicating that the second gene that confers anthracnose resistance in PI 207.262 and those present in Widusa and BAT 93 are alleles of *Co-9* and are distinct from the other anthracnose resistance genes previously characterized by allelism studies (Table 1). We propose the genetic symbols *Co-9*, *Co-9*$^2$ and *Co-9*$^3$ for the alleles of PI 207.262, BAT 93 and Widusa, respectively.

We have identified the two anthracnose resistance genes present in cultivar PI 207.262 and also the anthracnose resistance genes present in Widusa and BAT 93. In Brazil it has been shown that PI 207.262 is resistant to 41 and Widusa to 25 out of 46 pathotypes of *C. lindemuthianum* identified (Rava et al., 1994; Andrade et al., 1999; Thomazella et al., 2000; Sartorato, 2002). These results are useful for understanding the behavior of this cultivar and for analyzing the implications of the broad use of PI 207.262 as an anthracnose resistance source in breeding programs.

**Table 1.** Resistance (R)/susceptibility (S) reactions to *Colletotrichum lindemuthianum* pathotype 65 of $F_2$ plants derived from crosses involving cultivars PI 207.262, BAT 93, Widusa and TO

| $F_2$ Population | Reaction | Gene | Observed ratio[1] | | Expected ratio | $\chi^2$ | P value |
|---|---|---|---|---|---|---|---|
| | | | R | S | | | |
| **BAT 93 x PI 207.262** | RxR | **Co-4[3] *and* ?** | 208 | 0 | --- | --- | --- |
| BAT 93 x Widusa | RxR | **?** | 78 | 0 | --- | --- | --- |
| BAT 93 x TO | RxR | **Co-4** | 195 | 11 | 15:1 | 0.29 | 58.94 |

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# CHARACTERIZATION OF THE ANTHRACNOSE RESISTANCE IN THE ANDEAN BEAN CULTIVAR JALO EEP558

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## Introduction:

Many anthracnose resistance loci have been characterized in *Phaseolus vulgaris*. Most of the anthracnose resistance genes identified are of Middle American origin. Only one anthracnose resistance gene, *Co-1*, has been characterized from the Andean gene pool; thus, new Andean sources of resistance are constantly being sought. A multi-allelic series has been reported at the *Co-1* locus (Melotto and Kelly, 2000). The differential cultivars Michigan Dark Red Kidney (MDRK) (*Co-1*), Kaboon ($Co\text{-}1^2$), and Perry Marrow ($Co\text{-}1^3$), Widusa ($Co\text{-}1^5$) (Vidigal et al., 2003) all carry distinct alleles at the *Co-1* locus that confer resistance to a different spectrum of races. Another allele has recently been identified in the Andean line AND277 denoted $Co\text{-}1^4$ (Alzate-Marin et al., 2003). In the Andean gene pool of domestication, the choice of resistance specificities is among alleles rather than genes. The best strategy for broad-based control of anthracnose is through the deployment of cultivars in which Andean and Middle American anthracnose resistance genes are pyramided. The limited number of Andean anthracnose resistance genes available limits the possible gene combinations for these resistance pyramids. Other Andean sources of resistance must be investigated to identify new resistance genes or alleles that can be exploited in breeding programs. The objective of this study was to characterize the anthracnose resistance in the Andean cultivar JaloEEP558 (Jalo). Jalo is one of the parents used to construct the bean integrated linkage map (Freyre, 1998) and therefore can be useful in mapping resistance gene loci.

## Materials and Methods:

To determine if Jalo carries a resistance allele at the *Co-1* locus, Jalo was crossed with MDRK for an allelism test. Two-hundred progeny from the resulting $F_2$ population were inoculated with race 73, which produces an RxR reaction in both parents.

To characterize and compare the resistance spectrum of Jalo, a small group of cultivars of Andean origin in the differential series and Jalo were screened with various races of *Colletotrichum lindemuthianum*, causal pathogen of bean anthracnose.

The presence of *Co-1* in Jalo was further validated by the STS marker linked to *Co-1* locus (Vallejo and Kelly, 2002), which mapped to linkage group B1 (Vallejo and Kelly, unpublished data).

## Results and Discussion:

No segregation of resistant to susceptible individuals was observed in the $F_2$ population from the cross between Jalo and MDRK when inoculated with race 73. This indicates that the gene that confers resistance to race 73 in Jalo is not independent of *Co-1* and, therefore, could be an allele at that locus or another gene very closely linked to that locus. Since Jalo showed allelism to *Co-1*, no further allelism tests were conducted with other anthracnose resistance genes.

The inoculation study, the results of which are displayed in table 1, indicates that the anthracnose resistance gene in Jalo is indistinguishable from the reaction pattern of MDRK. These data suggest

that Jalo carries the same allele of *Co-1* as does MDRK and differs from other recognized alleles at the *Co-1* locus including *Co-1*$^4$ allele in AND 277 (Alzate-Marin et al., 2003).

**Table 1.** Disease reaction of Jalo EEP558 and differential cultivars MDRK (*Co-1*), Perry Marrow (*Co-1*$^3$), Kaboon (*Co-1*$^2$) and Widusa (*Co-1*$^5$) to various races of anthracnose.

| Race | Jalo EEP558 | MDRK | Perry Marrow | Kaboon | Widusa |
|---|---|---|---|---|---|
| *2* | S | S | R | R | R |
| *7* | S | S | S | R | R |
| *38* | S | S | S | S | R |
| *47* | S | S | S | S | R |
| *73* | R | R | R | R | R |
| *80* | R | R | R | R | S |
| *88* | R | R | R | R | S |
| *128* | R | R | R | R | R |
| *357* | R | R | S | S | R |
| *1545* | R | R | R | R | R |

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# NEW SOURCES OF RESISTANCE, RACE IDENTIFICACTION AND VIRULENCE AND RESISTANCE INDEXES IN ANTHRACNOSE RESEARCH.

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**Introduction**. Anthracnose, caused by the fungus Colletotrichum lindemuthianum, is the most important disease of common bean (Phaseolus vulgaris L.). in the state of Rio Grande do Sul, Brazil. The state is an important dry bean producer, with a farmer's contingent of about 200.000 growers. Fluctuation on race prevalence is a common phenomenon which reflects the permanent adaptation of the fungus population to the cultivars under use, on its struggle for survival. The result from this scenery, is the eventual appearance of novel recombinants which turn out to be characterized as new pathotypes, which eventually may cause serious losses in production. Breeding programs should monitor these possible occurrences in order to prevent diseases to reach epidemic character, through the identification of resistance sources that could be incorporated to the production systems.

In search for anthracnose resistance sources, race identification from samples collected in common bean growing areas, and the correspondent reaction of bean germoplasm under controlled conditions are described in the present paper.

**Material and Methods**. Anthracnose samples were collected in production areas of the state of Rio Grande do Sul in cropping years 2000/01 and 2001/02. The germplasm tested comprised the international anthracnose common bean differential cultivar set (twelve cultivars), a susceptible check (the cv. IPA 7419), eleven black-seeded cultivars released by official research programs, four local cultivars and eight promising breeding lines. Twelve seeds of each genotype were placed in trays and subjected to greenhouse conditions. The methodology used for anthracnose resistance determination, follows Rava et al (1993). The genotype reaction was evaluated 7 days after inoculation by using a 0 – 9 scale, where 1 , 2 and 3 were considered an incompatible reaction (resistant); 4, 5 and 6 intermediate and 7, 8 and 9, compatible (susceptible). A second evaluation was based on McKinney disease index (EMBRAPA, 1976).

**Results and Discussion.** It can be observed in Table 1 that nine of the twelve differential genotypes showed resistant reaction to all isolates. Resistance to all isolates was also displayed by the cultivar Soberano, among the black seed cultivars; by one of the four local cultivars, none of the other-seed-coat-color cultivars and, a very important finding, by four of the eight promising breeding lines. The isolates presented a maximum virulence percentage index (VI) of 25.0 for the differential cultivars, indicating that the genes for resistance present in these cultivars could be effective. In relationship to the already released black bean cultivars, VI`s ranged from 45.4 to 90.9, indicating a high compatibility of the anthracnose races to the available cultivars. For the breeding lines, the VI's were 37.5 for all isolates indicating a great progress of the breeding program for anthracnose resistance. For other-seed-coat-color cultivars the VI ranged for 42.8 to 85.7, whereas for local cultivars from 50.0 to 75.0. As an overall VI, the isolates 01/01 and 11/02, showed the highest values, 58.13%.
It can be concluded that for the studied anthracnose sampling, resistance in the available cultivars is low, whereas, based on the assumption that some of the breeding lines will be released as a new cultivar, a significant progress in anthracnose resistance can be expected, since four of these lines are resistant to all isolates.

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**Table 1**. Bean germplasm reaction to five *Colletotrichum lindemuthianum* isolates, collected in Rio Grande do Sul state, Brazil, 2003.

| Isolate / Genotype | 01/01 | | 23/01 | | 53/01 | | 11/02 | | 44/01 | | RI[2] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *R*[1] | *I*$_{MK}$ | R | $I_{MK}$ | R | $I_{MK}$ | R | $I_{MK}$ | R | $I_{MK}$ | (%) |
| Differential cultivar | | | | | | | | | | | |
| MICHELITE | S | 1,00 | S | 1,00 | S | 1,00 | S/I | 0,78 (4-8) | S/I | 0,68 (4-9) | 0 |
| MDR KIDNEY | R | 0,12 (1,2) | R | 0,11 | R | 0,11 | R | 0,14 (1,2) | R/I | 0,28 (1-6) | 100 |
| PERRY MARROW | R/I | 0,38 (1,5) | R | 0,11 | R | 0,11 | R | 0,11 | R | 0,11 | 100 |
| CORNELL 49-242 | S | 1,00 | R | 0,27 (1-3) | R | 0,22 (1,2) | S/I | 0,85 (6-8) | S | 1,00 | 60 |
| WIDUSA | R | 0,11 | R | 0,11 | R | 0,11 | R | 0,11 | R | 0,11 | 100 |
| KABOON | R | 0,16 (1,2) | R | 0,11 | R | 0,11 | R/I | 0,18 (1-6) | R | 0,11 | 100 |
| MÉXICO 222 | S | 1,00 | S | 1,00 | S | 1,00 | S | 1,00 | R | 0,11 | 0 |
| PI 207 262 | R | 0,11 | R | 0,11 | R | 0,11 | R | 0,11 | R | 0,11 | 100 |
| TO | R | 0,13 (1,2) | R | 0,25 (2,3) | R | 0,11 | R | 0,11 | R | 0,11 | 100 |
| TU | R | 0,11 | R | 0,11 | R | 0,11 | R | 0,11 | R | 0,11 | 100 |
| AB 136 | R | 0,11 | R | 0,11 | R | 0,11 | R | 0,11 | R | 0,11 | 100 |
| G 2333 | R | 0,11 | R | 0,11 | R | 0,11 | R | 0,11 | R | 0,11 | 100 |
| IPA 7419 | S | 1,00 | S | 1,00 | S | 1,00 | S | 0,99 ( 8,9) | S | 1,00 | 0 |
| *Race N◦* | **73 (α -Brazil)** | | **65 (alfa)** | | **65 (alfa)** | | **73 (α-Brazil)** | | **9 (α-Brazil)** | | |
| *VI*[2]*- Differential Cvs.* | **25** | | **16,7** | | **16,7** | | **16,7** | | **16,7** | | **80,0** |
| **Black-seeded cultivar** | | | | | | | | | | | |
| RIO TIBAGI | S | 1,00 | S | 1,00 | S | 1,00 | S | 1,00 | S | 1,00 | 0 |
| GUATEIAN 6662 | S | 1,00 | S | 0,98 (7,9) | S | 1,00 | S | 1,00 | S | 1,00 | 0 |
| MACANUDO | S | 1,00 | S/R | 0,88 (2,9) | S/I | 0,72 (5-9) | S | 1,00 | R | 0,11 | 20 |
| MINUANO | S | 1,00 | S | 1,00 | S/I | 0,78 (6-9) | S | 1,00 | R | 0,11 | 20 |
| IAPAR 44 | S | 1,00 | R | 0,12 (1-3) | R | 0,12 (1,2) | S | 1,00 | S | 1,00 | 40 |
| MACOTAÇO | S | 1,00 | S | 1,00 | S/I | 0,81 (6-8) | S | 1,00 | R | 0,11 | 20 |
| GUAPO BRILHANTE | S | 0,93 (7-9) | S | 0,89 (7,9) | S/R | 0,55 (1-9) | S/R | 0,91 (1,9) | R/S | 0,33 (1,9) | 20 |
| FT NOBRE | S | 1,00 | S | 0,89 (7-9) | S | 1,00 | S | 0,98 (7,9) | S | 1,00 | 0 |
| DIAMANTE NEGRO | S/I | 0,67 (5-9) | S/I | 0,67 (4-8) | S/I | 0,83 (6-9) | S/R | 0,66 (1-4) | S | 1,00 | 0 |
| VALENTE | S | 1,00 | S | 1,00 | R/I | 0,21 (1-5) | S | 1,00 | R | 0,11 | 40 |
| SOBERANO | R | 0,12 (1,2) | R | 0,11 | R | 0,13 (1,2) | R | 0,11 | R | 0,11 | 100 |
| *VI – Black-seeded cvs.* | **90,9** | | **81,8** | | **72,7** | | **90,9** | | **45,4** | | **23,6** |
| **Breeding line** | | | | | | | | | | | |
| TB 94-01 | R/S | 0,23 (1-9) | R | 0,11 | R/I | 0,19 (1-4) | R | 0,11 | R | 0,11 | 100 |
| TB 96-11 | R/I | 0,22 (1-5) | I/R/S | 0,43 (2-9) | R/S | 0,34 (1-9) | R | 0,11 | R/I | 0,18 (1-5) | 100 |
| TB 96-13 | R/I | 0,34 (2-5) | R/S | 0,23 (1-9) | R/I | 0,14 (1-4) | R | 0,11 | R | 0,11 | 100 |
| TB 97-13 | R/S | 0,33 (2-8) | R | 0,11 | R | 0,11 | R | 0,17 (1-6) | R | 0,15 (1,2) | 100 |
| CNFP 8104 | S/R | 0,89 (1-9) | S/I | 0,80 (4-9) | S | 1,00 | S | 0,83 (1,9) | S/R | 0,88 (1-9) | 0 |
| TB 98-20 | S | 1,00 | S | 1,00 | S/I/R | 0,77 (2-9) | S | 1,00 | R/I | 0,37 (2-5) | 20 |
| TB 99-13 | R/S | 0,28 (1-9) | R/I | 0,19 (1,4) | R | 0,15 (1,2) | R | 0,11 | S/R | 0,61 (1,9) | 80 |
| TB 00-10 | S | 1,00 | S | 1,00 | S | 0,95 (7-9) | S | 1,00 | S | 1,00 | 0 |
| *VI – Breeding lines* | **37,5** | | **37,5** | | **37,5** | | **37,5** | | **37,5** | | **62,50** |
| **Other-seed-coat-color cultivar** | | | | | | | | | | | |
| CAROCA | ***S*** | ***1,00*** | S | 1,00 | S | 1,00 | S | 1,00 | R | 0,11 | 20 |
| CARIOCA MG | S | 1,00 | R/I | 0,39 (1-4) | R | 0,27 (1-3) | S | 1,00 | I/S/R | 0,54 (1-7) | 40 |
| IAPAR 31 | S | 1,00 | I/S | 0,36 (4,9) | R | 0,22 | S | 1,00 | R | 0,11 | 60 |
| PÉROLA | I/S | 0,69 (5-7) | S | 0,94 (8,9) | S | 1,00 | S/I | 0,72 (2-9) | R | 0,24 (2,3) | 20 |
| MAGNÍFICO | S/R | 0,88 (3-9) | S/R/I | 0,81 (1-9) | S | 0,70 (7-9) | S/R/I | 0,71 (3-9) | S/I/R | 0,63 (2-9) | 0 |
| IRAÍ | R/I | 0,20 (1-4) | S | 0,92 (7-9) | S | 0,86 (7,8) | R | 0,12 | R | 0,11 | 60 |
| RADIANTE | S | 0,87 (7,8) | S | 0,95 (8,9) | S | 0,91 (8,9) | S | 0,86 (7,8) | S/R | 0,79 (1-9) | 0 |
| *VI-Oth. seed-coat-col cv* | **85,7** | | **71,4** | | **71,4** | | **85,7** | | **42,8** | | **28,57** |
| **Local cultivar** | | | | | | | | | | | |
| PRETO COMPRIDO | S | 1,00 | S | 1,00 | S | 1,00 | S | 1,00 | S | 1,00 | 0 |
| GUABIJU | S | 0,98 (7,9) | S | 1,00 | S | 1,00 | S | 1,00 | S | 1,00 | 0 |
| VERMELHO ITAJAÍ | R/S | 0,22 (1,9) | R/S | 0,29 (1-9) | R/S | 0,29 (8,9) | R/S | 0,19 (1-7) | R | 0,11 | 100 |
| C. SOBRADINHO | R/S | 0,26 (1-9) | S | 0,91 (7-9) | S | 0,93 (7-9) | R/S | 0,18 (1,8) | R | 0,11 | 60 |
| *VI – Local cultivars* | **50,0** | | **75** | | **75** | | **50** | | **50** | | |
| *VI - Overall* | **58,13** | | **53,49** | | **51,16** | | **58,13** | | **34,78** | | |

**1: R:** Genotype reaction based on a 0-9 scale, where 1,2 and 3 represent resistance (R); 4,5 and 6 intermediate (I); 7,8 and 9, susceptibility (S); $I_{MK}$: McKinney disease index where values above 0.5 represent susceptibility and below, resistance. Numbers within parenthesis represent the grades displayed by individual seedling, when separated by commas, and the range of grades when separated by hyphen. **2: VI:** Virulence index; **RI**: Resistance Index.

# DROUGHT STRESS EFFECTS ON CHARCOAL ROT SEVERITY AND GRAIN YIELD OF COMMON BEANS1

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The fungus *Macrophomina phaseolina* (Mp) causes charcoal rot on common beans, under drought and high temperature stresses and both tropical and arid regions of México. A high variability on genotype and pathogenicity in Mexican Mp isolates have been found and populations from tropical lands are more aggressive than those from arid lands. A 12 common bean differential cultivar set have been proposed to improve the characterization of pathogenic variability and clarify the biology of populations and host specialization of Mp (3). This work was conducted in order to characterize the response to drought stress of differential cultivars set under field conditions.

Four experiments were conducted at Isla (18°06' N, 95°53' W, 25 masl) and Cotaxtla, Veracruz, México (18°44' N, 95°58' W, 16 masl). Trials were planted on October 19, 2000 and February 14, 2001 (Isla) and October 20, 2000 and January 30, 2001 (Cotaxtla). The differential set includes twelve common bean cultivars, six classified as resistants and six classified as susceptibles to Mp (Table 1) (3). At each trial, a RCB design with six replications was used, and germplasm was established under two soil moisture levels: irrigation and drought stress (irrigation was stopped when 50 % of the germplasm was flowering until harvest). Plots of all experiments were infested at sowing using 5 g $row^{-1}$ of rice seeds colonized by local isolates of Mp. Plots were one row 3 m length and 0.61 m wide (30 seeds $row^{-1}$). Data of charcoal rot disease severity (DS) were registered at 21, 42, and 63 days after sowing (das). At maturity , seed yield was determined by harvesting all plants present in each plot. Data of each experiment were subjected to ANOVA and means compared using LSD test (P=0.05). The effect of drought stress on grain yield was estimated as suggested Fischer and Maurer (1).

Drought stress was greater in 2001 than 2000, due Drought Intensity Indexes (DII) were 0.12 (2000) and 0.37 (2001) at Isla, while DII were 0.18 (2000) and 0.26 (2001) at Cotaxtla. Charcoal rot resistant germplasm showed lower Mp severity and Drought Susceptibility Index (DSI) than susceptible genotypes, while resistant germplasm showed the highest grain yield. Mesoamerican germplasm had lower charcoal rot severity than other gene pools. Thus, the most-yielded genotypes showed the lowest Mp infection rates and the lowest DSI, such as TLP 19, BAT 477, SEQ 12, and Negro 8025. The exception was Pinto Villa that showed intermediate reaction to charcoal rot and intermediate grain yield, but its DSI was similar to resistant germplasm (Table 1). Pinto Villa was identified as resistant to drought stress under highlands of México previously (7), but susceptible to Mp at tropical lowlands (4). Negro 8025 and TLP 19 showed lower Mp severity than BAT 477 as been found previously at Honduras (2). Our data confirmed the resistance to Mp and high grain yield in Mesoamerican germplasm (4, 6) and the relationship between resistance both charcoal rot and drought stress (5).

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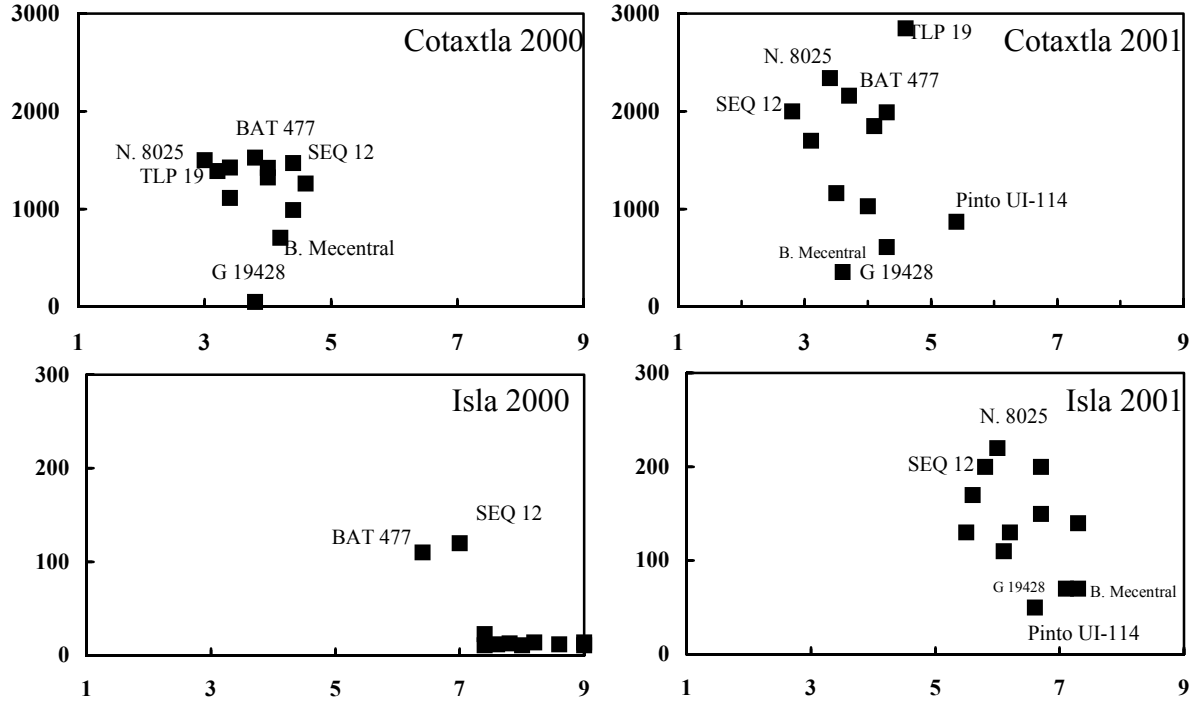
Table 1. Average Mp severity, grain yield and drought susceptibility index of 12 common bean cultivars.

| Genotype | Mp severity | Grain yield (kg $h^{-1}$) | DSI | **Genotype** | Mp severity | Grain yield (kg $h^{-1}$) | DSI |
|---|---|---|---|---|---|---|---|
| **Resistants** | | | | **Susceptibles** | | | |
| BAT 477 | 3.6 | 1031 | 0.91 | B. Mecentral | 6.2 | 358 | 1.77 |
| TLP 19 | 3.5 | 1114 | 0.97 | B. Durango | 4.6 | 780 | 1.60 |
| G 4523 | 5.7 | 653 | 1.74 | A. Tapatío | 4.5 | 828 | 1.16 |
| SEQ 12 | 3.8 | 990 | 0.99 | P. Villa | 4.1 | 633 | 0.92 |
| N. 8025 | 3.3 | 1020 | 0.93 | P. UI-114 | 5.7 | 490 | 1.53 |
| G 19428 | 5.1 | 155 | 1.73 | R. Tibagí | 4.4 | 935 | 1.23 |
| **Mean** | **4.2** | **827** | **1.21** | | **4.9** | **671** | **1.37** |
| LSD (P=0.05) | | | | | 0.9 | 202 | 0.22 |

Figure 1. Relationship between charcoal rot severity and grain yield in 12 bean genotypes. (X-axis=Charcoal rot severity; Y-axis=Grain yield (kg $h^{-1}$).



# IMPROVEMENT OF THE RUST RESISTANCE OF DRY BEANS IN SOUTH AFRICA

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Rust, caused by *Uromyces appendiculatus*, is the most serious fungal disease of the common bean in South Africa. The standard small white canning (SW) cultivar Teebus is highly susceptible to rust. In spite of the recent releases of several high yielding SW cultivars Teebus continues to be the most popular, due to its superior canning quality and wide adaptation. Red speckled sugar (RSS) (cranberry) types are widely planted by both the commercial and small scale farming communities. A number of widely adapted RSS cultivars with improved rust resistance have been released during the past 10 years. However, most of these share a narrow genetic base, and show signs of losing their rust resistance. The aim of this project is the improvement of the rust resistance (RR) of popular cultivars, while retaining yield, canning quality and other positive agronomic characteristics. For this purpose, the backcross method was chosen, and a similar policy was followed for other diseases.

## METHODS

***Identification of pathogenic groups:*** Using standard purification, inoculation and evaluation methods (Harter *et al.* 1935; Stavely, 1983a, 1984b, Stavely *et al.* 1989b), single pustule isolates of *U. appendiculatus* were inoculated on the set of 19 differential- and other important cultivars in the greenhouse. Ratings were made on the basis of pustule size- and type (Stavely *et al.* 1983), as well as rust intensity (Van Schoonhoven & Pastor-Corrales, 1987: scale 1).

***Race identification:*** Isolates differentiating important resistance genes were selected for re-isolation from single pustules (which was done at least one more time) and inoculations on differential- and other cultivars were repeated at least once.

***Field evaluations***: The same set of cultivars were planted in the field at <30 localities in summer and one locality in winter over the past eight years. Evaluation of rust susceptibility was done as in the greenhouse. At the same time, cultivars were also evaluated for possible race-non-specific resistance and other important characteristics.

***Back cross breeding:*** Resistance sources showing the broadest resistance (both in Southern Africa at that stage, as well as in other parts of the world) were selected as donor parents for use in back cross breeding programmes. Small white cvs. Teebus, and Helderberg, as well as RSS cvs. Kranskop, Jenny and Monati were chosen as recurrent parents.

## RESULTS AND DISCUSSION

***Identification of pathogenic groups and races:*** Over 300 single pustule isolates were inoculated in the greenhouse. From these, 35 pathogenic groups were identified. Eleven of these were re-purified and re-inoculated and used in various combinations for the screening of breeding material and germplasm.
***Backcross breeding:*** The most important donor parents chosen were BelNeb RR 1 (containing at least *Ur-5* and *Ur-6*) and BelMiDak RMR 8 and 9 (containing *Ur-11* and *Ur-4*) (Stavely *et al.* 1989a, Stavely *et al.* 1992; Stavely *et al.* 1994; Kelly *et al.* 1996). Several others , including CIAT line A 286, were also used. The CIAT line CAL 143 was chosen as a possible source of race non-specific resistance. Breeding lines were screened in the greenhouse after each backcross (BC) with appropriate races (usually four) available at the time of screening. Backcrossing was continued to $BC_1$ to $BC_5$,

(usually $BC_4$) depending on the parents involved. Progeny arising from the BCs were screened in the field at one to four summer and one winter locality.

To date, one SW cultivar (Teebus RR-1) has been released from this programme and has performed well in the National Cultivar trials, yielding more than two and a half times that of Teebus under moderately high rust pressure. During the present season, 51 small seeded and 52 large seeded lines from this programme, including lines with dual resistance to rust and angular leaf spot (ALS) have been entered in the check row and yield trials of the main breeding programme of the ARC - Grain Crops Institute. Crosses to pyramid RR genes in a Teebus background have reached the $F_3$ generation. Improved Teebus RR lines have also been crossed with improved Teebus lines with common bacterial blight (CBB) resistance from a separate BC breeding project (managed by D. Fourie). These are now in the $F_5$ generation.

This programme is still ongoing and future priorities are the pyramiding of RR genes and the attaining of multiple disease resistance in suitable backgrounds. In conjunction with the biotechnology section, work is also in progress to evaluate the possibilities of using molecular markers in this programme. The strong emphasis placed on team work between the two resistance breeders, main bean breeder and biotechnologist is already paying dividends and it is hoped to continue this approach to achieve stable multiple disease resistance.

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# RAPD Markers Tightly Linked to the *Ur-6* Gene of Andean Origin Controlling Specific Resistance to Rust in Common Bean

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Bean rust, caused by *Uromyces appendiculatus*, is an important disease of common bean. The most economic and environmentally sustainable method for controlling bean rust is the use of host plant resistance (Mmbaga et al., 1996). Bulked segregant analysis is an efficient method to rapidly identify molecular markers linked to a specific gene using DNA bulks from $F_2$ plants. This technique was used to detect four different genes (*Ur-3*, *Ur-5, Ur-7* and *Ur-11*) of Middle American origin and two genes (*Ur-4* and *Ur-9*) of Andean origin for rust resistance in beans using RAPD markers (Miklas et al., 2002). Miklas et al. (2002) reported integration of identified rust resistance genes in the core bean map. Three important rust resistance genes such as *Ur-3*, *Ur-7* and *Ur-11* of Middle American origin were located on linkage group 11 (Miklas et al., 2002; Park et al., 2003), while other rust genes *Ur-9*, *Ur-5* and *Ur-4* are located on linkage groups 1, 4 and 6 (Miklas et al., 2002). Pyramiding monogenic resistance genes into a single bean cultivar is a strategy recommended to obtain durable rust resistance (Mmbaga et al., 1996). Molecular markers linked to rust resistance genes are useful to pyramid these genes into a single cultivar (Kelly, 1995).

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=. On the basis of this result Miklas et al. (2002) mapped the *Ur-6* gene on linkage group B11 of the core bean map. However, markers tightly linked to the *Ur-6* gene of Andean origin for specific resistance to rust present in >Olathe= have not been reported.

Our objective was to identify RAPD markers tightly linked to the *Ur-6* gene for specific resistance to race 51 using bulked segregant analysis in an $F_2$ population from the Middle American common bean cross >Olathe= (resistant) x GN Nebr. #1 sel. 27 (susceptible).

## Materials and Methods

**Inoculation.** One hundred $F_2$ plants and 88 $F_3$ families (12 to 16 plants per $F_3$), along with their parents >Olathe= and GN Nebr. #1 sel. 27, were planted in a greenhouse on 16 February 1998 and 18 August 1998, respectively. All plants were inoculated by a spore suspension of race 51 sprayed on the abaxial leaf surfaces of the unifoliate leaves. Rust reaction was recorded on all plants as resistant (no disease symptom) or susceptible (uredinia>300 Fm in diameter) at 14 days after inoculation.

**RAPD.** Total genomic DNA was extracted from lyophilized leaf tissue of 100 $F_2$ plants along with their parents. PCR was performed on 96-well plates in a MJ Research thermalcycler (model PTC-0100; MJ Research, Waltham, MA). Two different DNA bulks were prepared from equal volumes of standardized DNA (10 ng/ul) from eight homozygous resistant and eight homozygous susceptible $F_2$ plants selected on the basis of $F_3$ phenotypic data for the reaction to race 51, respectively. A total of 680 primers were used to screen between two different DNA bulks from resistant and susceptible $F_2$ plants, and between the parents >Olathe= and GN Nebr.#1 sel. 27. The name of each RAPD marker is derived from an >O= prefix for Operon primers, the letters identifying the Operon kit, Operon primer number, and the approximate length of the marker.

**Linkage Analysis.** Due to the dominant feature of RAPD markers, the linkage analysis of seven coupling- or five repulsion-phase markers with the *Ur-6* locus for specific resistance to rust was separately performed on the data for 100 $F_2$ plants of the cross >Olathe= x GN Nebr.#1 sel. 27 using MAPMAKER version 3.0. Map distances (cM) between ordered loci of marker and gene were calculated using recombination fractions and the Kosambi mapping function.

## Results and Discussion

A goodness-of-fit to a 3:1 ratio of number of resistant to susceptible $F_2$ plants to rust race 51 was observed. It was hypothesized that a single dominant gene controlled specific resistance to race 51. This hypothesis was confirmed in the $F_3$ based on a satisfactory fit to a 1:2:1 ratio of number of families non-segregating for resistance, segregating for resistance and susceptibility and non-segregating for susceptibility.

Twelve RAPD markers were detected to be linked to the *Ur-6* gene for specific resistance to rust using bulked segregant analysis. Of these 12 markers, seven 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. A goodness-of-fit to a 3:1 ratio for band presence to band absence for each of these12 markers was observed in 100 $F_2$ plants.

The integrated location of the *Ur-6* locus for specific resistance to rust and the loci of the seven coupling-phase markers is developed. This linkage group included eight loci spanning a length of 63 cM. The *Ur-6* gene was located between markers OBC06.300 and OAG15.300 on the linkage group. Thus, two 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. These flanking markers would be more effective than a single linked marker in selecting the *Ur-6* gene.

The combined location of the *Ur-6* locus for specific rust resistance and the loci of the five repulsion-phase markers is also developed. The linkage group included six loci spanning a length of 66 cM. Repulsion-phase marker OAY15.200 was the most closely linked to the *Ur-6* gene among the five markers at a distance of 7.7 cM. The repulsion-phase markers linked to the *Ur-6* gene were classified and located on linkage group B11 of the existing linkage map. Those coupling- and repulsion-phase markers linked to the *Ur-6* gene of Andean origin identified here, along with other independent rust resistance genes from other germplasm, could be utilized to pyramid multiple genes into a bean cultivar for more durable rust resistance.

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Park, S.O., D.P. Coyne, J.R. Steadman, and P.W. Skroch. 2003. Mapping of the *Ur-7* gene for specific resistance to rust in common bean. Crop Sci. 43: in press.

# Development of A SCAR Marker Linked to the *Ur-6* Gene for Specific Rust Resistance in Common Bean

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Bean rust, caused by *Uromyces appendiculatus*, is an important disease resulting in reduced bean yield and quality in many parts of the world. The use of host plant resistance is the most economic and environmentally sustainable method for controlling bean rust (Mmbaga et al., 1996). Pyramiding monogenic resistance genes into a single bean cultivar is a strategy recommended to obtain durable rust resistance (Mmbaga et al., 1996). Park et al. (1999) also suggested that recombining resistance genes from both Andean and Middle American gene pools should provide more durable resistance to rust.

Grafton et al. (1985) reported a single dominant resistance gene $Ur_a$ of Andean origin present in >Olathe= of the Middle American background. It is known to be independent of other rust resistance genes and is expected to be resistant to some virulent races. Stavely et al. (1983) reported that >Golden Gate Wax= also possessed the $Ur_a$ gene for rust resistance. Kelly et al. (1996) subsequently reassigned the $Ur_a$ gene as *Ur-6* present in >Olathe= and >Golden Gate Wax=.

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. (2002) subsequently reported twelve 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 common bean cross >Olathe= (resistant) x GN Nebr. #1 sel. 27 (susceptible). Of these markers, seven 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 a distance of 7.7 cM. On the basis of this result Park et al. (2002) mapped the *Ur-6* gene on linkage group 11 of the core bean map.

A disadvantage of RAPD is that some of amplified DNA fragments per primer are difficult to reproduce. Reproducibility of RAPD marker can be enhanced by the use of SCAR (Paran and Michelmore, 1993). Therefore, our objective was to convert the RAPD marker OBC06.300 tightly linked to the *Ur-6* gene for specific rust resistance in the $F_2$ population from the cross >Olathe= x GN Nebr. #1sel. 27 into a robust and reproducible SCAR marker on the basis of a specific forward and reverse 24-mer primer pair.

## Materials and Methods

**RAPD.** Identification of the RAPD marker OBC06.300 tightly linked to the *Ur-6* gene for specific rust resistance in the $F_2$ population from the cross >Olathe= x GN Nebr. #1sel. 27 was conducted as described previously by Park et al. (2002).

**SCAR.** To develop a SCAR marker for the RAPD marker OBC06.300, 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 24-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). The composition of the final volume of reactants was similar to those described by Rubio et al. (2001). After an initial denaturation step at 94EC for 2 min., PCR was performed in a MJ Research thermalcycler (model PTC-0100; MJ Research, Waltham, Massachuset) for 30 cycles. The profile for each cycle was 30 s at 94EC, 1 min. at 59EC, and 2 min. at 72EC, followed by a final extension step at 72EC for 7 min.

## Results and Discussion

The RAPD marker OBC06.300 tightly linked to the *Ur-6* gene for specific rust resistance in the $F_2$ population from the cross >Olathe= x GN Nebr. #1sel. 27 was converted into a robust and reproducible SCAR marker on the basis of the specific forward and reverse 24-mer primer pair. The size of the RAPD fragment is 308 bp based on the its sequence data. For generating the SCAR marker for the RAPD marker OBC06.300, we designed the specific forward and reverse 24-mer primer pair containing the original 10-mer sequence (the below bold) of the OBC06 primer. The sequence of the forward 24-mer primer was 5'-**GAAGGCGAGA**AGAAAAAGAAAAAT-3', while that of the reverse 24-mer primer was 5'-**GAAGGCGAGA**GCACCTAGCTGAAG-3'.

The SCAR marker for the RAPD marker OBC06.300 was developed based on the specific forward and reverse 24-mer primer pair. The SCAR marker was present in the resistant parent >Olathe= and the DNA bulk from resistant $F_2$ plants, whereas it was absent in the susceptible parent GN Nebr.#1 sel. 27 and the DNA bulk from susceptible $F_2$ plants. The SCAR marker showed no recombination with the RAPD marker OBC06.300 in the $F_2$ population from the cross >Olathe= x GN Nebr. #1sel. 27, indicating that the SCAR and RAPD markers were observed at the same locus on the linkage group. The results confirm and support the identity of the SCAR marker with the RAPD marker OBC06.300. The SCAR marker for the RAPD marker OBC06.300 linked to the *Ur-6* gene of Andean origin developed here, along with other independent rust resistance genes from other germplasm, could be utilized to pyramid multiple genes into a bean cultivar for more durable rust resistance.

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# Mapping of the *Ur-7* Gene for Specific Resistance to Rust in Common Bean

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Bean rust, caused by *Uromyces appendiculatus*, is a major disease of common bean (*Phaseolus vulgaris* L.). Bulked segregant analysis is an efficient method to rapidly identify molecular markers linked to a specific gene using bulked DNA from $F_2$ plants. This technique has been chosen to detect four different genes (*Ur-3*, *Ur-5*, *Ur-7* and *Ur-11*) of Middle American origin and three genes (*Ur-4*, *Ur-6* and *Ur-9*) of Andean origin for rust resistance in beans using RAPD markers (Miklas et al., 2002; Park et al., 1999a, 1999b, 2002). Miklas et al. (2002) reported integration of identified rust resistance genes in the core bean map.

Park et al. (1999b) 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 GN 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 a distance of 7.6 cM. However, mapping of the *Ur-7* gene of Middle American origin for specific resistance present in >GN1140= has not been reported.

The objective of this study was to position the *Ur-7* gene for specific rust resistance on a genetic linkage map previously constructed using recombinant inbred lines from the Middle American cross GN BelNeb-RR-1 x A 55. This recombinant inbred line population was previously used to construct a genetic linkage map using RAPD markers. Using this map, QTL and genes for resistance to common bacterial blight, halo blight, Fusarium wilt, and bean common mosaic have been identified (Ariyarathne et al., 1999; Fall et al., 2001).

## Materials and Methods

**Plant Materials.** Seventy-eight recombinant inbred lines derived from the common bean cross BelNeb-RR-1 x A 55 were previously developed using single-seed-descent (Ariyarathne et al., 1999). Stavely et al. (1989) developed the GN BelNeb-RR-1 line with at least three different rust resistance genes such as *Ur-5*, *Ur-6* and *Ur-7*. This BelNeb-RR-1 parent also is resistant to common bacterial blight, halo blight and bean common mosaic (Ariyarathne et al., 1999) but is susceptible to Fusarium wilt (Fall et al., 2001). The A 55 parent is susceptible to common bacterial blight, halo blight and rust but is resistant to bean common mosaic and Fusarium wilt.

**RAPD.** Total genomic DNA was extracted from lyophilized leaf tissue of 78 recombinant inbred lines and the parents (BelNeb-RR-1 and A 55). Polymerase chain reactions were performed on 48-well plates in a Perkin-Elmer thermalcycler (model 480; Perkin-Elmer Co., Norwalk, Conn.). Eight 10-mer primers (Operon Technologies, Alameda, Calif.) were tested in the recombinant inbred line population of the cross BelNeb-RR-1 x A 55. The name of each RAPD marker is derived from an >O= prefix for Operon primers, the letters identifying the Operon kit, Operon primer number, and the approximate length of the marker.

**Linkage Analysis.** The recombinant inbred line population marker data was tested for goodness-of-fit to a 1:1 ratio to detect segregation distortion of markers. The linkage analysis of 90 markers containing 87 RAPD markers, one sequence characterized amplified region, the *I* gene for bean common mosaic resistance and a halo blight resistance gene previously mapped by Ariyarathne et al. (1999) with nine new RAPD markers identified in our study was executed on the data for the recombinant inbred line population of the cross BelNeb-RR-1 x A 55 using MAPMAKER version 3.0. Map distances (cM) between ordered loci of marker and gene were calculated using recombination fractions and the Kosambi mapping function.

## Results and Discussion

The nine markers linked to the *Ur-7* gene in the $F_2$ population from the cross >GN1140= x GN Nebr. #1 were also polymorphic between the parents BelNeb-RR-1 and A 55 that were utilized to create the recombinant inbred line population from which the RAPD marker-based genetic linkage map was constructed. Six coupling-phase markers displayed an amplified DNA fragment in the BelNeb-RR-1 parent possessing at least three different rust resistance genes *Ur-5*, *Ur-6* and *Ur-7*. Three repulsion-phase markers displayed an amplified DNA fragment in the A 55 parent susceptible to rust. All nine markers also segregated in the recombinant inbred line 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 nine markers was observed in the recombinant inbred line population. Based on linkage analysis of 90 formerly mapped markers with nine new RAPD markers identified here, all nine markers linked to the *Ur-7* gene were classified into one linkage group and located within a distance of 8.1 cM on linkage group 11 of the existing linkage map.

Important rust resistance genes such as *Ur-3*, *Ur-6* and *Ur-11* are located on linkage group B11 of the core bean map, whereas other rust genes *Ur-9*, *Ur-5* and *Ur-4* are located on linkage groups B1, B4 and B6 (Miklas et al., 2002; Park et al., 2002). However, the *Ur-7* gene is located in a different region on linkage group B11 with respect to the other important rust resistance genes, based on the result of Miklas et al. (2002). Also, the *Ur*-BAC 6 gene is located on linkage group 11, but the distance between the *Ur*-BAC 6 and the *Ur-7* genes is more than 50 cM.

Four QTL for common bacterial blight resistance on linkage groups 1, 2, 9, and 10 were identified (Ariyarathne et al., 1999). They reported six QTL for halo blight resistance on linkage groups 2, 3, 4, 5, 9,and 10. They also mapped the *I* gene for resistance to bean common mosaic on linkage group 2. A major QTL on linkage group 10 for Fusarium wilt resistance was found by Fall et al. (2001). Thus, there was no genetic relationship between the *Ur-7* gene and the previously mapped common bacterial blight, halo blight, Fusarium wilt, and bean common mosaic genes.

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# Survey of Molecular Markers Linked to the *Ur-7* Gene for Specific Rust Resistance in Diverse Bean Cultivars and Breeding Lines

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BEAN RUST, caused by *Uromyces appendiculatus*, is an important disease resulting in reduced bean yield and quality in many parts of the world. The use of host plant resistance is the most economic and environmentally sustainable method for controlling bean rust (Mmbaga et al., 1996). Bulked segregant analysis was used to detect four different genes (*Ur-3*, *Ur-5*, *Ur-7* and *Ur-11*) of Middle American origin and three genes (*Ur-4*, *Ur-6* and *Ur-9*) of Andean origin for rust resistance in beans using RAPD markers (Miklas et al., 2002; Park et al., 1999a, 1999b, 2002a). Pyramiding monogenic resistance genes into a bean cultivar is a strategy recommended to obtain durable rust resistance (Mmbaga et al., 1996). Those markers linked to rust resistance genes could be useful to pyramid these genes into a single cultivar, as suggested by Kelly (1995).

Park et al. (1999b) reported nine RAPD markers linked to the *Ur-7* gene for specific rust resistance using bulked segregant analysis in an $F_2$ population from the Middle American common bean cross >GN1140= (resistant) x GN Nebr. #1 (susceptible). Six RAPD markers were detected in a coupling phase linkage with the *Ur-7* gene. Cosegregating coupling-phase markers OAD12.550 and OAF17.900 were found in the $F_2$ population. Among three repulsion-phase markers, marker OAB18.650 was the most closely linked to the *Ur-7* gene at 7.6 cM. All linked markers detected in the $F_2$ population also segregated in recombinant inbred lines from the cross GN BelNeb-RR-1 x A 55, and were located on linkage group 11 of the existing linkage map (Park et al., 2002b).

The objective of this study was to determine the presence or absence of two coupling-phase markers OAD12.550 and OAF17.900 linked (no recombination) to the *Ur-7* gene for specific rust resistance in 21 Middle American and 12 Andean common bean cultivars/breeding lines resistant or susceptible to rust race 59.

## Materials and Methods

**Plant Material and Inoculation.** Thirty-three Middle American and Andean common bean cultivars/breeding lines were planted in a completely randomized design with two replications in a greenhouse on 27 August 1998. All plants were inoculated by a spore suspension of race 59 sprayed on the abaxial leaf surfaces of the unifoliate leaves. Resistant reactions included no visible symptom (immune), necrotic spots without sporulation (a hypersensitive reaction), and/or small uredinia less than 300Fm in diameter. A susceptible reaction produced uredinia larger than 300Fm in diameter.

**RAPD.** Total genomic DNA was extracted from lyophilized leaf tissue of 33 Middle American and Andean bean cultivars/lines. Polymerase chain reactions were performed on 48-well plates in a Perkin-Elmer thermalcycler (model 480; Perkin-Elmer Co., Norwalk, Conn.). Two 10-mer primers (Operon Technologies, Alameda, Calif.) were tested in 33 bean cultivars/breeding lines to determine the presence or absence of coupling-phase markers. The name of each RAPD marker is derived from an >O= prefix for Operon primers, the letters identifying the Operon kit, Operon primer number, and the approximate length of the marker.

## Results and Discussion

The presence or absence of two coupling-phase markers OAD12.550 and OAF17.900 linked to the *Ur-7* gene for specific rust resistance in the $F_2$ population was investigated in 21 Middle American and 12 Andean common bean cultivars/breeding lines. The presence of these markers was associated with all Middle American cultivars/lines resistant to rust race 59 except BelDakMi-RR-1 and >Chase=. All Middle American susceptible cultivars/lines lacked the marker fragments. Two resistant lines BelDakMi-RR-1 and >Chase= possessed a different rust resistance gene. All Andean cultivars/lines, regardless of response to race 59, lacked the marker fragments. This would be expected because the *Ur-7* gene is from the Middle American gene pool, and these markers are closely linked with the gene. Thus, these coupling-phase markers OAD12.550 and OAF17.900 could be useful in breeding and selecting for rust resistance in the Middle American bean germplasm.

The rust resistant pinto US-5 and US-14 dry bean lines were developed and released by the USDA in 1946. The rust resistant >GN1140= was selected from progenies of the cross GN UI 123 (susceptible) x pinto US-5 (resistant). Thus, the rust resistance gene of >GN1140= originated from pinto US-5. Four plants of the pintos US-5 and US-14 used in this study possessed the coupling-phase RAPD markers linked to the *Ur-7* gene. Because repulsion-phase markers were obtained from the susceptible parent GN Nebr. #1, the rust resistant pintos US-5 and US-14 did not have any repulsion-phase RAPD markers. These results are consistent with the hypothesis that the rust resistance *Ur-7* gene of >GN1140= was originally derived from pinto US-5, and the rust resistance gene present in pintos US-5 and US-14 is the same.

Kelly et al. (1996) indicated that two independently identified rust resistance genes, the *Ur-6* gene present in >Olathe= and the *Ur-7* gene present in >GN1140=, might be the same. >Golden Gate Wax= also possessed the *Ur-6* gene for rust resistance (Stavely et al., 1983). Both >Olathe= and >Golden Gate Wax= were resistant to race 59 in our tests. Thus, we needed to determine if coupling-phase markers linked to the *Ur-7* gene identified here were also present in the two cultivars containing the *Ur-6* gene. All the coupling-phase markers were present in >Olathe= as well as pintos BelDak-RR-1 and >Bill Z=, another source of *Ur-6* (M. Brick, Colorado State University, personal communication). They were absent in >Golden Gate Wax= and another source of *Ur-6*, BelDakMi-RR-1. These coupling-phase markers also segregated in an $F_2$ population of the bean cross >Olathe= x GN Nebr.#1 sel.27. On the basis of linkage analysis of the coupling-phase markers with the *Ur-6* gene, the markers were not linked to the *Ur-6* gene. These results support the hypothesis that the *Ur-6* and *Ur-7* genes are not the same.

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# BACKCROSS ASSISTED BY RAPD MARKERS TO DEVELOP COMMON BEAN LINES WITH CARIOCA TYPE GRAINS CONTAINING THE *Ur-11* RUST RESISTANCE GENE

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Rust, caused by the fungus *Uromyces appendiculatus*, may cause serious losses to the common bean culture mainly in regions with mild temperatures and high humidity (Paula Júnior & Zambolim, 1998). The BIOAGRO/UFV bean breeding program has been using cv. Ouro Negro as the only source for rust resistance. However, more recently new sources were characterized and cultivar Belmidak RR-3 (*Ur-11*) has been shown to be an important resistant source to *U. appendiculatus* races prevalent in the state of Minas Gerais, Brazil (Faleiro et al.,1999; Faleiro et al., 2001). Allelism studies involving Ouro Negro and Belmidak RR-3 showed that these cultivars possess distinct rust resistance genes that can be pyramided to develop bean materials adapted to Central Brazil with complementary resistant (Alzate-Marin et al., 2002).
In our backcross breeding program for the creation of common bean cultivars resistant to rust we usually develop near-isogenic lines containing the individual resistance genes and then they are intercrossed. Rudá, the recurrent parent that we have been using, is a "carioca" type cultivar, with good yield potential but susceptible to rust. Here we report on the transfer of *Ur-11* from Belmidak RR-3 to Rudá, in a process assisted by molecular markers.

## Materials and Methods

Common bean cultivar Belmidak RR-3 (pollen donor) was crossed with Rudá, in the greenhouse under controlled environmental conditions. In each backcross generation $BC_nF_1$ plants were inoculated with spore suspensions of a mixture of races of *U. appendiculatus* (2 x $10^4$ spores/ml). The plants were then incubated for two days in a mist chamber kept at 20 - 22 $^oC$ and 100% relative humidity. After this period, each plant was scored visually for the disease symptoms using a 1-to-6 scale (Stavely et al., 1983) in which 1 was attributed to plants with no visible symptoms and 6 to severely diseased or dead plants. Leaf DNA was extracted from the progenitors and the resistant $BC_nF_1$ plants by a mini-prep procedure based on Doyle and Doyle (1990). RAPD amplification reactions were according to Williams et al. (1990) using different primer sets in each backcross cycle. Genetic similirities based on the RAPD data and cluster analyses were determined by the SPSS program and the Euclidian method for binary data (Wilkinson et al., 1992).

## Results and Discussion

The relative genetic similarities between the $BC_2F_1$ resistant plants and the recurrent parent varied between 75.8 and 97%. The plants genetically closer to the recurrent parent were used in the next backcross cycle. The genetic similarities between the $BC_3F_1$ resistant plants and Rudá

varied from 82.1 to 97.4%. Three $BC_3F_1$ lines bearing the *Ur-11* gene which were phenotypically undistinguishable from the recurrent parent (Figure 1) will now be selfed to select homozygous plants to be used in the pyramidation of different rust resistance genes in the background Rudá

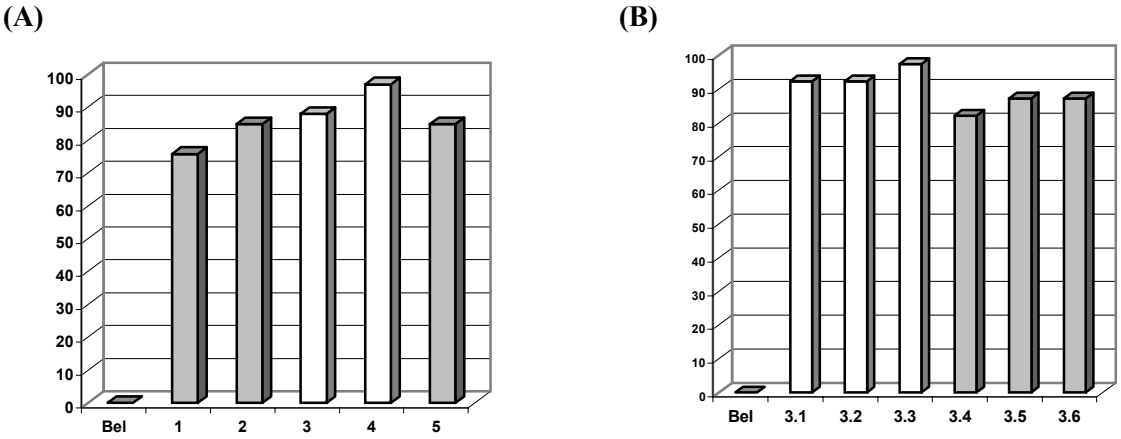


**Figure 1.** Relative genetic similarity (%) between $BC_2F_1$ (A) and $BC_3F_1$ (B) resistant plants and the recurrent parent Rudá. The relative distance between the recurrent and the donor parent Belmidak RR-3 (Bel) was considered to be 0%. Plants represented by white bars were selected based on their higher similarities in relation to Rudá**.**

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# Leaf and pod reaction of VAX lines to Bulgarian *Xanthomonas axonopodis pv. phaseoli* strains

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Breeding for resistance to *Xanthomonas axonopodis* pv. *phaseoli* is one of the major goals of the Dry Bean Breeding Program at Dobroudja Agricultural Institute, General Toshevo, Bulgaria (DAI). A number of bean accessions with reported resistance to common bacterial blight (CBB) were introduced in the DAI collection during the past years. Some of them (XAN 159, G.N. Jules and BAC 6) are the most used resistant donors in many beans breeding program worldwide. Recently, VAX 1-6 lines with pyramidal resistance to CBB have been reported (Singh and Muñoz, 1999, Crop Sci. 39:80-89 ) .

The resistance of common bean to CBB is under polygenic control and closely related to plant organ specificity, environmental conditions and virulence of bacterial strains. This imposes the necessity to test resistant donors at the environments used for breeding of new cultivars. The aims of the present study were: i- to evaluate leaf and pod reaction of VAX lines and some of their resistant parents to Bulgarian *X.a. pv. phaseopli* strains; ii- to select bacterial strains for markers to further usage of these donors in DAI bean breeding programs.

## Material and Methods

The VAX 1-6 lines and some of their parents were grown in the field in 1 m randomized rows (10 plants in the row, two replications for strain x line) during 2002. Ten bacterial strains from different regions of Bulgaria were included in the study. The strains were grown on YDC at 27°C for 48 h, and bacterial suspension ($10^8$ cfu/ml) was prepared in sterile tap water. The leaves were inoculated at flowering stage by the multiple needle method (Andrus, 1948, Phytopathology 38:757-759). A hypodermic needle and 1 ml syringe were used for inoculation of the pods. The disease reaction was recorded 14 days after inoculation. A nine-degree scale (1 - no visible symptoms; 9 - over 85% of inoculated tissue with blight symptoms) was used for the leaves, and size of lesions (mm) was recorded for the pods.

## Results and Discussion

High resistance of leaves to the used strains was observed in lines VAX 3, 4, 5 and 6 and XAN 159 (tab. 1). An insignificant variation of leaf reaction to some strains was registered in VAX 5 and XAN 159. Lines VAX 1 and 2 developed more severe blight symptoms to XB013.1F and XB015.1F (fuscans strains) in comparison to the other strains. These strains were considerably more aggressive to A 769 and G.N. Jules than the others.

Lines VAX 3, 4, 5 and 6 and XAN 159 showed a higher pod resistance to the strains included in the study (tab.2). The pod resistance of VAX 1 and 2, A769 and G.N. Jules were lower than in the other lines.

The results obtained showed that lines VAX 3, 4, 5 and 6, as well as XAN 159 are the most suitable donors for resistance to Bulgarian *X.a. pv. phaseoli* populations.

**Table 1.** CBB leaf reaction of lines VAX 1-6 and some of their parents to 10 Bulgarian strains of *X.a.pv. phaseoli*

| Strain | Dobr.7* | XAN 159 | Jules | A 769 | VAX 1 | VAX 2 | VAX 3 | VAX 4 | VAX 5 | VAX 6 |
|---|---|---|---|---|---|---|---|---|---|---|
| XB 9622.1 | 8.6 | 1.3 | 3.2 | 3.3 | 1.8 | 1.4 | 1.0 | 1.0 | 1.4 | 1.0 |
| XB 9625.1 | 9.0 | 1.1 | 2.4 | 3.8 | 1.4 | 1.3 | 1.0 | 1.0 | 1.3 | 1.0 |
| XB 9913.2 | 8.0 | 1.3 | 2.9 | 3.5 | 1.6 | 1.4 | 1.0 | 1.0 | 1.4 | 1.0 |
| XB 011.1 | 8.0 | 1.2 | 2.1 | 2.0 | 1.5 | 1.6 | 1.0 | 1.0 | 1.3 | 1.0 |
| XB 014.1 | 7.0 | 1.0 | 2.2 | 1.8 | 1.7 | 1.1 | 1.0 | 1.0 | 1.0 | 1.0 |
| XB 013.1F | 8.2 | 1.0 | 4.1 | 4.8 | 4.3 | 2.7 | 1.0 | 1.0 | 1.0 | 1.0 |
| XB 006.1 | 7.8 | 1.0 | 2.4 | 2.8 | 1.8 | 1.3 | 1.0 | 1.0 | 1.5 | 1.0 |
| XB 015.1F | 8.3 | 1.0 | 3.9 | 5.5 | 3.5 | 2.6 | 1.0 | 1.0 | 1.0 | 1.0 |
| XB 019.1 | 8.6 | 1.5 | 1.3 | 2.2 | 1.2 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| XB 007.1 | 8.6 | 1.0 | 3.2 | 3.3 | 1.5 | 1.4 | 1.0 | 1.0 | 1.3 | 1.0 |

*Dobroudjanski 7 -susceptible check

**Table 2.** CBB lesion size (mm) on pods of lines VAX 1-6 and some of their parents after inoculation with 10 Bulgarian strains of *X.a. pv. phaseoli*

| Strain | Dobr.7* | XAN 159 | A 769 | Jules | VAX 1 | VAX 2 | VAX 3 | VAX 4 | VAX 5 | VAX 6 |
|---|---|---|---|---|---|---|---|---|---|---|
| XB 9622.1 | 7.5 | 2.7 | 4.1 | 3.4 | 4.7 | 5.7 | 1.4 | 1.2 | 2.3 | 1.7 |
| XB 9625.1 | 5.7 | 1.7 | 2.7 | 2.8 | 4.3 | 4.1 | 1.6 | 1.2 | 2.7 | 1.6 |
| XB 9913.2 | 4.8 | 3.1 | 3.8 | 3.9 | 4.8 | 4.9 | 1.4 | 1.9 | 1.9 | 1.8 |
| XB 011.1 | 5.5 | 2.3 | 4.0 | 2.6 | 4.5 | 4.9 | 2.5 | 0.9 | 2.3 | 1.7 |
| XB 014.1 | 5.5 | 1.9 | 2.3 | 2.3 | 3.1 | 3.0 | 0.8 | 1.3 | 1.9 | 1.2 |
| XB 013.1F | 4.2 | 2.4 | 4.3 | 4.3 | 4.7 | 5.4 | 2.2 | 1.9 | 1.9 | 2.9 |
| XB 006.1 | 6.2 | 1.8 | 3.2 | 3.4 | 4.7 | 4.8 | 1.6 | 1.6 | 2.7 | 1.8 |
| XB 015.1F | 5.1 | 2.6 | 3.3 | 3.6 | 3.7 | 4.2 | 1.7 | 1.8 | 2.0 | 1.9 |
| XB 019.1 | 7.0 | 2.1 | 1.8 | 1.4 | 3.1 | 3.3 | 1.9 | 1.2 | 1.8 | 1.4 |
| XB 007.1 | 9.0 | 2.2 | 4.6 | 3.5 | 6.1 | 6.1 | 1.6 | 1.6 | 2.5 | 1.1 |

*Dobroudjanski 7 -susceptible check

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# Breeding for Common Bacterial Blight Resistance in Cranberry Beans

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Common bacterial blight (CBB) caused by *Xanthomonas axonopodis pv. phaseoli* is major bacterial disease of common bean. In Ontario, 25% of the crop may be affected (Hall, 1989) causing reduced marketability of seed and reduction of seed production. OAC Rex is a recently released CBB resistant white bean developed at the University of Guelph. SVM Taylor Horticulture is an established variety of cranberry bean that is susceptible to CBB. The objective of this study was to introgress the CBB resistance from OAC Rex into SVM Taylor Hort.

Crosses were made between OAC Rex and SVM Taylor Hort in a growth room at the University of Guelph. $F_1$ seed was advanced to the $F_5$ stage by single seed descent. The population of 159 lines was evaluated at both the $F_4$ and $F_5$ stages using a simple lattice design with two replications for CBB resistance at the Elora research station (Elora, Ontario). Lines were inoculated at the 3-4 trifoliate stage with a tractor mounted high-pressure sprayer (180 psi). The plants were rated visually on a scale of 0-4 (0 = no symptoms, 4 = spreading necrotic lesions on most leaves). A line with a score of 2 or less was considered resistant.

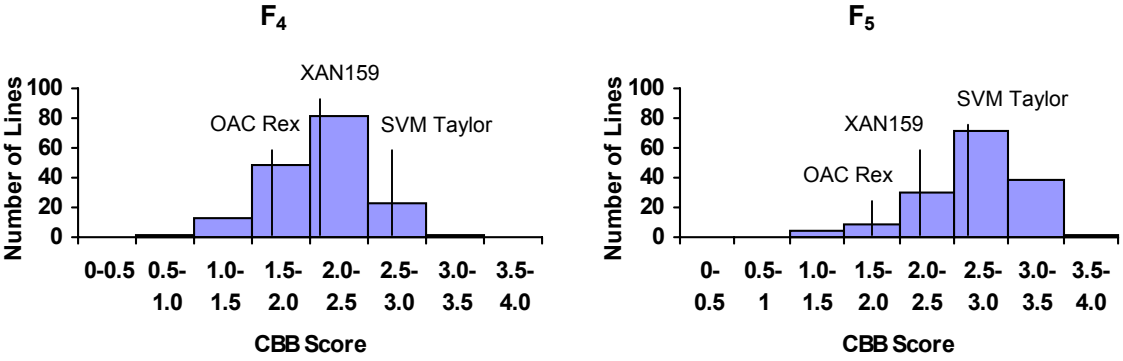



Figure 1: Distribution of CBB Scores for $F_4$ (left) and $F_5$ (right) lines with parental lines and XAN159, a resistant check.

The CBB disease scores for the population were continuously distributed and transgressive segregants with better resistance than OAC Rex were observed in both years of testing (Figure 1).

Molecular analysis was performed on DNA isolated from two plants from each $F_4$ line. Fifty-four polymorphic markers were scored including: nine RAPD's, six STS's, 21 SSR's, 14 SCAR's and four phenotypic markers. A linkage map was created with MAPMAKER 3.0 consisting of 29 markers and five linkage groups. All of the linkage groups included markers to link to the core map (Nodari *et al*., 1992). Quantitative trait loci (QTL) were identified using Mapmaker QTL 2.0 and QTL Cartographer. QTL for CBB resistance were identified on linkage groups corresponding to core linkage groups B8 and B7 (Figure 2). Several markers were found to be significantly associated with CBB resistance in the cross (Table 1, Figure 2).

Coloured bean breeding is difficult because many genes determine the seed coat colour. Testcrosses were made between tester lines identified by Bassett, (1992) and OAC Rex and SVM Taylor Hort. Examination of the $F_1$s from the testcrosses identified the seed coat genotypes of OAC Rex and SVM Taylor Hort. to be: [CR] G B V J and [$CR^{st}$] g b v J respectively. STS markers linked to colour genes G and V (McClean et al., 2002), were also mapped (Figure 2).

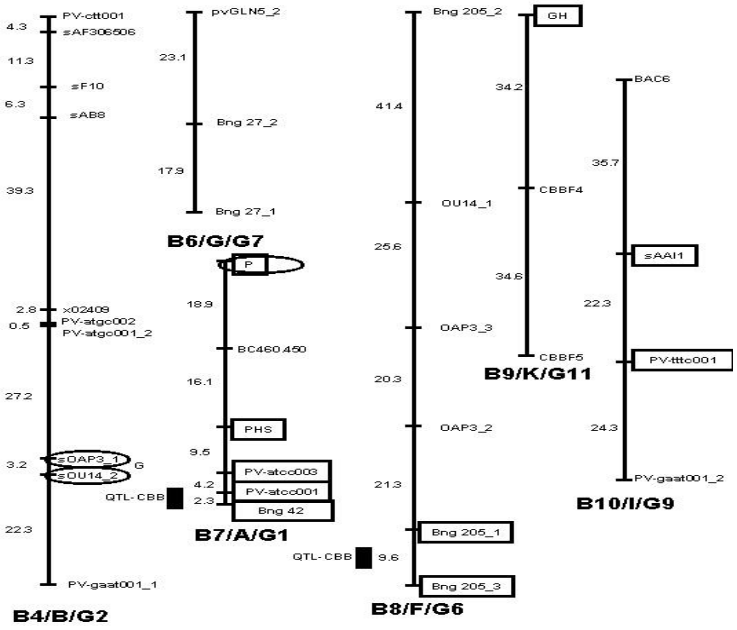
In total, five $F_5$ lines had seed with cranberry phenotypes. Using molecular and phenotypic markers a coloured line with a solid purple seed coat and a line with a white background with purple striping were identified that were more resistant than OAC Rex.

Table 1: List of a Selection of Molecular and Phenotypic Markers linked to CBB resistance in SVM Taylor x OAC Rex RI population.

| Marker | $R^2$ value | Linkage Group(Core/Florida/Guelph) |
|---|---|---|
| Pigment Locus (P) | 0.07 | B7/A/G1 |
| Growth Habit (GH) | 0.07 | B9/K/G11 |
| ATPase | 0.08 | unlinked |
| Bng 42 | 0.06 | B7/A/G1 |
| PV-atcc001 | 0.06 | B7/A/G1 |
| Bng205_1 | 0.03 | B8/F/G6 |
| Bng205_3 | 0.06 | B8/F/G6 |
| PV-cca001 | 0.04 | unlinked |
| Bng 21 | 0.05 | unlinked |

Table 2: Mapped SCAR markers. *Medicago truncatula* ATPase marker was taken from Thoguet *et al*., 2002. BMC Plant Biology 2:1-13.

| Marker | Primer sequence (5'-3') | size(bp) |
|---|---|---|
| ATPase (*Medicago truncatula*) | F: GGGTTTTTGATCCAGATCTTT<br>R: AAGGTGGTCATACGAGCTCC | 500 |
| sAAI1 | F: TGGCTTCCTCCAAGTTACTCA<br>R: GGATTTGGTTGAGGAGGATG | 800 |
| sAF306506 | F: AACCCTCTTCCCATTCGTTC<br>R: GACGTCACGGAATTCAAATG | 1200 |




Both of these lines have at least 3 colour and patterning genes from SVM Taylor that were not present in OAC Rex. The two lines are presently, being used for a cranberry bean breeding project at the University of Guelph. The materials will be screened with markers for CBB resistance and seed coat colour to facilitate the selection of a resistant cranberry line.

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Figure 2: Linkage map of common bean. Markers in boxes are significantly linked to CBB resistance and QTL for CBB are indicated by a black box. Markers in ovals are linked to seed colour genes sOAP3_1 and sOU14_2 are linked to colour gene 'G' (McClean *et al.* 2002). P is Pigment locus.

# Comparison of Aspersion and Multiple-Needles Inoculations for Selection in the Field for Halo Blight Resistance in Common Bean Populations

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## Introduction

Halo blight [caused by *Pseudomonas syringae* pv. *phaseolicola* (Burkh.)] is one of the most important production problems reducing yield and quality of common bean (*Phaseolus vulgaris* L.) in Spain, especially in Castilla and Leon. This seed transmitted disease is endemic in most production regions. One of the objectives of SITA's breeding program is the introgression of halo blight resistance in cultivars.

Different inoculation methods have been used in common bean. For example, rubbing primary leaves with carborundum (Hubbeling, 1973), leaf water soaking with an atomizer (Schuster, 1955), submergence and partial vacuuming of bean seeds in bacterial solution (Goth, 1966), and inoculating primary leaf with multiple-needles (Andrus, 1948). In greenhouse, Zaiter and Coyne (1984) found that the leaf water soaking caused the most severe reactions, followed by rubbing primary leaves with carborundum, spraying, and multiple-needles. The objective of this study was to compare aspersion and multiple-needles inoculation methods in the field in $F_2$ populations.

## Materials and Methods

Seven parental genotypes and approximately 130 $F_1$–derived $F_2$ families from four populations were evaluated. The populations were:

ZARA III=Wilkinson 2/'Montcalm'//'Casasola'/'Harris',
ZARA IV='Edmund'/Wilkinson 2//Casasola/BRB 131,
ZARA XI='Moradillo'/Montcalm//Wilkinson 2/Montcalm, and
ZARA V=Casasola/3/Wilkinson 2/Montcalm//Casasola/Harris.

Casasola, Edmund, and/or Harris were halo blight resistant parents used in the four populations. Each plot consisted of a single row, ten cm long, without replication.

The Race 6 of *P. syringae* pv. *phaseolicola* was used to inoculate the first trifoliolate leaf with multiple-needles. A backpack solo sprayer was used for the aspersion method. Evaluations were made 14 to 21 days after inoculation according to Innes et al. (1984) and Aggour et al. (1989). Individual evaluated plants with disease scores 1 to 3 were considered resistant, 4 to 6 intermediate, and 7 to 9 susceptible. Subsequently, the mean disease scores were calculated for each $F_{1:2}$ family, population, and inoculation method. Also, simple correlation coefficients were determined between the two methods.

## Results and Discussion

Disease scores for seven parental genotypes tended to be higher for the multiple-needles compared to the aspersion method of inoculation (Table 1). Similarly, the range and mean disease scores for each population were higher for the multiple-needles compared to the aspersion method (Table 2). Significant positive correlation was observed between the two methods for all populations (Tabla 3). However, Zaiter and Coyne (1984) reported higher disease scores for the spraying method in the greenhouse. Differences between the two results could be due to the environment (greenhouse versus field) and bacterial strains used for inoculation. Also,

differences between the common bean genotypes used (advanced breeding lines and cultivars versus segregating populations) may influence results.

Because of positive correlations between both methods, selection among $F_{1:2}$ families can be made with either inoculation method. However, the aspersion method will be cheaper, easier, faster, and less labor intensive than the multiple-needles method. Thus, for screening of large germplasm and early generation populations and families, the use of aspersion method is advisable, provided the inocula production is not a limiting factor. This should be followed by evaluation of advanced generation lines with the aspersion method, especially for selection of highly resistant cultivars for areas with severe halo blight problems.

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Table 1. Reaction of common bean genotypes to halo blight inoculations with aspersion and multiple-needles in the field at Valladolid, Spain in 2001.

| Genotype | Aspersion[1] | | Multiple-needles[1] | |
|---|---|---|---|---|
| | Mean | Range | Mean | Range |
| BRB 131 | 7.4 | 4-9 | 8.1 | 7-9 |
| Casasola | 2.5 | 1-6 | 5.2 | 3-6 |
| Edmund | 1.0 | 1 | 2.4 | 1-4 |
| Harris | 1.5 | 1-6 | 3.0 | 1-6 |
| Montcalm | 4.0 | 1-7 | 4.2 | 1-6 |
| Moradillo | 7.7 | 6-9 | 7.8 | 3-9 |
| Wilkinson 2 | 6.8 | 1-9 | 6.7 | 1-9 |

[1]Evaluated on a 1 to 9 scale, 1=no symptoms, 9=highly susceptible.

Table 3. Simple correlation coefficients between aspersion and multiple-needles inoculation methods in common bean populations evaluated in the field at Valladolid, Spain in 2001.

| Population | Correlation |
|---|---|
| ZARA V | 0.68* |
| ZARA XI | 0.72* |
| ZARA III | 0.81* |
| ZARA IV | 0.68* |

Table 2. The minimum, maximum, and mean halo blight scores for four $F_2$ populations of common bean inoculated with aspersion and multiple-needles in the field at Valladolid, Spain in 2001.

| **Population** | **Inoculation method** | **No. of families** | **Minimum** | **Maximum** | **Mean** |
|---|---|---|---|---|---|
| ZARA V | Aspersion | 35 | 2.1 b | 5.4 a | 3.9 b |
| | Multiple-needles | 33 | 4.0 a | 6.3 a | 5.1 a |
| ZARA XI | Aspersion | 20 | 3.1 a | 6.9 a | 5.4 a |
| | Multiple-needles | 20 | 4.0 a | 7.4 a | 5.9 a |
| ZARA III | Aspersion | 44 | 2.1 b | 6.5 a | 4.4 a |
| | Multiple-needles | 44 | 3.3 a | 6.6 a | 5.0 a |
| ZARA IV | Aspersion | 36 | 1.5 b | 5.8 a | 3.4 b |
| | Multiple-needles | 35 | 2.8 a | 6.1 a | 4.4 a |

[1]Evaluated on a 1 to 9 scale, 1=no symptoms, 9=highly susceptible.

# IDENTIFICATION AND DEVELOPMENT OF MOLECULAR MARKERS LINKED TO COMMON BACTERIAL BLIGHT RESISTANCE GENES FROM WILK 2

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Common bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *phaseoli* (Xap) is a major disease limiting dry bean production in South Africa. All locally grown commercial cultivars are susceptible to CBB and thus improvement of cultivars by introducing stable resistance is important. Two resistance sources, GN Nebr.#1 sel 27 and XAN 159 each contribute 2 independent quantitative trait loci (QTL) with major effects on common blight resistance. Sequence characterized amplified region (SCAR) markers linked to these four QTL are available for DNA marker-assisted breeding (Miklas *et al*., 2000). Wilk 2 has shown superior resistance to that in XAN 159 in greenhouse testing under South African conditions (Fourie, 2002). The aims of the study were to determine if Wilk 2 contributes any additional resistance QTLs and if so, develop flanking markers for the QTL. Development of suitable markers will assist in the improvement of resistance in Teebus, a commercially important local navy bean that is highly susceptible to CBB.

The AFLP technique was employed as molecular tool to identify markers linked to CBB resistance from Wilk 2. A total of 20 *Eco*RI/*Mse*I primer pair combinations were screened and 53 putative markers identified. These were tested on a segregating $F_2$ population and mapped using MAPMAKER-EXP along with four existing SCARs, BC420, BC409, SU91 and SAP6. One linkage group was obtained and three markers were unlinked. Using StatGraphics the highest linkages to the resistance trait obtained were 74.93% (Marker #25) and 70.15% (Marker #17) (Table 1). The existing SCARs showed lower linkages than expected (BC420 and BC409 both 58.44%, SU91 18% and SAP6 5.84%). SAP6 is present in the susceptible cultivar Teebus and is thus not useful in the local breeding programme. It is furthermore not closely linked to the resistance trait emphasizing the need for a closer flanking marker to the resistance QTL.

Six putative markers were selected, cloned and sequenced for further development of new SCARs and five SCAR primers were developed from the obtained sequence data. The clone with fragment #18's sequence contained two fragments with different sequences in tandem. Primers were developed for both sequences. Table 1 shows the linkage data of AFLP markers and developed SCAR primers. Two SCAR primers did not show polymorphisms between the two parents (SN4 and SN6), while the other four were polymorphic. The polymorphic SCARs were tested on the $F_2$ population and mapped onto a linkage map. SN2A was the only SCAR that mapped close to the original AFLP marker, i.e. 2.2 cM from marker #18 (Fig. 1) and showed a higher linkage to CBB resistance than the AFLP marker (from 63% to 65%). SCARs developed from fragments # 17 and 25 did not map close to the AFLP fragment from which it was derived, indicating that the wrong fragment was sequenced. Cloning of these two AFLP markers was repeated and two clones of each selected for sequencing. Each clone had a unique sequence and primers were developed for each clone. These clones are currently being tested for closer linkage to the original AFLP fragment.

Table 1: Linkage data of AFLP fragments and developed SCAR markers

| AFLP marker | Linkage phase | $R^2$ % | SCAR | Linkage phase | $R^2$ % |
|---|---|---|---|---|---|
| 17 | C | 70 | SN1 | R | 16 |
| 18 | C | 63 | SN2A | C | 65 |
| | | | SN2B | C | 44 |
| 25 | C | 75 | SN3 | R | 57 |
| 53 | C | 62 | SN4 | - | - |
| 69 | C | 44 | SN6 | - | - |

$R^2$: Coefficient of determination C: Coupling phase
R: Repulsion phase

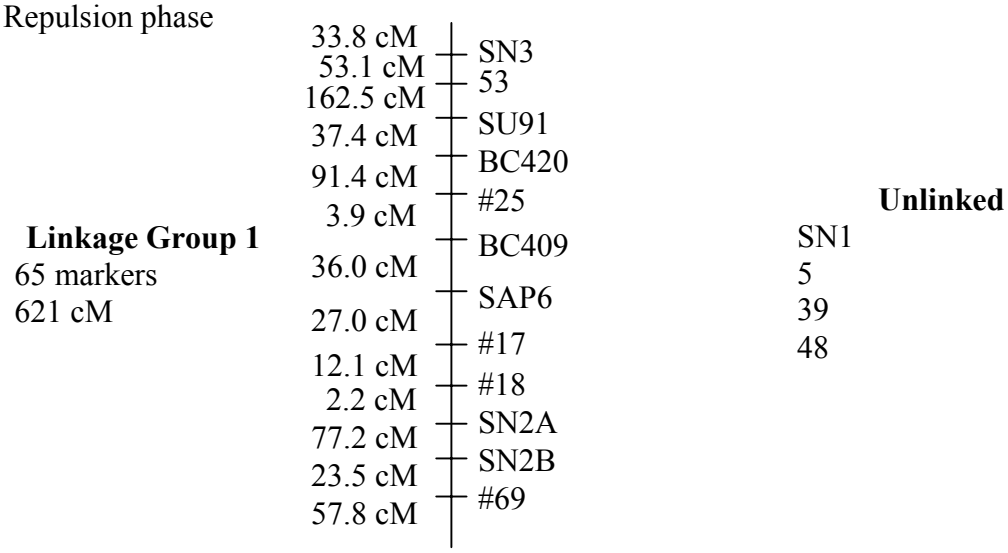


Figure 1: Partial linkage map of relevant AFLP markers and SCARs

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# Reactions of resistant and susceptible bean genotypes using three inoculation methods with *Xanthomonas campestris* pv *phaseoli* from Salta (Argentina)

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## • Introduction

The development of cultivars improved for their performance against diseases requires a prior evaluation of lines to be used as parents in the improvement process. The performance of each genotype regarding the pathogen varies with the strain, the inoculation concentration and the inoculation used (Leyna et al., 1982; Leyna et al., 1985; Opio et al., 1994). The choice of the inoculation method is defined according to the uniformity of the results obtained and the practicability of its application (Opio et al., 1994; Kinakov et al., 1994).

## Objective

The objective of this study was to evaluate the three more used inoculation methods in leaves, regarding reproducibility and discrimination capacity, using one local isolate of *Xcp* (Ac) and genotypes with known reactions to other isolates.

## Methods and Materials

Three inoculation methods were used:

a) Double cut. Consisting of making two parallel cuts in the foliar surface using blades soaked in the inoculum set into handles. In order to ensure the pathogen entered, the cut was made holding the blade over a sponge moistened with the bacterial suspension.
b) Aspersion. Consisting of spraying the underside of the leaves using an airbrush at a pressure of 15 psi.
c) Multiple needles. Several entomology needles are set up on a wooden support and the leaves pricked. The leave to be positioned on a sponge soaked in the inoculum.

Six bean lines were used: Alubia Selección Cerrillos INTA and TUC 180 (EEAOC), field susceptible; Perla INTA and SI 56 (advanced line in the INTA Improvement Programme), intermediate field resistant; and HR-45 (provided by Dr.Park) and VAX 4 (provided by CIAT), resistant.

The assay was carried out in greenhouses under controlled conditions using a completely random design with 9 repeats and 3 controls for each material and method. The controls were inoculated with sterile distilled water. Seeding took place on April 4$^{th}$.

The plants were inoculated before flowering on young fully open leaves with a bacterial suspension of 1 x $10^7$ ufc/ml. Inoculation date: May 21$^{st}$. Evaluation date: June 1$^{st}$.
The scales used were the following:

**Double Cut (DC)**

**1**. Controls.
**2**. Absence of symptoms, dry tissue in the area around the cut.
**3**. Chlorosis with slight necrosis in the area around the cut (less than 50%).
**4**. Chlorosis with necrosis in the area around the cut (up to 90%).
**5**. Necrosis in the area around the cut (more than 90%) extending beyond the incision with chlorosis.

**Aspersion (A)**

**1**. Controls.
**2**. Dry and wrinkled tissue (hypersensitivity).
**3**. Dry and wrinkled tissue with dark edges (hypersensitivity).
**4**. Slight chlorosis.
**5**. Chlorosis with abundant necrotic spots.
**6**. Typical symptom.

**Multiple Needles**

**1**. Controls.
**2.** Chlorosis with necrotic spots.
**3.** Chlorosis with widespread necrosis.

- The results were analyzed through an ANOVA, with the LSD test for the comparison of averages.

**Results:** The three methods used are easily repeatable.

The multiple needle method was the most reliable in discriminating the materials, grouping them in accordance with the resistance / susceptibility characteristics known previously. The other two methods were very aggressive and had low discrimination capacities.

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## Acknowledgements


We would like to thank Mr. Mario Chocobar for the technical assistance.

# PROBLEMS FACED IN THE CHARACTERIZATION OF THE *Pseudomonas syringae* pv *phaseolicola* RACES PRESENT IN THE MAIN BEAN GROWING AREAS OF THE CENTRAL REGION OF SPAIN.

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## Introduction

Halo blight, caused by *Pseudomonas syringae* pv *phaseolicola* (*Psp*), is one of the three bacterial blights that affects dry edible beans in the central region of Spain (Castilla y León). Detailed analysis of pathogenic variation among population of *Psp* in Castilla y León, is a fundamental prerequisite for the ongoing bean breeding program at SITA, Valladolid

Based on their interaction with eight bean differential cultivars, nine races of *Psp* were identified (6). Nonetheless, it has been reported the existence of isolates whose race could not be established (4,6). In this work, we explain the problems we found trying to characterise the races of *Psp* present in Castilla y León.

## Material And Methods

*Strain Collection:* A total of 84 *Psp* isolates from the strain collection at SITA were selected on the basis of their geographical origin, to represent the mayor dry bean growing areas in Castilla y Leon. Pathogenicity test, biochemical identification (test LOPAT) (3) and DNA amplification with specific primers (5), firstly confirmed the identities of these isolates.

*Race Identification:* Isolates were identified to race level using eight standard differential bean cultivars according to the method described by Taylor *et al.* (6). These differentials were planted in 7 cm plastic pots containing turf and maintained in a growth chamber at 21 º C. Inoculation was conducted as describe above. At least 6 replicate plants were used for each combination of isolate and cultivar. Inoculated plants were kept in a humidity chamber at 20 º C, 12-h day length and 100 % HR. Plants were rated for infection 7 and 14 days after inoculation on a 1 to 5 scale (6). A rating of 1-2 was considered resistant and 3-5 susceptible.

## Results

Three races of *Pseudomonas syringae* pv *syringae* were identified in Castilla y Leon. From the 84 isolates examined, twenty-three were identified as race 7, nineteen as race 6 and two as race 2. Both, race 7 and 6, were widely distributed and occurred in all the regions surveyed. Race 2 was restricted to only one region.

Race of the remaining 47 isolates (56 %) was not possible to determine. From these, 13 isolates correspond to isolates that did not match those of any known race. Description of their reactions on the differential set and number of isolates included in each pattern (patterns A, B, C, D y E) is illustrated in Table 1. The remaining 27 isolates have been grouped into two categories designated as races: “1-like” (8 isolates) and “7-like” (19 isolates) (Table 1). They seemed to be race 1 and 7, but it was not possible to determine definitively its race due to a contradictory reaction within the cultivar Guatemala 196-B. On this cultivar we scored, for the same isolate, reactions ranging from highly resistant up to fully susceptible.

**Table 1.** Pattern of reaction of the isolates that did not match those of any known race and that of races seemed to be race 1 ("1 like") and race 7 ("7 like") on eight differential cultivars.

| | *Differential cultivar* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **PATTERN** | **CW** | **A52** | **TG** | **UI3** | **1072** | **A53** | **A43** | **196-B** | **N º Isolates** |
| **A** | + | + | + | - | - | - | - | - | 6 |
| **B** | + | + | + | - | - | + | + | + | 2 |
| **C** | + | + | + | - | - | + | + | - | 2 |
| **D** | + | + | + | - | - | + | - | + | 2 |
| **E** | + | + | + | - | + | + | + | + | 1 |
| **"1 like"** | + | + | + | - | + | + | + | +/- | 8 |
| **"7 like"** | + | + | + | - | - | + | - | +/- | 19 |

CW = Canadian Wonder, A52 = ZAA 54 (A52), TG = Tendergreen, UI3 = Red Mexican, 1072 =*Ph. acutifolius*, A53 = ZAA55, A43 = ZAA12, 196-B = Guatemala 196- B.
(+) susceptible reaction; (-) resistant reaction; (+/-) reaction ranging from highly resistant up to fully susceptible.

## Discussion

Races 7 and 6 were the most prevalent in the region. This result is not consistent with that of race 6 considered the most predominant worldwide (6).

Regarding the isolates classified as "race 1-like" and "race 7-like" due to the controversial results on the differential cultivar Guatemala 196-B (GU196-B) (Table 1), we observed this strange reaction in both, seed coming from CIAT (Centro Internacional de Agriculltura Tropical, Cali, Colombia), and the Horticultural Research International in UK. This response was observed only when scoring races seemed to be 1 or 7. Also, we found phenotypic differences in the differential cultivar such as seeds with, both, dull and bright coat. No previous report on that was found. At present we are investigating the possibility of genetic variation in the cultivar, in order to find out whether it is a problem of race split in the pathogen or a genetic variation in the differential cultivar.

The remaining 13 isolates with unknown races, were grouped into five different patterns according to their reaction on the differential set (Table 1). Ariyarathene *et al.* (1) identified two new races in Nebraska, however, to our knowledge, no description of their reactions on the differential set has been published. Also, Lamppa *et al.* (2) reported another two different patterns. Neither of them matches any of these that are reported here. Additional tests must be carried out to further investigate these new patterns. Also, since this is a problem already reported by different authors working on *Psp* race characterisation, we think this topic should be matter of discussion.

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# APPLICATION OF EFFICACY PROFILE ANALYSIS TO MEASURING CONTROL OF RHIZOCTONIA DAMPING-OFF OF COMMON BEAN.

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Efficacy profile analysis was introduced using as an example the control of Pythium damping-off of white beans with seed treatments (Hall 2003). This study tests the concept using Rhizoctonia damping-off of white beans. The seed treatments were Apron Maxx RTA (fludioxonil 7.7 g ai/L, metalaxyl-M 11.4 g ai/L) at 3.28 mL/kg seed, ICIA5504 100FS (azoxystrobin 100 g ai/L) at 2.0 mL/kg seed, and Crown (carboxin 92 g ai/L, thiabendazole 58 g ai/L) at 6.0 mL/kg seed. *Rhizoctonia solani* was grown on potato dextrose agar in 10-cm petri dishes incubated at room temperature (20-22EC). These cultures were used to inoculate rye grains in erlenmeyer flasks (Acharya et al. 1984). The infested grain was ground and incorporated into Pro-Mix BX potting mix (Plant Products Co. Ltd.) at the rate of 0.5, 1.0, 5.0, and 10 g infested rye/L mix. Seeds of the white bean cultivars Envoy, Premiere, Stingray, and Vista were sown 2.5 cm deep in the potting mix at the rate of 4 seeds per 8.5 x 8.5 cm pot. Seeded pots were placed in a greenhouse and stand and fresh shoot weight per pot were determined 28 days after seeding. Seed treatment products were tested at each level of infestation. The two checks used were uninfested untreated (C1) and infested untreated (C2). In the analysis below, fungicidal seed treatments are labelled T. The experiment was arranged as a randomized complete block with four replications. The data were analyzed initially by ANOVA and means comparisons (REGWQ, P = 0.05). The combination of 4 cultivars and 4 inoculum concentrations provided 16 comparisons per seed treatment and measure of plant productivity. For Apron Maxx RTA, ICIA5504 and Crown, T significantly exceeded C2 in 8, 12 and 12 comparisons of stand and in 7, 12 and 12 comparisons of shoot weight, respectively.

Efficacy profiles were obtained by plotting (T/C1 - C2/C1) against disease pressure (C2/C1) (Figs. A to F). The higher C2/C1, the lower the disease pressure. When plotted against C2/C1, the values for T/C1 - C2/C1 were arranged along a trajectory. This trajectory, called the efficacy profile, is analyzed here as a straight line. Complete efficacy (T = C1) would be expressed by the line $x + y = 1$. Measures of profile efficacy include the intercept with the ordinate, the slope of the line, the value of T/C1 - C2/C1 when C2/C1 is 0.5 (E50), and the area under the line. When the area under the efficacy profile between abscissa values of 0 and 0.7 (to avoid extrapolation) is expressed as a percentage of the corresponding area under the line for complete efficacy, the values for efficacy in protecting stand and shoot weight are 60%, 65%; 82%, 65%; and 84%, 76% for Apron Maxx RTA, ICIA5504, and Crown, respectively. Intercepts with the ordinate and slopes of the efficacy profile (Figs. A to F) as well as E50 values (0.31, 0.33; 0.42, 0.33; 0.39, 0.36) also place these products in the same increasing order of efficacy. The profiles had similar intercepts with the abscissa (1.09, 1.08; 1.03, 0.99; 0.88, 0.89). This study supports the concept of an efficacy profile (Hall 2003) and illustrates its use to provide synoptic measures of efficacy, such as degree (area under the curve, ordinate intercept, E50) and extent (abscissa intercept, E50, slope), over a range of conditions.

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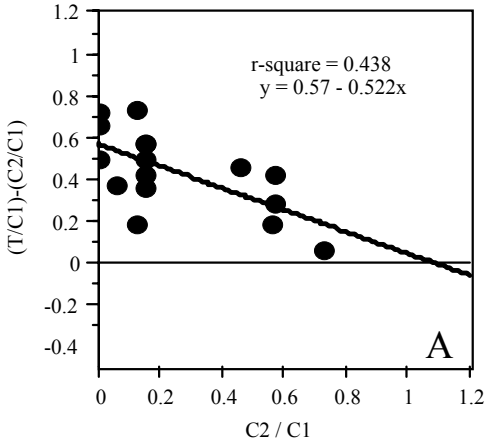
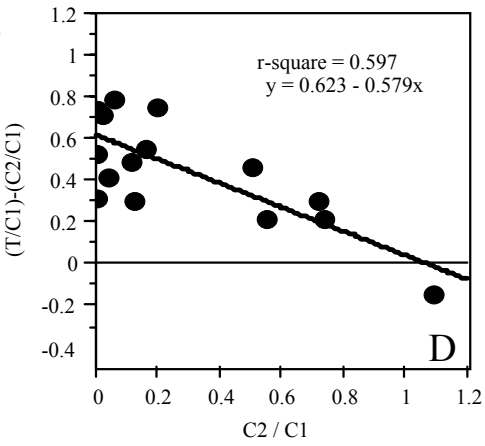
Rhizoctonia

Stand, Day 28

Fresh shoot weight, Day 28

Apron Maxx RTA

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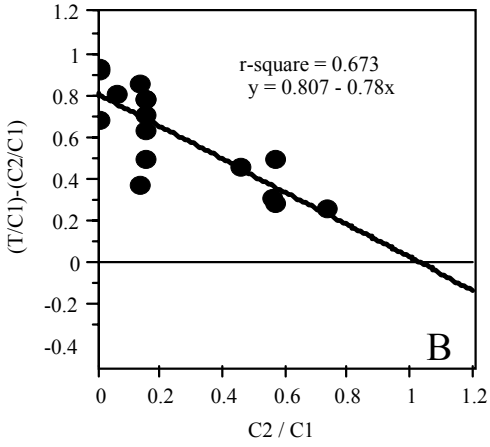


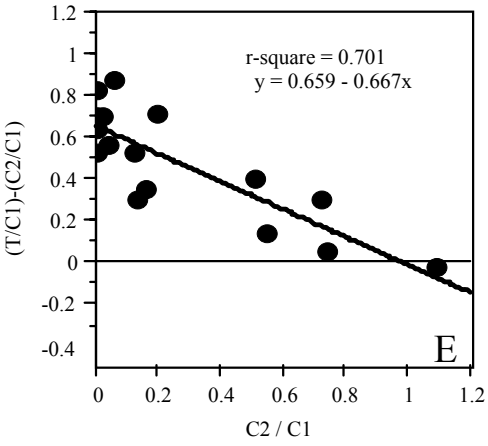


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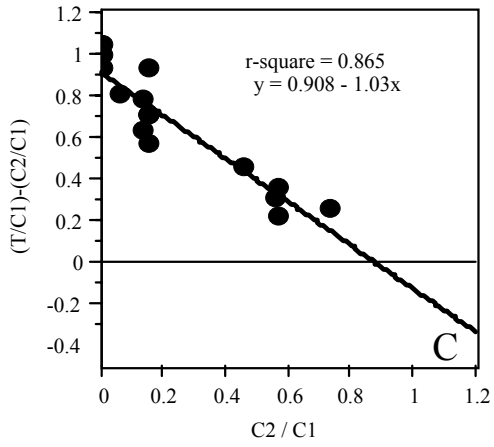


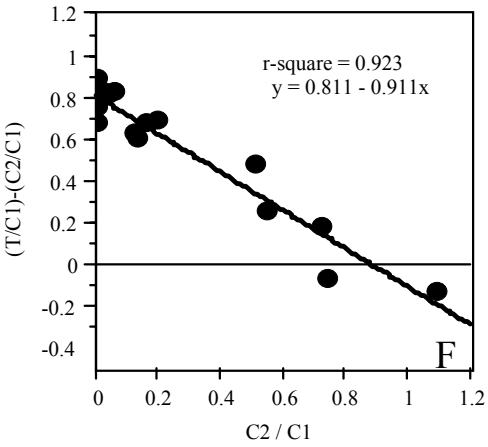

# EFFICACY PROFILE ANALYSIS: A NOVEL METHOD OF MEASURING EFFICACY OF PLANT DISEASE CONTROL

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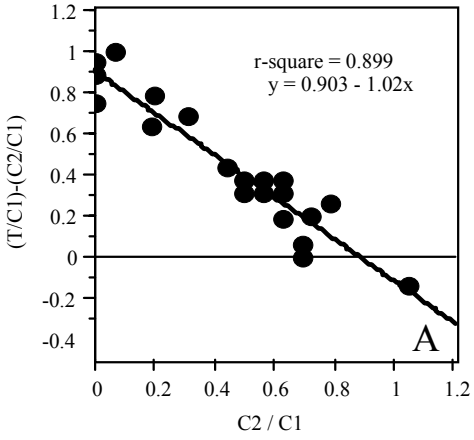
The relation of efficacy of fungicidal seed treatments to the severity of damping-off of white beans caused by *Pythium ultimum* was determined. The seed treatments were Apron Maxx RTA (fludioxonil 7.7 g ai/L, metalaxyl-M 11.4 g ai/L) at 3.28 mL/kg seed, ICIA5504 100FS (azoxystrobin 100 g ai/L) at 2.0 mL/kg seed, and Crown (carboxin 92 g ai/L, thiabendazole 58 g ai/L) at 6.0 mL/kg seed. Seeds of the white bean cultivars Envoy, Premiere, Stingray, and Vista were sown 2.5 cm deep in Pro-Mix BX (Plant Products Co. Ltd.) at the rate of 4 seeds per 8.5 x 8.5 cm pot. To infest potting mix, cultures of *P. ultimum* growing on V-8 juice agar (100 mL V-8® Juice, 2 g calcium carbonate, 15 g Difco agar, 900 mL distilled water) in 10-cm petri dishes were briefly macerated in a blender then incorporated into the potting mix at the rate of 1/8, 1/4, ½, 1, or 2 dishes/L of potting mix. Seeded pots were placed in a greenhouse and stand and fresh shoot weight per pot were determined 21 days after seeding. Seed treatment products were tested at each level of infestation. The two checks were uninfested untreated (C1) and infested untreated (C2). In the analysis below, fungicidal seed treatments are labelled T. The experimental design was a randomized complete block with four replications. Data were analyzed initially by ANOVA and means comparisons (REGWQ, P = 0.05).

A common method of determining efficacy is to show that T is statistically superior to C2. A single comparison provides one assessment of efficacy, here called point efficacy. The combination of 4 cultivars of bean and 5 levels of infestation provided 20 assessments of point efficacy per product for each measure of plant productivity. Because cultivar affected stand and shoot weight, the data were normalized by dividing T and C2 values by the corresponding C1 value. The magnitude of point efficacy was calculated as T/C1 - C2/C1. For Apron Maxx RTA, Crown, and ICIA5504, T/C1 significantly exceeded C2/C1 in 9, 1 and 0 tests on stand and in 8, 1 and 1 tests on shoot weight, respectively. Point efficacies of Apron Maxx RTA on stand and shoot weight were significant at inoculum concentrations of 1/8 to 2 and 1/4 to 2 cultures/L potting mix, respectively. Efficacy profiles were produced for each seed treatment for each measure of plant response by plotting point efficacy (T/C1 - C2/C1) against disease pressure (C2/C1) (Figs. A to F). The values for T/C1 - C2/C1 were not distributed randomly but were arranged along a trajectory, called the efficacy profile, analyzed here as a straight line. Profile efficacy could be expressed as the intercept with the ordinate, the slope of the line, the value of T/C1 - C2/C1 when C2/C1 is 0.5 (E50), and the area under the line. For example, when the area under the efficacy profile between abscissa values of 0 and 0.8 (to avoid extrapolation beyond the highest measured abscissa value) is expressed as a percentage of complete efficacy (the area under the line $x + y = 1$) between the same x values, the levels of efficacy in protecting stand and shoot weight are 83% and 58%, 27% and 4%, and 18% and 4% for Apron Maxx RTA, Crown and ICIA5504, respectively. The efficacy profile suggests efficacy is related to the amount of disease, which is in turn conditioned by the amount of inoculum and the resistance of the host. Profile efficacy is consistent with point efficacy in evaluating the seed treatments and provides simple, direct and quantitative measures of composite efficacy across disease levels, cultivars and inoculum concentrations. It could also be used to select disease levels for testing efficacy, and might prove useful in expressing or predicting efficacy in trials conducted under different conditions.

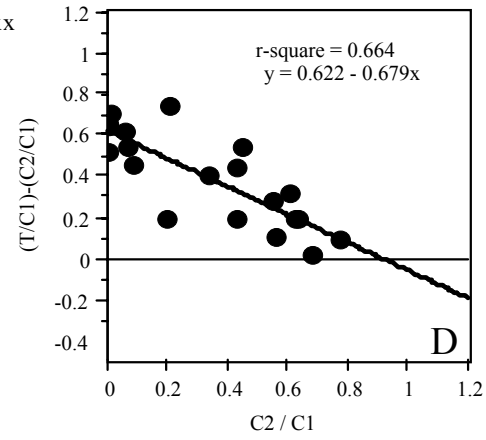
Pythium

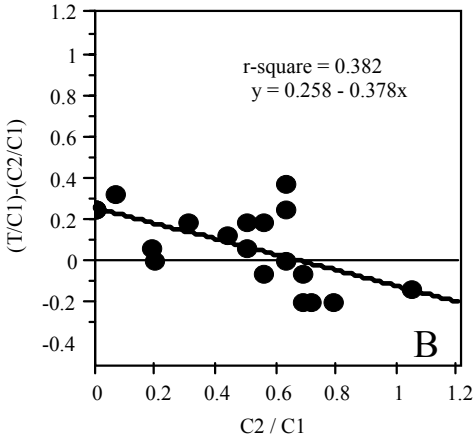
Stand, Day 21

Fresh shoot weight, Day 21

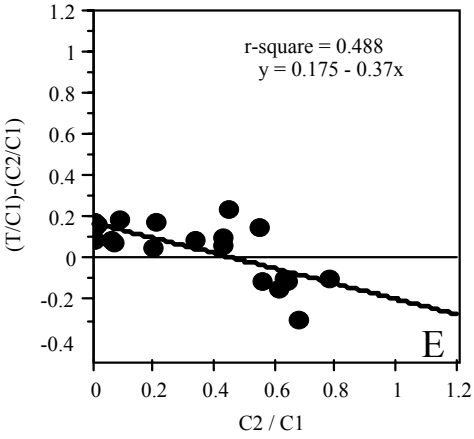


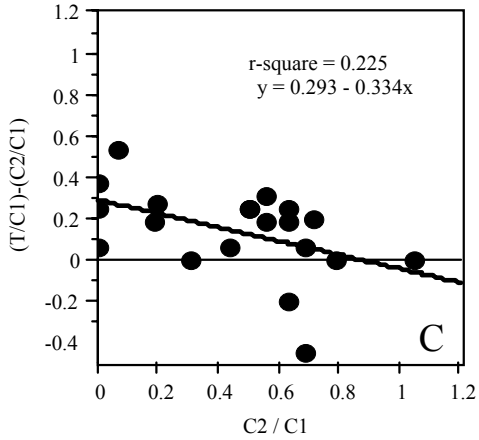

Apron Maxx RTA



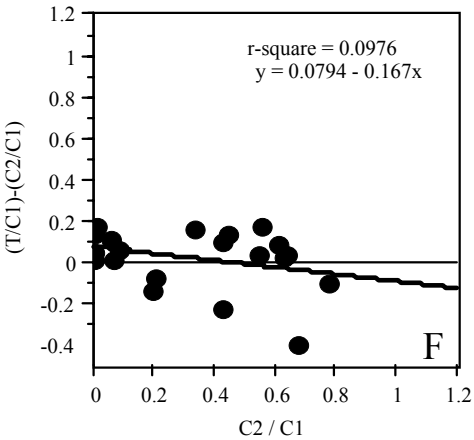




ICIA5504




Crown



# IDENTIFICATION AND MAPPING BEAN ROOT ROT RESISTANCE IN A POPULATION OF MESOAMERICAN X ANDEAN ORIGIN[2]

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## Introduction

Processing snap beans (*Phaseolus vulgaris* L.) is an important industry in the Midwest with over 100,000 acres of production in Wisconsin, Illinois and Michigan in 2001 (USDA, 2002). The production restraints are primarily attributed to weather and pest problems, among the most important of which is root rot. The bean root rot disease complex is caused by several organisms from the genera *Aphanomyces*, *Fusarium, Pythium* and *Rhizoctonia*. Resistance is the best long-term solution to the root rot disease complex. To search for sources of resistance our lab is characterizing germplasm of both Mesoamerican and Andean origin.

## Materials and Methods

**Plant Material and Design:** During the summers of 2001 and 2002, we carried out field experiments at the Hancock, WI Agricultural Research Station (ARS). We evaluated three replicates of 81 recombinant inbred lines (RIL) derived from a cross between the root rot susceptible snap bean cultivar 'Eagle' and 'Puebla 152', a root rot resistant, black seeded, Mesoamerican dry bean variety. The plot size consisted of 22 plants planted in 1.52m rows separated 0.91 m apart. The test site has a history of 11 years consecutive snap bean production thus maintaining root rot pressure mainly associated with the *Pythium* and *Aphanomyces* genera.

**Disease Evaluation:** Root rot severity was measured using a 1 to 9 scale (1= no symptoms, 9=extreme severity). Among the variables taken into account were initial plant stand, number of dying plants two weeks after germination and foliage chlorosis. Plants were evaluated for vigor 17 day after planting (dap) and again at flowering.

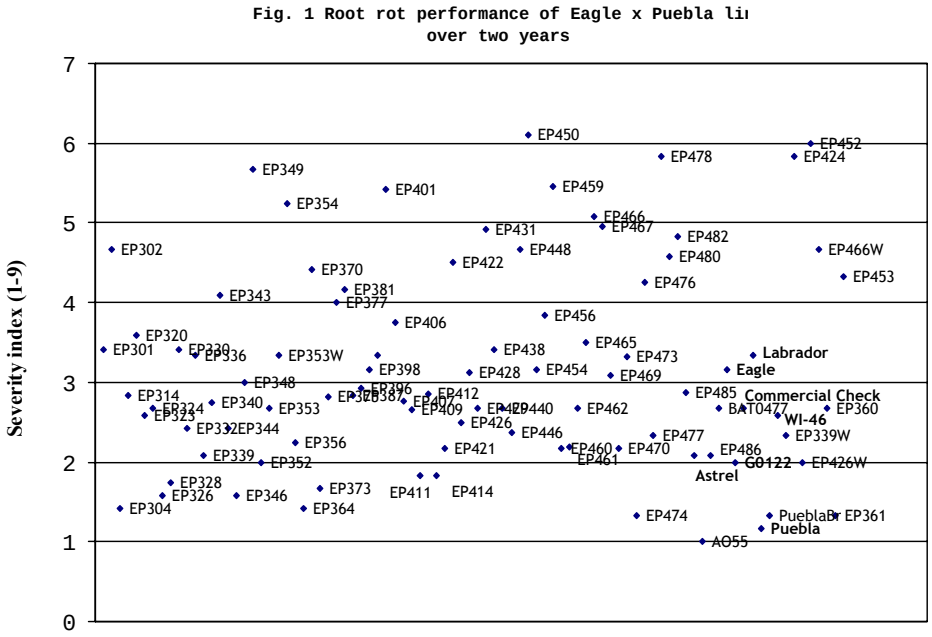
**Marker Analysis:** A Random Amplified Polymorphic DNA (RAPD) map including 364 markers ordered in 11 linkage groups, spanning 852.8cM (Skroch et al., 1996) was used for the search of QTL associations to root rot. Linkage between marker and QTL was analyzed using QTL Cartographer (Basten et al. 2000). Composite interval mapping was set to scan every 2 cM, using a LOD score threshold of 2.5.

## Results and Discussion

Root rot conditions were highly conducive in 2001 and favorable in 2002. The mean performance of the 81 lines and checks tested at Hancock is illustrated in Figure 1. Several Eagle x Puebla RIL showed consistent root rot resistance (< 2 severity index) over the two years tested (Fig. 1). High root rot resistance was also shown for Puebla in 2002 as previously reported (Navarro and Nienhuis, 2002).

The W13.850 RAPD marker was consistently associated over both years with root rot resistance with LOD scores of 3.99 and 3.02 for 2001 and 2002 respectively. Other markers associated to root rot resistance in 2001 were S18.1500 (LOD score 3.89) and U12.500 (LOD score = 4.55). For 2002, other markers associated to root rot included F08.1250 (LOD score = 5.75), O10.650 (LOD score = 3.55) and R02.1400 (LOD score 3.44). These marker associations to root rot will be further confirmed so they can be used for marker facilitated selection programs.

[2] 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.




Based in the results of this experiment over two years, several EP lines can be identified which represent a range of compromise between root rot resistance, plant type and pod quality (data not shown). Lines with lesser resistance but good quality could be used to introgress QTL associated to a lesser degree of root rot resistance if speed is the primary objective. In contrast, if a higher level of root rot resistance is desired, lines with high levels of resistance could be used; however, it is anticipated that snap bean quality would be achieved more slowly.

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# VARIABILITY OF *FUSARIUM* sp. ISOLATES INFECTING COMMON BEANS IN AGUASCALIENTES, MÉXICO[1]

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In Aguascalientes, México, common bean (*Phaseolus vulgaris* L.) production is affected by root rot incidences caused by fungi as *Fusarium* sp. *Fusarium* increase the percentages of seedling death and reduce the crop stand. These negative effects reduce grain yields and decrease crop profits. The high variability of *Fusarium oxysporum* has been characterized by different methodologies as morphology, symptomatology, epidemiology and pathogenicity tests. However, these thechniques are time consuming, labor intensive, and subject to varying environmental conditions (5). Molecular marker methodologies could overcome these limitations and will provide additional information for fungal characterization in order to improve the knowledgement of the genetic composition of the pathogen populations. This work was conducted to characterize pathogenicity and AFLP genotype of *Fusarium* isolates from Aguascalientes, México.

Plants infected with root rots were collected through different locations of Aguascalientes, México. Samples were desinfested with NaOCl 2 % and then cultured on potato-dextrose-agar medium acidified with lactic acid. Petri dishes were incubated for 7 days at 28 °C. Sixteen isolates of *Fusarium* were obtained from Aguascalientes and one isolate from the following states: México, Guanajuato, and Veracruz were included. The pathogenicity of isolates was evaluated on 10 common bean cultivars (Azufrado Tapatío, Bayo Mecentral, Bayo Durango, Pinto Villa, Pinto UI-114, SEQ 12, BAT 477, TLP 19, Negro 8025, Rio Tibagi). Treatments (isolate x cultivar) were randomized on a completely randomized design with two replications. Experimental unit was 10 seeds growing on Petri dishes with 8 days-old *Fusarium* isolates, and then incubated for 4 days to 28 °C. At fourth day, root rot severity was evaluated using an arbitrary scale with five values (1 to 5), where 1=< under 20 % 2=21-30, 3=31-50, 4=51-80, and 5=> above 80 % of seed coat and/or roots infected by the fungus. DNA of each isolate was obtained (3) and AFLP was based on Vos *et al.* (4). AFLP products were separated by electrophoresis on 6 % acrylamide gels and revealed by silver nitrate staining (PROMEGA$^{R}$). Data from pathogenic and genetic analyses were subjected to cluster analysis using UPGMA method and SAS 6.12 and Statistica 6.0 softwares.

Most of common bean cultivars tested were susceptible to the majority of *Fusarium* isolates. Rio Tibagí, BAT 477 and SEQ 12 were resistant to 8 or more isolates; while Bayo Durango, Azufrado Tapatío and Pinto Villa were resistant to three or less isolates (Table 1). Cluster analysis of *Fusarium* isolates on basis of pathogenicity data in 10 common bean cultivars showed two major groups, one that included AGS05, AGS14, AGS10, AGS15, MEX01, and VER01 while the other included 13 isolates (Fig. 1a). Isolates were analized by using four combinations of AFLP oligonucleotides. Only 1.3 % of amplified products (459) were monomorphic; while 98.7 % were polymorphic. Cluster analysis of AFLP data showed two major groups of isolates, one included 9 isolates and other included 8 (Fig. 1b). No clear association among pathogenicitiy and AFLP genotype was found, although a high genetic variability on *Fusarium* populations from Aguascalientes was detected, since similarity ranged from 70 to 95 %. In addition, no identical isolates of *Fusarium* were detected on basis of

pathogenicity or genotype. High pathogenic and genetic variability was found on *Fusarium* populations from Aguascalientes, despite the identical host (common beans). Similar results were reported in *Fusarium* (1, 5). Results indicate the high levels of genetic variability in *Fusarium*, produced by the presence of heterocaryosis and parasexualism as genetic exchange mechanisms between vegetatively compatible isolates. This research confirmed the diverse and heterogeneous nature of the genus. Further research that includes traditional and molecular methodologies will improve the knowledge and understanding of *Fusarium* biodiversity. All isolates were pathogenic on common beans. Resistant germplasm belonged to Mesoamerican race, while susceptible cultivars were classified as Durango and Jalisco races. Similar results were reported in the pathosystem *Macrophomina phaseolina*-common beans (2). Results suggest that resistance to root rot pathogens in common beans could be operating as a resistance gene cluster that controls similar strategies to defend roots and stems against root rot fungi. Further research could confirm this suggestion.

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Table 1. Resistance/susceptibility relationship of 10 common bean cultivars to 19 *Fusarium* isolates.

| Cultivar | Race | Resistance | Susceptibility |
|---|---|---|---|
| Rio Tibagi | Mesoamericana | 10 | 9 |
| BAT 477 | Mesoamericana | 9 | 10 |
| SEQ 12 | Mesoamericana | 8 | 11 |
| TLP 19 | Mesoamericana | 7 | 12 |
| Bayo Mecentral | Jalisco | 6 | 13 |
| Negro 8025 | Mesoamericana | 5 | 14 |
| Pinto UI 114 | Durango | 5 | 14 |
| Pinto Villa | Durango | 3 | 16 |
| Bayo Durango | Durango | 1 | 18 |
| Azufrado Tapatío | Jalisco | 1 | 18 |

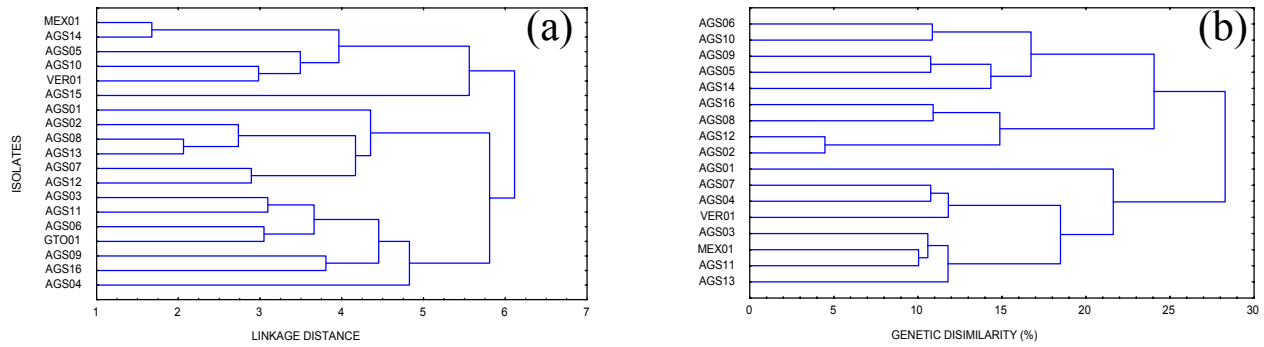


Fig. 1. Dendrograms of *Fusarium* isolates on basis of pathogenic (a) and genetic (b) analyses.

# REACTION TO ROOT ROT PATHOGENS OF COMMON BEAN GERMPLASM IN AGUASCALIENTES, MÉXICO[1]

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In Aguascalientes, México drought stress affects frequently common bean production. The drought stress is frequently aggravated by high incidences of soilborne fungi as *Fusarium* sp. and *Rhizoctonia solani* (3). Both factors, alone or combined reduce grain yields due increase the percentages of seedling death and consequently reduce the crop standing. These negative effects reduce grain yields and decrease crop profits. We consider that production of common bean germplasm with combined resistance to drought stress and diseases could be an appropriate strategie for grain yield improvement. This research was proposed in order to characterize the reaction to root rot pathogens of common bean germplasm under field conditions.

Experiments were divided in two sets. The first set consisted on two experiments: Experiment I (E-I)=70 genotypes and experiment II (E-II)=36, both cultivated under rainfed conditions at Sandovales, Aguascalientes (22° 09' N, 102° 18' W, 2000 masl), and established in june 27 (E-I), and july 11 (E-II), 2001. Germplasm of E-I was separated on three groups of genotypes based on color seed coat: 25 pinto, 25 Flor de Mayo, and 20 black. Each group was randomized on RCB design with three replications. E-II included 36 common bean genotypes randomized on 6x6 lattice design with three replications. In both experiments, experimental unit (EU) was 2 rows 6 m long. The second set included 49 genotypes evaluated under two levels of soil moisture at Sandovales (june 25, 2001) and Chapingo, México (july 16, 2002; 19° 28' N, 98° 52' W; 2250 masl). Germplasm was randomized on 7x7 lattice design with four replications. Two replications received supplemental irrigation, while the other two were conducted under rainfed conditions (irrigation was stopped when the most of germplasm initiated flowering until harvest). EU at Chapingo was 2 rows 5 m long, while at Sandovales was 1 row 5 m long. In both sets of experiments root rot severity ratings were determined at 28 and 56 das. Five plants were randomly picked off from each experimental unit and root rot severity was evaluated by using a 1-9 scale (1). Fungi associated to root rots were identified at laboratory. Days to flowering and to maturity were registered in each EU. At harvest, grain yield (kg $h^{-1}$) was registered at all experiments. Data were subjected to ANOVA, and LSD test (P=0.05)) was used for mean comparisons.

*Fusarium* sp. and *R. solani* were found to be associated frequently (88 % of the samples) to root rots of common beans at both locations, as been reported by others (2, 5). None genera were found to be associated to any cultivar in each location. A high variability on reactions to root rot pathogens was found in Sandovales (E-I and E-II) but not on grain yields; therefore no clear association between root rot severity and seed yield or phenology was found (Table 1). In E-I, resistance to root rots was more frequent on black beans (Negro Otomí, Negro Altiplano, Negro 8025), while susceptibility was common on pinto genotypes (Pinto Mestizo, Pinto Zapata) (Fig. 1a). Similar results have been reported for other *Macrophomina*-bean pathosystem (4). Black beans from Mesoamerican race could be a good source of resistance to root rot pathogens of common bean in México. BAT 477, RAB 636 and SEA 20 showed high seed yields and resistance to root rot pathogens in E-II (Fig 1b). BAT 477 has showed a consistent resistance to root rot pathogens (4, 6, 7). Rainfed conditions reduced seed yields and increased root rot

severity in common bean germplasm compared to irrigated conditions, since the low water availability increase physiological stress in the host. Therefore, host defence mechanisms was not efficient for arrest fungal infection or to slow fungal pathogenesis. Seed yield and root rot incidences were greater in Chapingo than Sandovales (Table 1). In both locations, G 2494, G 4258, 97RS101 and Negro 8025 showed the lowest root rot severities and high seed yield, while G 801, SEA 5, G14538 and G 4523 were susceptible and exhibited low seed yields (data no shown).

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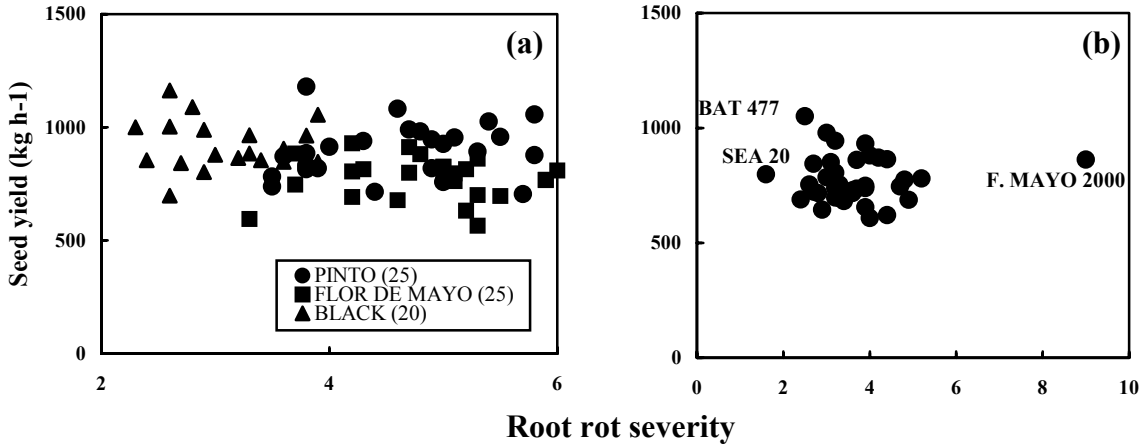


Fig. 1. Relationship between root rot severity and seed yield in 70 (E-I) (a) and 36 (E-II) (b) common bean genotypes under field conditions. Sandovales, México.

Table 1. Characteristics on common bean germplasm under rainfed-irrigated conditions.

| Experiment | Days to flowering | Seed yield (kg/h) | Root rot severity (56 das) |
|---|---|---|---|
| Sandovales | | | |
| Rainfed | 49 | 622 | 5.7 |
| Irrigated | 48 | 1141 | 4.6 |
| Chapingo | | | |
| Rainfed | 53 | 940 | 6.3 |
| Irrigated | 54 | 1730 | 5.4 |
| LSD (P=0.05) | 1 | 109 | 0.7 |

# GENETIC MODE OF RESISTANCE IN BEAN GENOTYPE MLB 49-89A TO *Pythium* ROOT ROT

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## Background

Bean root rot caused by several *Pythium* Species is relatively recent problem in east and Central Africa that is increasing in importance. Yield losses of up to 70% in popular commercial bean cultivars have been reported in Rwanda and Kenya (Buruchara and Rusuku 1992; Otsyula et al., 1980). In order to curb the effects of this disease, identification of resistance of resistance to bean genotypes preferred by resource poor small holder farmers is an important consideration.

Resistant Varieties (Table 1) have been identified and are being grown by farmers in Western Kenya southern Uganda and Rwanda. These varieties are however not of preferred market classes. Mode of inheritance of these sources is also unknown and this limits their use in breeding program. This study was undertaken to determine the nature of inheritance to Pythium root resistance in a bean genotype MLB 49-89A.

Crosses between MLB 49-89A and several popular market oriented commercial varieties grown in Kenya, Rwanda and Uganda (Table1) were made in screen house at CIAT Kawanda. Parental lines and F2 population were tested in a root rot hot spot in Vihiga district western Kenya in August 2002 in RCBD with three replications. Severity of root rot was based on root rot lesion and plant survival. (Otsyula et al., 1998) 50 plants from each plot were sampled for the parents while 150 plants were sampled F2 generations. They were scored for number of plant resistant and susceptible. $X^2$ was calculated based on actual number of planted counted (observed) and expected

Bean root rot score of F2 plants from cross from a cross of MLB 49-89A and the commercial types segregated in the ration approximately 3:1 (Table 2). This indicates that the resistant gene in MLB 49-89A is most likely a single dominant gene. Further screen house and molecular characterization involving the parents, F1, F2 and backcrosses is underway and will provide useful information for mechanism of resistance in the studied genotype. More field screening of the F2-3 families of resistant progenies derived from MLB 49-89A to check goodness of fit for the ratio 1:2:1 (resistant: segregating: susceptible) would be confirmatory for the mechanism of resistance in the cultivar that could be utilized in breeding program

**Results**

Table 1. Mean root reacrion of cultivars used in this study.

| Cultivar | Seed Type | Status |
|---|---|---|
| GLP 2 | Large Red Mottle | 8 ± 6 |
| GLP 585 | Small red haricot type | 7 ± 0.8 |
| Urugezi | Large Red Mottle | 8 ± 0.6 |
| CAL 96 | Large Red Mottle | 9 ± 0.7 |
| MLB-49/89A | Large Black | 2.5 ± 0.5 |

[x] = root rot reaction based on CIAT scale of 1 –9 where 1= resistant and 9 susceptible

Table 2. Number of plants that showed resistant and susceptible reaction and calculated Chi-square values for segregation ratios of the F2 populations derived from MLB 49-89 A

| Susceptible | Progeny | Resistant | Susceptible | Ratio tested | $X^2$ | *P* |
|---|---|---|---|---|---|---|
| | | 50 | | | | |
| GLP 2 | P2 | 6 | 44 | | | |
| GLP 585 | P2 | 8 | 42 | | | |
| CAL 96 | P2 | 3 | 47 | | | |
| URUGEZI | P2 | 4 | 46 | | | |
| GLP 2 | F2 | 103 | 29 | 3:1 | 3.05** | 0.05 – 0.10 |
| GLP 585 | F2 | 98 | 46 | 3:1 | 3.71** | 0.05 – 0.01 |
| CAL 96 | F2 | 107 | 32 | 3:1 | 1.28** | 0.10 – 0.20 |
| URUGEZI | F2 | 115 | 30 | 3:1 | 1.74** | 0.010 – 0.02 |

P2 = susceptible parents, F2 progenies

[xx] = significant value at 1 degrees of freedom, 95% confidence level and $X^2 < 3.84$

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# IN VITRO CONTROL OF Fusarium oxysporum f. sp. phaseoli WITH VEGETAL ESSENTIAL OILS.

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Plants produce and exude secondary compounds, which play an important role as defense mechanisms against different organisms. Those compounds are mainly phenols (flavonoids, tanins, alkaloids, etc.), most of the essential oils can be extracted from aromatic and medicinal plants, and could be used for biologic control against plant pathogens (4). The aim of this work was the evaluation of the activity of eleven essential oils upon the growth and development of *Fusarium oxysporum* f. sp. *phaseoli, in vitro* and *in vivo.*

**Materials and methods.**

*In vitro a*ssay. To evaluate the activity of essential oils from: mint, eucalyptus, laurel, clove, sweet marjoram, rosemary, origanum, thyme, cinnamon, pepper, and grapefruit against *Fusarium oxysporum* f. sp. *phaseoli*, three concentrations 1,250, 2,500 and 3,750 ppm were chosen. Each oil was spread on Petri dishes, after a mycelium disc of 0.7 cm was placed on the center of the dish. The same procedure was made for each oil and replicated four times. The control treatment was the mycelium without any treatment. The Petri dishes were sealed and kept into hermetic plastic bags, and incubated a 25°C during 15 days. In those treatments that allowed the growth of the fungi, the conidial density was estimated three times using a hemocytometer.

*In vivo a*ssay. To discard the potential phytotoxicity of oils over *Phaseolus vulgaris* L. seeds, those essential oils that inhibited the growth of the fungus were used on bean seeds. Using the same concentration as tested *in vitro* the bean seeds were treated by immersion into the chosen oil. Afterwards an standard germination test using sterilized paper towels and water was carried out, determining the germination percentage after 12 days of incubation at 25°C. The same procedure was made for each treatment and for the control without essential oil.

Active principles identification. Using gas chromatographer and masses spectrometer the active principles of four essential oils were identified (1,3).

**Results.**

*In vitro a*ssay. Two oils inhibited completely the growth of the fungus, four oils were fungistatic at low or media concentration, but fungicides at high concentration (Table 1). Four essential oils promoted fungus growth and development in three days. The control treatment showed a good fungus growth, but in eight days. Some essential oils treaments had influence upon the fungus reproduction at low concentration (1,250 ppm) increased the conidia number, which was higher than the same treatments at high concentration (3,750 ppm). It is possible to control *Fusarium oxysporum* f. sp. *phaseoli* with essential oils. The control is due to active principles in the essential oils, like carvacrol in origanum and thyme, menthone in mint, and eugenol in clove, which can be used alone or mixed showing the same response (1).

*In vivo a*ssay. The essential oils did not showed phytotoxicity upon bean seeds and seedlings, since germination was enhanced as compared to the control treatment and non abnormal plantlets were observed.

The use of essential oils constitute an ecologic advantage because their degradation is easier than the agrochemical compounds (2), even in plants or soil and since the demand for organic food is growing, thus some essential oils can have a benefitial impact on organic food production.

Table 1. Growth of *Fusarium oxysporum* f. sp. *phaseoli* on PDA Petri dishes with essential oils, incubated during 15 days at 25°C.

| | Concentration (ppm) | | |
|---|---|---|---|
| Essential oils | 1,250 | 2,500 | 3,750 |
| Mint | 4.72 cd + | 1.00 d | 1.00 c |
| Eucalyptus | 48.78 a | 48.78 a | 4.54 c |
| Laurel | 5.05 c | 31.17 b | 1.00 c |
| Clove | 2.38 de | 1.00 d | 1.00 c |
| Sweet marjoram | 48.78 a | 48.78 a | 1.91 c |
| Rosemary | 48.78 a | 48.78 a | 6.60 bc |
| Origanum | 1.00 d | 1.00 d | 1.00 c |
| Thyme | 1.00 d | 1.00 d | 1.00 c |
| Cinnamon | 2.14 c | 1.00 d | 1.00 c |
| Pepper | 48.78 a | 48.78 a | 12.64 b |
| Grapefruit | 48.78 a | 48.78 a | 30.78 a |
| Control | 27.16 b | 19.00 c | 28.16 a |

+ growth in cm $^2$, different letters indicate significant statistical differences ($P< 0.05$)

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# INTEGRATION OF TILLAGE, SEED TREATMENT AND INOCULATION TO DECREASE DRY BEAN ROOT ROT.

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Field experiments were conducted in 2001 and 2002 at Park Rapids and Verndale, MN on grower's fields. The sites were selected for past history of root rot (*Fusarium solani* f. sp. *phaseoli*, *Rhizoctonia solani* and *F. oxysporum*). The soil type at each site is a Verndale, sandy loam (USDA classification). The field experiments were organized as a split-plot design with six replications. Main plots were tillage treatments consisting of either a shallow chisel (RT) or deep chisel (DT) and subsoiling (SS) with DMI. The subplots were a comparison between 1) the standard seed treatment (SST) consisting of Captan + Streptomycin + Lorsban and 2) the SST with Kodiak (Bacillus subtilis GBO3) and inoculation with HiStick N/T (*Bacillus subtilis* + *Rhizobium*). Plots consisted of twelve rows, 365 m in length, 1.5 m wide with row spacing of 76 cm. Each experiment was approximately 12 $ha^{-1}$. At Park Rapids in 2201 and 2002 spring tillage consisted of DT (28 cm depth) and SS to a depth of 45 cm. At Verndale tillage in 2001 consisted of RT and SS (15 and 45 cm depth, respectively). In 2002 tillage in DT and SS was 30 and 45 cm depth respectively. Plots were sown on June 7 and on June 14 in 2001 and 2002 at Park Rapids and Verndale, respectively. Dry bean cultivar 'Montcalm' was sown at a rate of 100 Kg $ha^{-1}$.

Dry bean disease severity (DS) was decreased by Kodiak and inoculation with HiStick N/T at Park Rapids in 2001 and 2002 when compared to the SST (3.3 vs 4.5), respectively (Figure 1 and 2). Dry bean yields were lower at Park Rapids in 2002 than in 2001. Grain yield of dry bean was affected significantly by Kodiak and HiStick N/T at Park Rapids in 2001, and by both tillage and Kodiak plus HiStick N/T in 2002 (Table 1). Kodiak and HiStick N/T increased grain yield of dry beans at Park Rapids consistently in both years (Table 1).

Cone index values above 2000 Kpa were present in the upper 300 mm at Verndale after RT. At Verndale DS was not affected significantly by treatment, tillage or the interaction tillage with treatment in 2001. Kodiak and HiStick N/T resulted in a non-significant increase in dry bean yield at Verndale in 2001 (Table 2). Subsoiling also produced a non-significant yield increase of 5%. Tillage and Kodiak plus HiStick N/T decreased DS significantly in 2002. Plots with the SST alone had greater DS than the SST plus Kodiak and HiStick N/T. In Verndale dry bean yields were increased 11% by SS in 2002. Yields of plots sown with SST Kodiak and inoculated with HiStick were 10% greater than yield of plots sown with seed treated with SST alone (Table 2). At Park Rapids in both 2001 and 2002 deep tillage increased yields significantly. The effect of tillage differed between the two locations. The combined treatment of SST plus Kodiak plus HiStick increased yield consistently at both locations at both years, although the yield increase at Verndale in 2001 was not significant. Subsoiling does not always increase yield.

Fig 1. Effect of tillage and seed treatment on dry bean root disease severity observed at flowering (V4) at Park Rapids in 2001 and 2002.

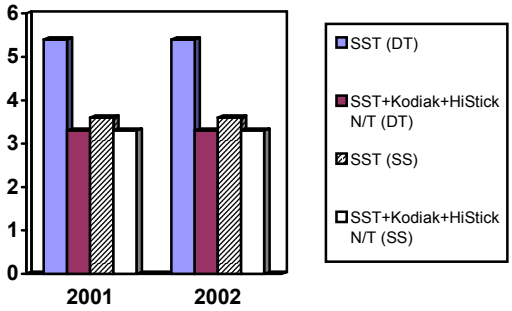


Fig 2. Effect of tillage and seed treatment on dry bean root disease severity observed at flowering (V4) at Verndale in 2001 and 2002.

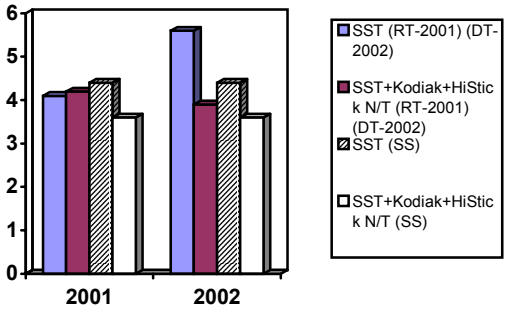


Table 1. Effect of tillage and seed treatment on dry bean yields obtained at Park Rapids in 2001 and 2002.

----------Yield (Kg/ha)----------

| | --------Park Rapids 2001---- | | | --------Park Rapids 2002------ | | |
|---|---|---|---|---|---|---|
| | ----------Tillage---------- | | | ----------Tillage---------- | | |
| Treatment | Deep Chisel (DT) 28 cm | Subsoiling (SS) 45 cm | Avg. | Deep Chisel (DT) 28 cm | Subsoiling (SS) 45 cm | Avg. |
| SST | 2,871 | 2,842 | 2,857 b† | 2,451 | 2,287 | 2,369 b |
| SST + Kodiak + HiStick N/T | 3,060 | 2,828 | 2,944 a | 2,651 | 2,472 | 2,562 a |
| Average | 2,966 | 2,835 | | 2,551 a | 2,380 b | |
| Factor | LSD | P | | LSD | P | |
| Tillage | NS‡ | 0.09 | | 117 | 0.04 | |
| Treatment | 177 | 0.03 | | 141 | 0.02 | |
| TXTreat.§ | NS | 0.09 | | 72 | 0.91 | |

† Means followed by different letter in columns or in rows are significant different at $P = 0.05$.
‡ Not significant at $P = 0.05$.
§ Tillage by treatment interaction.

Table 2. Effect of tillage and seed treatment on dry bean yields obtained at Verndale in 2001 and 2002.

----------Yield (Kg/ha)----------

| | --------Verndale 2001---- | | | --------Verndale 2002------ | | |
|---|---|---|---|---|---|---|
| | --------Tillage---------- | | | ---------Tillage--------- | | |
| Treatment | Reduced Tillage (RT) 15 cm | Subsoiling (SS) 45 cm | Avg. | Deep Chisel (DT) 30 cm | Subsoiling (SS) 45 cm | Avg. |
| SST | 2,478 | 2,703 | 2,591 | 1,621 | 1,720 | 1,670 b |
| SST+Kodiak+HiStick N/T | 2,738 | 2,809 | 2,773 | 1,743 | 1,930 | 1,836 a |
| Average | 2,608 | 2,756 | | 1,682 b† | 1,825 a | |
| Factor | LSD | P | | LSD | P | |
| Tillage | NS† | 0.16 | | 89 | 0.00 | |
| Treatment | NS | 0.10 | | 57 | 0.00 | |
| TXTreat. § | NS | 0.44 | | NS | 0.18 | |

† Means followed by different letter in columns or in rows are significant different at $P = 0.05$.
‡ Not significant at $P = 0.05$.
§ Tillage by treatment interaction.

# IDENTIFICATION OF PARTIAL RESISTANCE TO *SCLEROTINIA SCLEROTIORUM* IN FIELD AND GREENHOUSE TESTS AT MULTIPLE LOCATIONS

J. R. Steadman, Coordinator and participant, K.M. Eskridge, and K. Powers, statistical analysis University of Nebraska. Data from C. Kurowski (California), R. Mainz (Minneosta), J. Kelly (Michigan), P. Griffiths (New York), K. Grafton (North Dakota), J. Myers (Oregon), P. Miklas (Washington), and K. Kmiecik (Wisconsin).

Developing resistant cultivars to *Sclerotinia sclerotiorum*, cause of bean white mold, is the most economical disease management strategy for growers and the bean industry. However, only partial resistance has been identified to date. The objective of the study was to identify bean genotypes with broad partial resistance to white mold by testing putative sources of resistance developed by bean breeders with greenhouse methods and field sites in different states.

Field tests consisted of two rows of each entry and a common susceptible genotype resulting in a three-row plot 4.6m (15 ft) long replicated three times in a randomized complete block design. The greenhouse tests were the straw test (Petzoldt and Dickson, 1996) and detached leaf test (Steadman et al, 1997). Twelve separate tests, seven field and five greenhouse were rated. Because of the differences in these data sets, e.g., field disease severity, length of stem and number of nodes affected and area of leaf lesion, the entries were ranked from most resistant (1) to most susceptible (12) in each test (Table 1). Spearman and Pearson correlation tests were used to compare entry rankings in each test. Thirteen entries were tested using greenhouse methods - the lines listed in Table 1 and a *P. coccineus* line dwarf bees.

The highest positive correlations were CA and NE fields ($r=0.983$, $p=0.007$), NE and WI fields ($r=0.966$, $p=0.01$) and MI and WI fields ($r=0.948$, $p=0.02$). Field and greenhouse (GH) correlations of some significance were field CA and GH WI ($r=0.939$, $p=0.02$), field ND and GH NY ($r=0.894$, $p=0.045$) and field MI and GH NE ($r=0.863$, $p=0.05$).

The rankings for each test are found in Table 1. When an ANOVA was used on ranking, with each test as a block and bean genotype (entry) as a treatment, there were significant differences ($p=0.001$) among genotypes (Table 2). AN 37 (P. Miklas), G122 and Cornell 501 (P. Griffiths) had the lowest mean rank (= most resistant). When greenhouse and field tests were analyzed separately, AN 37, G122 and Cornell 501 were ranked lowest in field tests, but Cornell 501 and dwarf bees replaced AN37 in the lowest rankings from greenhouse tests. There appears to be some disease escape or avoidance in addition to some low level of partial resistance in AN 37.

## References

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Table 1. Comparison of rankings* of 12 bean lines for white mold reaction at nine locations.

| | Field | | | | | | | Greenhouse | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cultivar | ND | MI | WI | CA | WA | OR | NE | MN | WI | OR | NE | NY |
| AN 37 | 4 | 1 | 3 | 7 | 6 | 3 | 3 | 7 | 7.5 | 5 | 12 | 7 |
| Beryl | 8 | 12 | 9 | 12 | 12 | 12 | 12 | 11.5 | 11 | 4 | 1 | 11 |
| Bunsi | 9 | 9 | 2 | 2 | 7 | 4 | 11 | 8 | 7.5 | 8 | 11 | 2 |
| CO75944 | 5 | 11 | 7 | 4 | 11 | 11 | 9 | 6 | 2 | 6 | 10 | 10 |
| Corn 501 | 2 | 2 | 4 | 1 | 5 | 5 | 4 | 1 | 5 | 3 | 3 | . |
| Corn 601 | 3 | 4 | 12 | 8 | 4 | 1 | 6 | 3 | 4 | 2 | 6 | 1 |
| G122 | 1 | 10 | 1 | 10 | 1 | 2 | 2 | 2 | 1 | 1 | 8 | 4 |
| G99750 | 7 | 5 | 10 | 5 | 2 | 8 | 5 | 11.5 | 6 | 10 | 5 | 9 |
| OT28724 | 12 | 6 | 8 | 6 | 3 | 7 | 8 | 9 | 9 | 12 | 9 | 3 |
| OT29657 | 11 | 8 | 6 | 3 | 10 | 9 | 7 | 5 | 11 | 9 | 2 | 5 |
| P99120 | 10 | 3 | 5 | 11 | 9 | 3 | 10 | 10 | 3 | 7 | 4 | 6 |
| USWA-6 | 6 | 7 | 11 | 9 | 8 | 6 | 1 | 4 | 11 | 11 | 7 | 8 |

*1-12 low to high disease.

Table 2. Mean ranking of bean lines for white mold reaction in 12 separate greenhouse and field tests.

| Cultivar | Mean Ranking | |
|---|---|---|
| Beryl | 11.1 | A |
| CO75944 | 8.0 | B |
| P99120 | 7.4 | B |
| OT9743-287-2-4 | 7.4 | B |
| OT9743-296-5-7 | 7.4 | B |
| G99750 | 6.7 | BC |
| Bunsi | 6.5 | B D |
| USWA-6 | 6.5 | BCD |
| Cornell 601 | 5.1 | BCDE |
| AN 37 | 4.3 | CDE |
| G122 | 3.6 | DE |
| Cornell 501 | 3.0 | E |

Means with the same letter are not significantly different.
LSD 0.05 =3.02

# PLANTING DENSITIES AFFECTING WHITE MOLD INCIDENCE AND SEVERITY, AND COMMON BEAN YIELD

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## Introduction

White mold (*Sclerotinia sclerotiorum*) is a serious disease of common beans in irrigated areas of Minas Gerais State, Brazil, during the fall-winter growing season. Beans are planted in rows spaced 0.5 m apart with 10 to 15 seeds per meter. However, some farmers use narrow row widths and/or up to 20 seeds per meter. It is known that low temperature, high humidity and wet plant canopy and/or soil surface favor pathogen spread. Therefore, wider row spacing and/or lower plant population can provide less favorable environmental conditions to white mold because of better light penetration into plant canopy and soil, and increased ventilation. Nevertheless, the effects of this technique on bean yield have not been quantified. The objective of this research was to quantify white mold intensity (incidence and severity) and bean yield using different planting densities.

## Material and Methods

A trial was installed in Viçosa, Minas Gerais State, on 26 May 2001 in a field naturally infested with sclerotia of *S. sclerotiorum*. Bean cv. Pérola (type III) was sown in rows spaced 0.5 m apart. Treatments were four levels of planting densities: 4, 8, 12, and 16 plants per meter, with or without fungicide (fluazinam). At planting, a high seedling rate was used to ensure that enough seeds would germinate. Ten days after emergence (DAE), seedlings were thinned to the desirable planting densities (initial stand). Fluazinam (0.5 L $ha^{-1}$) was applied at 45 and 55 DAE with 667 L $ha^{-1}$ of water. At 45 DAE, 40% of plants had at least one open flower. The trial was laid out on a randomized complete block design with six replications. Each plot had four 5m-long rows. Weeds were chemically controlled with metolachlor (preemergence) and after emergence with fomesafen + fluazifop-p-butyl. Insects were controlled when necessary. Weekly plants were sprinkler irrigated with a volume of 50 mm of water. An area of 1 $m^2$ of each plot was separately harvested for disease assessment and quantification of bean yield components. White mold severity was assessed using a rating scale of grades 0, 1, 2, 3, and 4 which correspond to 0, 1-25, 26-50, 51-75, and 76-100% of stem and branches area with disease symptoms, respectively. Disease incidence was calculated as the percentage of plants with symptoms on stem and branches.

## Results and Discussion

Environmental conditions were less favorable to white mold in 2001 than in the previous two years. There was no interaction between planting densities and fungicide treatments. Final stand was 2.5, 7.5, and 8.1% lower than the correspondent initial stand of 8, 12, and 16 plants per meter, respectively (Table 1). All variables were linearly related to planting densities. White mold incidence and severity increased with the number of plants per meter. Yield components and grain yield decreased as planting densities increased. Fungicide treatments did not affect white mold incidence, but disease severity was reduced by fluazinam ($P < 0.05$). Fungicide treatment increased bean yield components and grain yield ($P < 0.01$). These results indicate that

low planting densities may reduce white mold incidence and severity and increase common bean yield. White mold control was cost effective.

Table 1. White mold incidence and severity, yield components, and grain yield at four planting densities with or without fungicide (fluazinam) applications

| Treatments | Final stand | Incidence[1] | Severity | Pods/ plant | Seeds/ pod | 100-seed weight (g) | Grain yield (kg $ha^{-1}$) |
|---|---|---|---|---|---|---|---|
| **Plants per meter** | | (1) | (2) | (3) | (4) | (5) | (6) |
| 4 | 4.0 | 53.0 (59.0)[2] | 1.09 | 26.7 | 4.64 | 25.0 | 2,623 |
| 8 | 7.8 | 67.2 (79.4) | 1.53 | 17.7 | 4.48 | 23.8 | 2,612 |
| 12 | 11.1 | 72.8 (84.2) | 1.78 | 11.4 | 4.33 | 23.4 | 2,538 |
| 16 | 14.7 | 77.4 (88.7) | 2.02 | 10.3 | 4.20 | 22.3 | 2,396 |
| **Fungicide treatments** | | | | | | | |
| with | 9.5 | 65.0ns (76.2) | 1.38* | 17.9** | 4.50** | 24.3** | 2,873** |
| without | 9.4 | 70.2 (79.5) | 1.83 | 15.1 | 4.33 | 23.0 | 2,211 |

[1] Arc sine transformation. [2] Untransformed mean percentage of incidence.
** = significant at 1% level, * = significant at 5% level, ns = not significant.
(1) $y = 47.96 + 1.194x$ $r^2 = 0.92$
(2) $y = 0.850 + 0.076x$ $r^2 = 0.98$
(3) $y = 30.42 – 1.388x$ $r^2 = 0.91$
(4) $y = 4.784 – 0.037x$ $r^2 = 1.00$
(5) $y = 25.79 – 0.213x$ $r^2 = 0.97$
(6) $y = 2.731{,}1 – 18.9x$ $r^2 = 0.87$

# EARLY MATURITY AMONG COMMON BEAN LANDRACES OF THE WESTERN UNITED STATES

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The common bean was domesticated within 35$^{o}$ N and 35$^{o}$ S latitudes in the Americas. Cultivars are characterized as a short day species, meaning that most genotypes will go from seed to seed in an environment having approximately 12 or less hr of photoperiod. However, cultivation has expanded to environments with > 15 hr day lengths and >52$^{0}$N and 35$^{0}$S latitudes worldwide. Large differences for days to first flower, flowering duration, and first pod maturity occur among cultivars. Moreover, landraces occur that grow, flower, and produce seed across varying day lengths. These are often referred to as day-neutral or photoperiod insensitive. Examples of such landraces are found in the races Mesoamerica (e.g., Rabia de Gato and Pata de Zope) and Nueva Granada (Bola 60 Dias, Goiano Precose, and Jatu Rong). Similar early maturating landraces from race Durango in the Mexican highlands (e.g., 'Ojo de Cabra' and 'Ojo de Cabra Santa Rita') do not flower in the long summer days in the western U.S. Yet, Native Americans have grown dry bean in the western U.S. for thousands of years. Because wild beans are not reported from the north of "Rio Grande," the U.S. landraces were either selected from introductions or subsequently appeared from mutation and recombination.

Anasazi, great northern, pink, pinto, and red Mexican landraces occur. All have a Type III growth habit and medium seed. The latter four have typical characteristics of race Durango. Anasazi will require a closer examination to determine its race affiliation. However, despite a distinct seed type its plant, leaf, and flower are similar to race Durango. Until the advent of irrigation, landraces were grown as a dry land crop. While in some areas they may be grown under dry land or irrigation-assisted systems, in most of the western U.S. irrigated cropping predominates. High land cost, limited water availability, and increasing demand for high quality pathogen-free seed for Michigan, Minnesota, Nebraska, North Dakota, and other regions have contributed to adoption of disease resistant cultivars of increasingly more upright growth habits. Consequently, a fast disappearance of landraces has occurred.

For the past five years I have observed the performance of some landraces under well managed, 50 year-continual bean cropping, drought- and low soil fertility-stressed, and organic farming systems in Idaho. The latter were under intense competition from weeds for light, moisture, and nutrients. The landraces, in general, seem to possess high levels of tolerance to abiotic stresses, and compete favorably with weeds. Also, there are differences in days to maturity among landraces (Table 1): Common Red Mexican was 12 days latter than Common Pinto. Common Pinto was as early as LeBaron, Othello, UI 320, and others. Early maturing cultivars facilitate early availability of protein, calorie, and vitamin rich food to needy populations. Such cultivars permit growing a catch crop (i.e., a crop grown between the main crops, or before or after the main crop). They also contribute to the avoidance of abiotic and biotic stresses.

While the U.S. landraces are insensitive to photoperiod and adapted to higher latitude, what imparts 12 days earlier maturity to Common Pinto and similar cultivars compared to Common Red Mexican is not understood. Also, one could ask a broader question, what is it that facilitated the common bean production expansion into higher latitudes in the Americas and elsewhere in the world, and how could its production be expanded to yet higher latitudes? Similarly, what controls maturity differences among cultivars of similar growth habits at different elevations in the tropics and sub-tropics?

Cerna and Beaver (1990) and White and Singh (1991), among others, reported sources and inheritance of earliness among tropical and sub-tropical germplasm. Coyne (1966), Masaya and Wallace (1984), and Padda and Munger (1969) studied photoperiod, temperature, and genotype interactions and their inheritance. Earliness was controlled by partially to completely dominant alleles, and reversal of dominance was observed with a change of environments. Gu et al. (1998) reported two alleles, namely *ppd* that controlled insensitivity to photoperiod, and *Hr* enhancing its response. Could the differences at these two loci alone explain observed variations in maturity among landraces and improved cultivars across bean growing environments at higher latitudes and in the tropics and sub-tropics at different elevations? Does a multiple-allelic series occur at either locus? Are alleles at other loci also involved in controlling initiation and completion of the reproductive phase? We know that alleles *fin* controlling determinate growth habit and *ppd* are located on the linkage group B 1 (Freyre, et al., 1998). It is a common belief that alleles controlling phenological traits also are responsible for partitioning the photosynthate, and they interact with alleles at the *fin* locus affecting yielding ability of cultivars. Nonetheless, it is important to know the genetic basis of differences in maturity of genotypes such as Common Red Mexican and Common Pinto. Similarly, it is important to understand why early maturing cultivars from the tropics and sub-tropics exhibit differential response within and across varying latitudes. This knowledge should facilitate breeding for early maturing high yielding cultivars, and extend common been adaptation beyond its current limits.

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**Table** 1. Days to maturity of common bean genotypes at Kimberly, Idaho in 2002.

| ***Genotype*** | ***Days to maturity*** |
|---|---|
| Common Pinto | 87 |
| Common Red Mexican | 99 |
| LeBaron | 93 |
| Othello | 87 |
| UI 37 | 85 |
| UI 320 | 88 |

# PERFORMANCE OF THE CRANBERRY BEAN "BRS RADIANTE" IN BRAZIL

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The most popular common beans consumed in Brazil are represented by small-seeded varieties presenting, mostly, carioca or black grain types and, to a lesser extent, by the beige, pink and purple colored grain types. Some medium and large seeded beans are produced regionally and offered in local markets at higher prices. The production of these bean types, very well accepted in the international market, may contribute to diversify product availability to the Brazilian consumers as well as for exportation, in case of production surplus.

Line PR 93201472, developed at Embrapa Arroz e Feijão, belong to the cranberry bean class and is characterized by high yield potential, erect plant type, wide adaptation, earliness, good grain quality and resistant to lodging. It was released in 2002, denominated as BRS Radiante, and recommended for cultivation in the States of Goiás/Distrito Federal, Mato Grosso do Sul and Minas Gerais.

BRS Radiante was derived from a simple cross between Pompadour and Iraí. It was conducted in bulk from $F_2$ to $F_3$ generations and in $F_4$ plant population was inoculated with pathoptype 89 (alfa Brasil) of *Colletotrichum lindemuthianum*. Susceptible plants were eliminated and one pod per plant was harvested from the resistant plants to compose the following generation ($F_5$) when the same selection procedure (single pod descent) was performed to compose the F6 families. Plant selection was performed in F6 based on yield and erect plant type, originating line PR 93201472 that demonstrated superior grain yield (4.6%) as compared to the local check varieties (Table 1). In addition, BRS Radiante presents uniform grain appearance, seed weight around 43.5 g and excellent cooking and eating characteristics (Table 2).

Under artificial inoculation BRS Radiante was resistant to common bean mosaic virus and to anthracnose pathotypes: 89 (alfa Brasil); 585 (alfa Brasil TU susceptible); and 95 (capa). Under field conditions it showed intermediate reaction to rust and was tolerant to powdery mildew, but susceptible to common bacterial blight and angular leaf spot.

**Table 1.** Grain production of BRS Radiante compared to local checks (average from 14 trials) from 1997 to 1998.

| Region | State | BRS Radiante (kg ha$^{-1}$) | Checks* (kg ha$^{-1}$) | Relative yield (%) | Number of testing sites |
|---|---|---|---|---|---|
| Southeast | Minas Gerais | 2.601 | 2.559 | 101.6 | 4 |
| Central West | Goiás/Distrito Federal | 2.877 | 2.720 | 105.8 | 5 |
| | Mato Grosso do Sul | 1.697 | 1.586 | 107.0 | 5 |
| Mean | - | 2.440 | 2.332 | 104.6 | |

*Local checks: Iraí and Roxo 90.

**Table 2**. Cooking and nutritional quality of the BRS Radiante.

| Cultivar | Cooking time (minutes) | Water absorption (%) | Soluble solutes (%) | Non cracked grains (%) | Broth color | Protein (%) |
|---|---|---|---|---|---|---|
| BRS Radiante | 38,10 | 103,9 | 9,4 | 98 | Purple | 19,4 |

With a maturation period of 80 days, erect plant type and lodging resistance in a variety of cropping systems, soil types and planting seasons, according to results obtained in all trial sites, BRS Radiante presents an interesting option for producers. Genetic seed stocks are maintained by Embrapa Arroz e Feijão and basic seed is available at Embrapa Negócios para Transferência de Tecnologia.

Participating scientists and institutions are:

Embrapa Research Units: Arroz e Feijão; Milho e Sorgo; Cerrados; Negócios para Transferência de Tecnologia/ENT de Sete Lagoas and Goiânia;

State Organizations: Empresa de Pesquisa, Assistência Técnica e Extensão Rural de Mato Grosso do Sul (Empaer/MS); Agência Goiana de Desenvolvimento Rural e Fundiário (Agenciarural); Cooperativa Agropecuária da Região do Piratinga Ltda. (Coopertinga);

Universities: Universidade Federal de Viçosa; Fundação de Ensino Superior de Rio Verde (Fesurv/Esucarv).

**Reference**

Faria, L.C. de, M.J. Del Peloso, J.G.C Costa, C.A. Rava, G.E. de S. Carneiro, D.M. Soares and J.L. Cabrera Diaz. 2000. BRS Radiante: nova cultivar de feijoeiro comum com tipo de grão rajado. Goiânia, Embrapa Arroz e Feijão. (Comunicado Técnico, 45).

# PERFORMANCE OF THE BLACK BEAN "BRS VALENTE" IN BRAZIL

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The urgent demand for high yielding black bean varieties, resistant to some major diseases, with erect plant type and good grain quality, lead the breeding program of Embrapa Arroz e Feijão to develop the line LM 93204217, released as BRS Valente and recommended for planting in three main growing regions of Brazil: Central-West; Southeast; and South. These regions comprise 10 states such as Goiás/Distrito Federal, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Espírito Santo, Rio de Janeiro, São Paulo, Paraná, Santa Catarina and Rio Grande do Sul.

BRS Valente was derived from a triple cross among Emgopa 201-Ouro/Ônix/AN 512586, and conducted in bulk from F1 to F3. From F4 to F6 the populations were inoculated with *Colletotrichum lindemuthianum* pathotype 89 (alfa Brasil), and the susceptible plants were eliminated. The single pod descent method was utilized from F4 to F5 and from F6, single plant selection was applied. The line LM 93204217 was included in the adaptation and yield trials conducted in 10 states. The testing results comprising 74 experiments, are shown in Table 1.

BRS Valente possesses desirable grain appearance (size, form and 100 grain weight) and nutritional quality, as well as good cooking attributes, providing a thick and brown-purple broth (Table 2).

**Table 1.** Grain production of BRS Valente compared to local checks* (average from 74 trials) from 1995 to 2001.

| Region | State | BRS Valente (kg ha$^{-1}$) | Mean of local checks (kg ha$^{-1}$) | Relative yield[1] over the local checks (%) | Number of testing sites |
|---|---|---|---|---|---|
| | *ES* | 2.206 | 1.790 | 123,2 | 5 |
| **Southeast** | RJ | 1.946 | 1.540 | 126,4 | 7 |
| | MG | 2.998 | 2.461 | 121,8 | 6 |
| | SP | 2.464 | 2.372 | 103,9 | 23 |
| | GO/DF | 3.014 | 2.544 | 118,5 | 10 |
| **Central-West** | MS | 1.918 | 1.682 | 114,0 | 9 |
| | MT | 1.932 | 1.670 | 115,6 | 7 |
| | RS | 2.398 | 2.156 | 111,2 | 5 |
| **South** | SC | 2.161 | 1.910 | 113,1 | 9 |
| | PR | 2.382 | 1.996 | 119,3 | 13 |

*Iapar 44; Rio Tibagi; Capixaba Precoce; Serrano; Xamego; Diamante Negro; FT 120; FT Nobre; IAC Una; Macotaço; and Macanudo.

**Table 2**. Cooking and nutritional quality of the BRS Valente as compared to standard checks.

| Cultivar | Cooking time (minutes) | Water absorption (%) | Soluble solutes (%) | Non cracked grain (%) | Broth color | Protein (%) | Fiber (%) | Testa (%) |
|---|---|---|---|---|---|---|---|---|
| BRS Valente | 28,10 | 95 | 10,91 | 78 | *Clear** | 19,25 | 9,7 | 11,75 |
| FT Nobre | 28,48 | 104 | 11,05 | 70 | Clear* | 21,60 | - | 13,48 |
| Rio Tibagi | 36,00 | 102 | 12,40 | 97 | Dark | 20,00 | 12,5 | 13,10 |
| D. Negro | 34,02 | 104 | 11,20 | 97 | Clear* | 20,00 | 10,0 | 11,40 |
| Iapar 44 | 37,00 | 104 | 11,00 | - | - | - | 10,5 | - |

*Brown-purple.

Under artificial conditions BRS Valente is resistant to bean common mosaic virus and to 19 pathotypes of anthracnose. Under field conditions it presents moderate reaction to rust, common bacterial blight and angular leaf spot.

Furthermore BRS Valente has an erect plant type, resistant to lodging in any cropping system and under the different soil types and climate conditions prevalent in the testing sites. The maturation period varied between 80 and 94 days, depending on latitude and altitude.

Seed production is accomplished by Embrapa Arroz e Feijão and basic seed is commercialized by Embrapa Negócios para Transferência de Tecnologia.

Participating scientists and institutions are:

Embrapa Research Units: Arroz e Feijão; Milho e Sorgo; Cerrados; Trigo; Clima Temperado; Agrobiologia; Soja; Negócios para Transferência de Tecnologia: ENT de Ponta Grossa (PR); Sete Lagoas (MG) and Goiânia (GO);

State Organizations: Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural (Incaper); Empresa de Pesquisa Agropecuária do Rio de Janeiro (Pesagro); Empresa Mato-grossense de Pesquisa, Assistência e Extensão Rural (Empaer); Instituto da Terra (Idaterra); Agência Goiana de Desenvolvimento Rural e Fundiária (Agenciarural); Fundação Estadual de Pesquisa Agropecuária do Rio Grande do Sul (Fepagro); Instituto Agronômico de Campinas (IAC); Instituto Agronômico do Paraná (Iapar); Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (Epagri); Cooperativa Agropecuária da Região do Piratinga Ltda. (Coopertinga); Tec-Agro - Tecnologia em Agricultura Ltda;

Universities: Universidade Federal de Santa Maria (RS); Universidade Federal de Viçosa (MG).

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Del Peloso, M.J., J.G.C. Costa, C.A. Rava, G.E. de S. Carneiro, D.M. Soares, L.C. Faria, J.L. Cabrera Diaz, I.F. Antunes, E.P. Silveira and A.M. Mesquita. 2000. Feijão Preto BRS Valente: Extensão de Indicação para São Paulo, Paraná e Santa Catarina. Goiânia, Embrapa Arroz e Feijão. (Comunicado Técnico, 44).

**Sources, Genes for Resistance, and Pedigrees of 52 Rust and Mosaic Resistant Dry Bean Germplasm Lines Released by the USDA Beltsville Bean Project in Collaboration with the Michigan, Nebraska and North Dakota Agricultural Experiment Stations**

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## 25 PINTO LINES

**1988, 2 Releases: Beldak-RR-1, and -2**. Rust resistant, high yielding, type III, viny. Resistance sources and genes: BDK-RR-1 and -2 were developed for resistance to the 33 races of the rust pathogen available at the time of their release. They are resistant to all of the 90 races identified later. Their resistance is derived from Compuesto Negro Chimaltenango (CNC), Mexico 235 (*Ur-3*+), and Olathe (*Ur-6*+). The resistance in CNC has not yet been characterized but it is resistant to 83 of the 90 races of the rust pathogen maintained at Beltsville. Pedigree: BelDak-RR-1 and BelDak-RR-2 are selections from the cross Bel 4-2537/3/Bel 4-844*2//Olathe/Mexico 235. Bel 4-844 derived from the cross Fiesta*2//olathe*3/CNC and Bel 4-2537 from Fiesta*3//Olathe*3/CNC. USDA Release Note: Stavely, J. R., and Grafton, K. F. 1988. Release of two pinto dry bean germplasm lines, Beldak-rust resistance-1 and -2. U.S. Department of Agriculture and North Dakota Agricultural Experiment Station. Germplasm release Notice. 3pp. Published. Stavely, J. R., and Grafton, K. F. 1989. Registration of Beldak-rust resistance -1 and -2 dry bean germplasm. Crop Science 29(3): 834-835.

**1992, 4 Releases: BelDakMi-RR-1, -2, -3, and -4.** Rust resistant, narrow profile, upright, short vine, type II, relatively early maturing. **Resistance sources and genes:** These are the first type II pinto beans that derive their resistance to all 64 races of the bean rust pathogen available at the time of their release from PI 151388 or PI 181996 (*Ur-11*). The resistance in PI 151388 has not been characterized but it is similar to that of PI 181996. BDM-RR-1 is homozygous for the resistance genes present in PI 151388, PX-057 (*Ur-6*+, Olathe genes) and for the *bc-2*$^2$common mosaic recessive resistance gene. BDM-RR-2 is homozygous for the rust resistance genes in PI 181996 or 151388 but it is not homozygous for the second resistance gene in PX-057 that is present in BDM-RR-1. BDM-RR-3 is homozygous for the resistance genes in PI 181996 or 151388 and in PX-057. BDM-RR-4 is homozygous for the genes in PI 181996 or 151388 but has not additional rust resistance genes and it also has the *bc-1*. **Pedigree:** The pedigree of BDM-RR-1 is PX-004/4/PX-057/3/PX-010*2//Fiesta*3/PI 151388. The pedigree of BDM-RR-2 is PX-057//Bel 1-2547-1/Bel 1-2545-1. Bel 1-2547-1 was an $F_1$ plant from PX-010* 2//Fiesta* 2/PI 151388 and Bel 1-2545-1 is an $F_1$ plant from PX-010/4/86263* 2//Fiesta/PI 181996. BDM-RR-3 and BDM-RR-4 are derived from Sierra//Bel 1-2547-1/Bel 1-2545-1. **USDA Release Note**: Stavely, J. R., Grafton, K. F., and Kelly, J.D. 1992. Release of four upright, short vine pinto bean germplasm lines, BelDakMi-Rust Resistant-1, -2, -3, and -4. U.S. Department of Agriculture, North Dakota and Michigan Agricultural Experiment Stations. Germplasm release Notice. 3pp.

**1993, 5 Releases: BelDakMi-RR-5, -6, -7, -8, and -9.** Rust resistant, narrow profile, upright, short vine, type II, relatively early maturing. **Resistance sources and genes:** All five released lines derive their rust resistance to all 65 races of the rust pathogen in the Beltsville collection available at the time of their release from PI 181996 or 190078. Both PIs are sources of the *Ur-11* rust resistance gene that is effective against 89 of the 90 races presently maintained at Beltsville. BDM-RR-5, -6, and -7 are homozygous for the genes in PI 181996 (*Ur-11*), 87-039-34 (*Ur-6*+,

Olathe genes), *I*, and a recessive gene that provides partial protection of *I* against of BCMNV. The rust resistance of BDM-RR-8 and -9 is similar to that of BDM-RR-5, -6, -7, but the *Ur-11* rust resistance gene is derived from PI 190078. The BCMV resistance in BDM-RR-8 appeared diffuse green instead of local lesions and no systemic symptoms with NL3, plus apparent immunity to other strains but that of BDM-RR-9 is similar to that of BDM-RR-5, -6, -7. **Pedigree:** BDM-RR-5, BDM-RR-6, and BDM-RR-7 are derived from 87-039-34*2/Bel 2-2193. Bel 2-2193 is derived from P86263/4/Sierra/3/P86263//Fiesta*2/PI 181996. BDM-RR-8 and BDM-RR-9 are derived from 87-039-34/4/Sierra/3/PX004//Sierra/PI 190078. **USDA Release Note**: Stavely, J. R., Grafton, K. F., and Kelly, J.D. 1993. Release of five upright, short vine pinto bean germplasm lines, BelDakMi-Rust resistant –5, -6, -7, -8, and -9. U.S. Department of Agriculture, North Dakota and Michigan Agricultural Experiment Stations. Germplasm release Notice. 3pp.

**1995, 1 Release: BelDakMi-RMR-10.** Rust and mosaic resistant, high yielding, upright, short vine, type II. **Resistance sources and genes:** This is the first released pinto line to combine resistance from PI 190078 (*Ur-11*) to all 66 available races of the rust pathogen maintained at Beltsville at the time of their release and one of the rust resistance genes in Olathe (*Ur-6*), with the homozygous recessive *bc-u* and *bc*-$2^2$genes and the homozygous dominant *I* gene that together provide resistance to all strains of bean common mosaic virus (BCMV) and bean common mosaic necrosis virus (BCMNV). The resistance sources are PI 190078 (*Ur*-11), 87-039-34 (Ur-6, Olathe gene), and 92US-1006 (*bc-u, bc-*$2^2$, and *I*). **Pedigree:** BDM-RMR-10 has the pedigree 92 US-1006/8/88-011-03*2/6/Aztec/5/87-039-34*2/3/PX010//Fiesta/PI 190078. **USDA Release Note**: Stavely, J. R., Grafton, K. F., Kelly, J.D. and Silbernagel, M.J. 1995. Release of BelDakMi-RMR-10 erect, short vine, rust and mosaic resistant pinto bean germplasm. Department of Agriculture, North Dakota and Michigan Agricultural Experiment Stations. Germplasm release Notice. 3pp.

**1997, 3 Releases: BelDakMi-RMR-11, -12, and -13.** Rust and mosaic resistant, high yielding, upright, short vine, type II. **Resistance sources and genes:** These are the first pinto beans to combine resistance from PI 190078 (*Ur-11*) to 86 of 87 races of bean rust and the *Ur-6* rust resistance gene that is in Olathe, with homozygous recessive *bc-3* gene that provides resistance to all known strains of BCMV and BCMNV. The source for *Ur-6*, *bc*-3 and type II growth habit was pinto line P94232 from J. D. Kelly. None of these lines contain the dominant allele of the *I* gene for resistance to many strains to BCMV. **Pedigree:** The pedigree of BMD-RMR-11 (tested as 4-12223), BDM-RMR-12 (tested as 4-12228), and BDM-RMR-13 (tested as 4-12255) is 94232*2/8/92BR3-1084B/7/92 BR-1006/6/88-011-03*2/5/Aztec/4/87-039-34*2//3/PX010//Fiesta/PI 190078. **USDA Release Note**: Stavely, J. R., Grafton, K. F., and Kelly, J.D. 1997. Release of BelDakMi-RMR-11, -12, and -13 erect, short vine, rust and mosaic resistant pinto bean germplasm lines. Germplasm release Notice. 3pp.

**1998, 1 Releases: BelDakMi-RMR-14.** Rust and mosaic resistant, high yielding, upright short vine, type II. **Resistance sources and genes:** This is the first released bean to combine the *Ur-3*, *Ur-6* and *Ur-11* genes for rust resistance that provide resistance to all 90 races of the rust pathogen maintained at Beltsville and the *bc-3* and *I* for resistance to all strains of BCMV and BCMNV. PI 190078 is the source of U*r-11* that is effective against 89 of the90 races of the rust pathogen. The single race for which *Ur-11* is not effective (race 108) is controlled by *Ur-3* that is effective against 45 of the other races. Michigan pinto bean P94232 and Kodiak are the sources of *Ur-3*, *Ur-6*, and type II growth habit. Line P94232 is the source of *bc-3* and several pinto parents were the sources of *I*. **Pedigree:** The pedigree of BDM-RMR-14 is Kodiak/9/P94232*2/8/92 BR-3-1084B/7/92 BR3-1006B/6/88-011-03*2/5/ Aztec/4/87-039-34*2/3/P0X10//Fiesta/PI190078. **USDA Release Note**: Stavely, J. R., Grafton, K. F., and Kelly, J.D. 1998. Release of BelDakMi-

RMR-14 erect, short vine, rust and mosaic resistant pinto bean germplasm line. U.S. Department of Agriculture, North Dakota and Michigan Agricultural Experiment Stations. Germplasm release Notice. 3pp

**1999, 4 Releases; BelDakMi-RMR-15, -16, -17, and –18.** Rust and mosaic resistant, high yielding, upright short vine, type II. **Resistance sources and genes:** All four lines are resistant to all 90 races of the rust pathogen maintained at Beltsville and to all strains of BCMV and BCMNV. BDM-RMR-15, -16, -16 and -17 combine the *Ur-3*, *Ur-6*, and *Ur-11* rust resistance genes with the *bc-3* and *I* mosaic resistance genes. BDM-RMR-18 is the first bean of any class to have four genes for rust resistance. This is the first bean to combine the *Ur-4* rust resistance gene with *Ur-3*, *Ur-6* and *Ur-11*. BDM-RMR-18 also has *bc-3* and *I*. BDM-RMR-15, -16, -16, -17 and -18 have the same sources of rust, BCMV and BCMNV resistance as BDM-RMR-14. The source of the *Ur-4* rust resistance gene in BDM-RMR-18 is the great northern line BelMiNeb (BMN)-RMR-3 into which *Ur-4* had been introgressed from BelMiDak(BDM)-RR-2 navy line. The source of *Ur-4* in BDM-RR-2 was Early Gallatin. The single race of the rust pathogen for which *Ur-11* is not effective, is controlled by *Ur-3* in BDM-RMR-15, -16, and -17 and by *Ur-3* and *Ur-4* in BDM-RMR-18. **Pedigree:** BMD-RMR-15, BDM-RMR-16, and BDM-RMR-17 were selected from bulked $F_5$ generation seed from successive generations of single plant selections from different $F_3$ plants of line 3-7449 that had *Ur-6* and *Ur-3* recombined with *Ur-11*. The pedigree of 3-7449 and these lines is Kodiak/9/P94232*2/8/92 BR-3-1084B/7/BR3-1006/6/88-011-03*2/5/ Aztec/4/87-039-34*2/3/P0X10//Fiesta/PI190078. BDM-RMR-18 is from crossing an $F_5$ plant from a sib of the 3-7449 parents of BDM-RMR-15, -16, and -17 with a selected plant of line BMN-RMR-3 that has *Ur-4*, *Ur-11*, *bc-3* and *I* and the pedigree G94567/4/G91213*2/3/Starlight*2//Alpine*3/BelMiDak-RR-2. The pedigree of BDM-RR-2 is Mayflower/4/4-5753/3/Mayflower//NX-040/PI 181996. The pedigree of 4-5753 is C20*5/Early Gallatin. **USDA Release Note**: Stavely, J. R., Grafton, K. F., and Kelly, J.D. 1999. Release of BelDakMi-RMR-15, -16, -17, and -18 erect, short vine, rust and mosaic resistant pinto bean germplasm lines. U.S. Department of Agriculture, North Dakota and Michigan Agricultural Experiment Stations. Germplasm release Notice. 3pp.

**2003, 5 Releases; BelDakMi-RMR-19, -20, -21, -22, and –23.** Rust and mosaic resistant, high yielding, upright short vine, type II. **Resistance sources and genes:** BDM-RMR-19, -20, -21, -22, and -23 are resistant to all 90 races of the rust pathogen maintained at Beltsville and to all strains of BCMV and BCMNV. These five lines combine the *Ur-4* rust resistance gene with *Ur-3*, *Ur-6* and *Ur-11* and the *bc-3* and *I* genes for mosaic resistance. BDM-RMR-19, -20, -21, -22, and -23 have the same sources of rust, BCMV and BCMNV resistance as BDM-RMR-18. **Pedigree:** The pedigree of BMD-RMR-19 (Tested as 5-2455) is 5-3352//5-3374/BMN-RMR-3. The pedigree of both 5-3352 and 5-3374 is Kodiak/9/P94232*2/8/92 BR-3-1084B/7/BR3-1006/6/88-011-03*2/5/ Aztec/4/87-039-34*2/3/P0X10//Fiesta/PI190078. BDM-RMR-20 (Tested as 6-2285), BDM-RMR-21 (tested as 6-2073), BDM-RMR-22 (tested as 6-2188), and BDM-RMR-23 (tested as 6-2149) are derived from crossing an $F_5$ plant from a sib $F_3$ of the 3-7449 parents of BDM-RMR-15, -16, and -17 (with *Ur-6* and *Ur-3* recombined with *Ur-11* and *bc-3 and I*) with a selected plant of great northern line BMN-RMR-3 with *Ur-4*, *Ur-11*, *bc-3* and *I* and the pedigree 94567/4/G91213*2/3/Starlight*2//Alpine*3/BelMiDak-RR-2. The pedigree of BDM-RR-2 is Mayflower/4/4-5753/3/Mayflower//NX-040/PI 181996. The pedigree of 4-5753 is C20*5/Early Gallatin. **USDA Release Note:** Pastor Corrales, M. A., Grafton, K. F., and Kelly, J.D. 2003. Release of BelDakMi-RMR-19, -20, -21, -22, and - 23 erect, short vine, rust and mosaic resistant pinto bean germplasm lines. U.S. Department of Agriculture, North Dakota and Michigan Agricultural Experiment Stations. Germplasm release Notice. 3pp.

## 15 GREAT NORTHERN LINES

**1988, 2 Releases: Belneb-RR-1, and -2.** Rust, common and halo blight, and common mosaic resistant, high yielding, type 3, viny. **Resistance sources and genes**: These were the first great northern dry beans developed for resistance to the 33 races of the rust pathogen available at the time of their release. The sources of rust resistance are B-190 (*Ur-5*), Olathe (*Ur-6+*) and GN 1140 (*Ur-7*). GN Harris is a source of common bacterial and halo blight resistance. Belnebe-RR-1 and –2 are homozygous for *bc-1*$^2$ and *bc*-2$^2$ genes that condition resistance to BCM and BCMNV. **Pedigree:** Belneb-RR-1 and -2 were developed by crossing B-190 with GN 1140 and then backcrossing a rust resistant $F_1$ plant, in succeeding generations, with GN 1140, Olathe, and Harris as a recurrent parent. The pedigree is GN Harris*3/6/Olathe/3/GN1140//B190/GN1140. **USDA Release Note**: Stavely, J. R., Steadman, J. R., and Coyne, D.P. 1988. Release of two great northern dry bean germplasm lines, Belneb-Rust Resistant-1 and –2. U.S. Department of Agriculture and Nebraska Agricultural Experiment Station. Germplasm release Notice. 3pp. **Published.** Stavely, J. R., Steadman, J. R., Coyne, D. P., and Lindgren, D. T. 1989. Belneb Rust Resistance-1 and -2 Great Northern Dry Bean Germplasm. HortScience 24(2): 400-401.

**1993, 2 Releases: BelMiNeb-RR-1 and -2.** Rust resistant**,** high yielding, erect and narrow profile, short vine (type II), medium maturing. **Resistance sources and genes**: These are the first great northern beans to derive their resistance to all 65 races of the rust pathogen maintained at Beltsville at the time of their release, from PI 181996 (*Ur-11*). Early Gallatin was the source of *Ur-4*. BMN-RR-1 is homozygous for *Ur*-11, *Ur-4*, *I* and a recessive *bc* gene that provides partial protection to *I* gene against strains of BCMNV. BMN-RR-2 is homozygous for *Ur*-11; does not contain *Ur-4* or *I*, but it is resistant to Western, NY 15, and Mexican strains of BCMV. **Pedigree:** BMN-RR-1 and BMN-RR-2 have the same pedigree: Alpine*3/BelMiDak-RR-2. BMD-RR-2 is a selection from the cross Mayflower/4/4-5753/3/Mayflower//NX 040/PI 181996. The pedigree of Beltsville line 4-5753 is C20*5/Early Gallatin. **USDA Release Note**: Stavely, J. R., Kelly, J. D. Steadman, J. R., Coyne, D.P., and Lindgren, D. T. 1993. Release of two erect, short vine, great northern bean germplasm lines, BelMiNeb-Rust Resistant-1 and -2. U.S. Department of Agriculture, Michigan and Nebraska Agricultural Experiment Stations. Germplasm release Notice. 3pp.

**1996, 1 Release: BelMiNeb-RMR-3.** Rust and mosaic resistant, high yielding, upright short vine (type II). **Resistance sources and genes**: This is the first released great northern bean to combine resistance to all 87 races of the bean rust fungus, maintained at Beltsville at the time of their release, with resistance to all strains of BCMV and BCMNV. PI 1818996 is the source of U*r-11* that is effective against all but one race of the rust pathogen maintained at Beltsville. The single race for which *Ur-11* is not effective (race 108) is controlled by *Ur-4*. This gene is many snap beans cultivars and was introgressed from snap bean Early Gallatin to navy lines BelMiDak-RR-1, -2 and others released in 1991. BMN-RMR-3 is also homozygous for *bc-3* and *I.* Michigan great northern line G94567 has type II growth habit and was the source of the *bc-3* and *I* for mosaic resistance. **Pedigree:** G94567/8/G91213*2/6/Starlight*2/4/Alpine*3/BelMiDak-RR-2. See the pedigree of BMD-RR-2 in BMN-RR-1 and -2. **USDA Release Note**: Stavely, J. R., Kelly, J. D. Steadman, J. R., Coyne, D.P., and Lindgren, D. T. 1996. Release of BelMiNeb-RMR-3, erect, short vine rust and mosaic resistant great northern bean germplasm. U.S. Department of Agriculture, Michigan and Nebraska Agricultural Experiment Stations. Germplasm release Notice. 3pp.

**1998, 2 releases: BelMiNeb-RMR-4, and –5.** Rust and mosaic resistant, high yielding, upright short vine, type II, early maturing. **Resistance sources and genes**: These are the first great northern beans to combine three major genes that provide resistance to all 89 races of the rust pathogen and two genes for resistance to all strains of BCMV and BCMNV. The sources of resistance are PI 1818996 (*Ur*-11), Early Gallatin (*Ur-4*), G94567 (*Ur-6* and *I*) and G91213 (*bc-$1^2$*). **Pedigree:** The pedigree of both lines is G94567*2/4/G91213*2/3/Starlight*2/4//Alpine*3/BelMiDak-RR-2. **USDA Release Note**: Stavely, J. R., Kelly, J. D. Steadman, J. R., Coyne, D.P., and Lindgren, D. T. 1998. Release of BelMiNeb-RMR-4 and -5 erect, short vine rust and mosaic resistant great northern bean germplasm lines. U.S. Department of Agriculture, Michigan and Nebraska Agricultural Experiment Stations. Germplasm release Notice. 3pp.

**1999, 2 Releases: BelMiNeb-RMR-6 and –7.** Rust and mosaic resistant, high yielding, upright short vine, type II. **Resistance sources and genes:** Both lines are resistant to all 90 races of the rust pathogen maintained at Beltsville an to all strains of BCMV and BCMNV. BMN-RMR-6 has the same genes for rust and mosaic resistance that are in BMN-RMR-4 and -5. BMN-RMR-7 is the first released great northern bean to combine the *Ur-3* with the *Ur-4* and *Ur-11* rust resistance genes. The single race (108) of the rust pathogen for which *Ur-11* is not effective, is controlled by Ur-4 in BMN-RMR-6 and -7, as well as by Ur-3 in BMN-RMR-7. BMN-RMR-6: PI 1818996 (*Ur*-11), Early Gallatin (*Ur-4*); G91213 (*bc-$1^2$*) and *I*. BMN-RMR-7: PI 181996 and PI 190078 (*Ur*-11), Kodiak (*Ur-3* and *Ur-6*), Early Gallatin (*Ur-4)*, G94567 (*bc-3*) and *I.* Several parental lines were sources of *I* in BMN-RMR-6 and -7. **Pedigree:** The pedigree of BMN-RR6 is: G94567*2/4/G91213*2/3/Starlight*2//Alpine*3/BelMiDak-RR-2. BMN-RMR-7 is derived from crossing an $F_5$ pinto plant homozygous for the recombined *Ur-3* and *Ur-11* genes with pollen from great northern germplasm release BMN-RMR-3. The pedigree of the pinto parent is Kodiak/9/P94232*2/8/92 BR-3-1084B/7/BR3-1006/6/88-011-03*2/5/ Aztec/4/87-039-34*2/3/P0X10//Fiesta/PI190078. See the pedigree of BMN-RMR-3 above. **USDA Release Note**: Stavely, J. R., Kelly, J. D. Steadman, J. R., Coyne, D.P., and Lindgren, D. T. 1999. Release of BelMiNeb-RMR-6 and -7 erect, short vine, rust and mosaic resistant great northern bean germplasm lines. U.S. Department of Agriculture, Michigan and Nebraska Agricultural Experiment Stations. Germplasm release Notice. 3pp.

**2003, 6 Releases; BelMiNeb-RMR-8, -9, -10, -11, -12, and –13.** Rust and mosaic resistant, high yielding, upright short vine, type II. **Resistance sources and genes:** These are the first great northern bean lines to combine four genes for resistance to the common bean rust pathogen with two genes for resistance to BCMV and BCMNV. These lines are resistant to all known races of the bean rust pathogen and all strains of bean common mosaic (BCMV) and bean common mosaic necrosis (BCMNV) viruses. The sources of resistance are PI 181996 and 190078 (*Ur-11*), BDM-RR-2 (*Ur-4*, derived from Early Gallatin), Kodiak (*Ur-3*, *Ur-6)*, P94232 (*bc-3*), pinto parents (*I*). **Pedigree:** BMN-RMR-8, -9-10, -11, -12, and -13 were selected from bulked $F_5$ generation seeds derived from crossing an $F_5$ pinto plant homozygous for *Ur-6* and *Ur-3* recombined with *Ur-11* genes and for *bc-3* and *I* with pollen from a selected plant of the great northern germplasm released line BMN-RMR-3 that has *Ur-4*, *Ur-11*, *bc-3* and *I*. The pedigree of the $F_5$ pinto parent used in this cross to produce BMN-RMR-8, -9-10, -11, -12, and -13 is: Kodiak/9/P94232*2/8/92 BR-3-1084B/7/BR3-1006B/6/88-011-03*2/5/ Aztec/4/87-039-34*2/3/P0X10//Fiesta/PI 190078. The pedigree of BMN-RMR-3 is G94567/4/G91213*2/3/Starlight*2//Alpine*3/BMD-RR-2. The pedigree of BMD-RR-2 is Mayflower/4/4-5753/3/Mayflower//NX 040/PI 181996. The pedigree of 4-5753 is C-20*5/Early Gallatin. **USDA Release Note:** Pastor Corrales, M. A., J. R., Kelly, J. D. Steadman, J. R., Coyne, D.P., and Lindgren, D. T. 2003. Release of BelMiNeb-RMR-8, -9, -10, -

11, -12, and -13 erect, short vine, rust and mosaic resistant great northern bean germplasm lines. U.S. Department of Agriculture, Michigan and Nebraska Agricultural Experiment Stations. Germplasm release Notice. 3pp.

## 12 NAVY LINES

**1991, 7 Releases: BelMiDak-RR-1, -2, -3, -4, -5, -6, and –7.** Rust resistant, high yielding, erect, narrow profile, short vine, type II, relatively early maturing. **Resistance sources and genes:** BelMiDak-RR-1, -2**:** Bel 4-5753 (*Ur-4* from Early Gallatin), PI 181996 (*Ur-11*). BelMiDak-RR-3, -5, -6, and -7: PI 181996 (*Ur-1)* and *I.* BelMiDak-RR-4: *Ur-11* (PI 181996). **Pedigree:** The pedigree of BMD-RR-1, BDM-RR-2, and BMD-RR-3 is Mayflower/4/4-5753/3/Mayflower//NX-040/PI 181996. The pedigree of BMD-RR-4 is: Mayflower*3//NX-040/PI 181996. The pedigree of BMD-RR-5 and BMD-RR-6 is NX-040/4/4-5753/3/Mayflower//NX-040/PI 181996. The pedigree of BelMiDak-RR-7 is 4-5753*2/3/Mayflower//NX040/PI 181996. **USDA Release Notice**: Stavely, J. R., Kelly, J. D., and Grafton, K. F. 1991. Release of seven erect, short vine navy bean germplasm lines, BelMiDak-Rust Resistant-1, -2, -3, -4, -5, -6, and -7. U.S. Department of Agriculture, Michigan and North Dakota Agricultural Experiment Stations. Germplasm release Notice. 3pp. **Published.** Stavely, J. R., Kelly, J. D., and Grafton, K. F. 1994. BelMiDak-Rust Resistant Navy Dry Beans Germplasm Lines. HortScience 29(6): 709-711.

**1993, 2 Releases: BelMiDak- RR-8 and -9.** Rust resistant**,** high yielding, erect and narrow profile, short vine, type II, relatively early maturing. **Resistance sources and genes:** Bel 4-5753 (*Ur-4* from Early Gallatin), PI 181996 (*Ur-11*), and homozygous for the I gene and for a recessive *bc* that provides partial protection of the *I* gene against strains of BCMNV. BMD- RR-9 segregating for *bc*. **Pedigree:** The pedigree of BMD-RR-8 is Northstar/BelMiDak-RR-1 and the pedigree of BMD-RR-9 is Mayflower/BelMiDak-RR-2. **USDA Release Notice**: Stavely, J. R., Kelly, J. D., and Grafton, K. F. 1993. Release of two erect, short vine navy bean germplasm lines, BelMiDak-Rust Resistant-8, and -9. U.S. Department of Agriculture, Michigan and North Dakota Agricultural Experiment Stations. Germplasm release Notice. 3pp. **Published.** Stavely, J. R., Kelly, J. D., and Grafton, K. F. 1994. BelMiDak-Rust Resistant Navy Dry Beans Germplasm Lines. HortScience 29(6): 709-711.

**1994, 2 Releases: BelMiDak-RMR-10, and -11.** Rust and mosaic resistant, high yielding, narrow profile, upright, short vine, type II, early maturing. **Resistance sources and genes:** Early Gallatin (*Ur-4*), PI 181996 (*Ur-11*); I90-302 (*bc-3* and I). Both lines released were segregating for *I*. **Pedigree:** The pedigree of BMD-RMR-10 is I 90-302*2//NX041/BelMiDak-RR-1 and of BDM-RMR-11 is I 90-302*2//Norstar/BelMiDak-RR-1. **USDA Release Notice**: Stavely, J. R., Kelly, J. D., Grafton, K. F., and Silbernagel. M.J. 1994. . Release of two erect, short vine navy bean germplasm lines, BelMiDak-Rust Resistant-10, and -11. U.S. Department of Agriculture, Michigan and North Dakota Agricultural Experiment Stations. Germplasm release Notice. 3pp.

**1995, 1 Release: BelMiDak-RMR-12.** Rust and mosaic resistant, high yielding, narrow profile, upright, short vine, type II. **Resistance sources and genes**: Early Gallatin (*Ur-4*), PI 181996 (*Ur-11*), I90302 and N93018 (*bc-3* and *I*). **Pedigree:** The pedigree of BMD-RR-12 is: N93018/4/I 90-302*2//Norstar/BelMiDak-RR-1. **USDA Release Notice**: Stavely, J. R., Kelly, J. D., Grafton, K. F., and Silbernagel. M.J. 1995. . Release of erect, short vine navy bean germplasm line BelMiDak-Rust Resistant-12. U.S. Department of Agriculture, Michigan and North Dakota Agricultural Experiment Stations. Germplasm release Notice. 3pp.

# HISTORIC BEAN VARIETY TO BOOST EARLY MATURITY FOR WESTERN CANADA

Dr. Hans-Henning Mündel

Dry Bean Breeding, Lethbridge Research Centre, P.O. Box 3000, Lethbridge, Alberta, T1J 4B1.

A dry bean that helped sustain the Lewis & Clark expedition of the 1800s has been newly registered to benefit Western Canada's booming bean industry.

Arikara Yellow is a dry bean with excellent early maturity, says bean breeder Dr. Hans-Henning Mündel of Agriculture and Agri-Food Canada's Lethbridge Research Centre. It is named after the Dakota Indian tribe that Lewis & Clark encountered during their famous expedition to explore the uncharted western U.S. "This bean provides our growers with an early maturing option," says Mündel, who produced pedigreed seed of the variety in collaboration with David Gehl of the AAFC Indian Head Research Farm. "Most importantly, it provides a valuable breeding tool to improve early maturity in our future varieties."

Arikara Yellow is the first dry bean in Canada registered to the Canario mexicano (also called Mantequilla) market class, he says. It produces a sizeable bean, slightly larger than a pinto, and is widely adapted to the Prairies. In addition to early maturity, the variety features an upright and bushy growth habit. Though it yields lower than most modern varieties, Arikara Yellow shows excellent lodging resistance and moderate white mould resistance. "What interested us most about Arikara Yellow was its earliness," says Mündel. "Most beans grown in western Canada are U.S. varieties that are sensitive to latitude and agri-ecological niches, and have late maturity for our climate. We have already started crossing Arikara Yellow with other lines to transfer this characteristic."

Arikara Yellow was first maintained by Seed Savers Exchange, a U.S.-based organization that maintains heritage varieties of horticultural and field crops. The bean was brought to Ontario in 1986, and Seeds of Diversity Canada (SoDC), Canada's heritage seed program, has since offered Arikara Yellow to home gardeners through an annual seed list. Gehl obtained the seed from a SoDC member to grow in his own garden. Noticing its early maturity, he recommended the bean to Mündel and provided the first seed for the AAFC tests.

Replicated trials of the Arikara Yellow began in 1998 and the line was advanced to the Co-operative Registration Test in 2000 and 2001. As the cultivar had no obvious group it should be compared to, different market-class checks were used in each year of testing. Arikara Yellow was finally ascribed to the Canario mexicano market-class and registered in December 2002.

In researching the heritage of Arikara Yellow, the bean was described as "exceedingly hardy, drought resistant, and an excellent baking bean."

"Cooking quality parameters are not included in the co-operative testing of beans for the Prairies," says Gehl. "For this reason we cannot claim these qualities in the marketing of Arikara Yellow."

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AGRICULTURAL RESEARCH SERVICE
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# NOTICE OF NAMING AND RELEASE OF MERLOT, A NEW, UPRIGHT, DISEASE RESISTANT SMALL-RED BEAN (*PHASEOLUS VULGARIS*, L.) CULTIVAR

The Agricultural Research Service, United States Department of Agriculture and Michigan State University announce the joint release of Merlot, a new small-red dry bean (*Phaseolus vulgaris* L.) cultivar developed to improve the seed characteristics and bring disease resistance into the small-red market class.

Merlot, breeding line No. ARS-R98026, was derived from a cross made in 1994 between ARS-R94037 and ARS-R94161. Both parents of Merlot are upright, indeterminate Type IIa growth habit, mid-to full season small-red breeding lines. $F_1$ plants from the cross were grown and self-fertilized in the greenhouse in the spring of 1995. Seven single $F_2$ plant selections from the cross were made in a nursery grown near Saginaw, Michigan in the summer of 1995. The selections were based on upright Type IIa growth habit, mid-season maturity and seed characteristics commensurate with the small-red market class. Seed was advanced in nurseries in Michigan and Puerto Rico. The $F_{4:8}$ breeding line was given the permanent code No. ARS-R98026 and entered into replicated yields trials in Saginaw, MI in 1998. ARS-R98026 was extensively tested (18 locations) in mid-Michigan over 5 seasons (1998 to 2002) and compared with the small-red cultivars (checks), Brooks, Rufus, UI 239, NW63, and Garnet. Based on its yield in the respective trials, appearance in the field, overall agronomic performance, and canning characteristics, ARS-R98026 was cited for release and given the name Merlot.

Merlot yielded 2,708 kilograms per hectare (2,418 pounds per acre) over the 18 locations and ranged from a high of 3,323 kilograms per hectare (2,967 pounds per acre) in 2000 to a low of 1,219 kilograms per hectare (1088 pounds per acre) in 2001 under severe environmental stress. Merlot out-yielded all of the checks from 3 to 23%. Merlot's yield advantage was 3% over the upright growth habit, Brooks, in 14 tests, and 6%, 7%, 10%, and 23% over the prostrate Type III cultivars, Rufus, UI 239, NW63, and Garnet in 4 to 11 tests.

Merlot combines the upright and short-vine growth habit (Type IIa) characteristic of its parents with the preferred seed size, shape, and pigmentation characteristic of the small-red market class. The strong main stem and upright growth habit gives Merlot a superior lodging resistance to the viny and prostrate growth habit of small-red commercial cultivars. Plants of Merlot average 47 centimeters in height and have the narrow profile appearance characteristic of the dry bean architype. Merlot has white flowers and blooms 45 days after planting, which is similar to other small-red cultivars. Merlot matures, on average, 93 days after planting and ranges in maturity from 87 to 100 days, thus, making it a mid-to-full season bean. Merlot matures uniformly and displays an attractive “dry-down”appearance. On a 1 to 9 scale where 1=the least and 9=the most

desirable, Merlot had a desirability score of 3.7. This value was slightly lower than Brooks (4.0), but considerably higher than the prostrate, Type III commercial small-red cultivars used as the checks.

Merlot has an attractive garnet seed color, which is characteristic of small-red commercial cultivars, and a noticeable black hilum ring. Individual seeds are oval, . 1.2 x 0.8 centimeters in length, and widths plump at the surface tangential to the hilum, and gently rounded at the apices, giving them a more attractive appearance than Rufus, Garnet, NW63, and UI 239, which have rhomboid-shaped seeds. Merlot has a more intense seed color, as indicated by the hue angle color criterion, than the other small-red cultivars to which it was compared. Seed mass of Merlot averaged 39.2 grams per 100 seed in eight locations in which seed mass data were taken and ranged from 37.5 to 39.8 grams per 100 seed for this trait over the 10 locations. Merlot exhibited a consistent and remarkable canning quality in canning trials conducted in the Michigan State University Pilot Processing Laboratory and stood out among the numerous breeding lines in the canning evaluations. In the canning trials, a panel of judges subjectively rated Merlot as having an above average visual appearance with a score of 5.0 on a scale from 1 to 7 ( 1=most undesirable, 4=neither desirable nor undesirable, and 7= most desirable). Merlot's cooked bean texture estimated with a Kramer Shear Press was 64 kilograms force per 100 grams cooked beans and was within the 55 to 85 kilograms force per 100 grams cooked beans considered desirable for cooked beans of the small-red market class. There were no differences between Merlot and the check cultivars for the Washed Drained Ratio, and Hydration Ratio.

Merlot carries the bc-$1^2$ gene for resistance to bean common mosaic virus and the Ur-3 gene for resistance to bean rust [Uromyces appendiculatus (Pers.:Pers.) Unger] disease making it the first commercial small-red cultivar to have rust resistance. Merlot is susceptible to bean common blight [Xanthomonas campestris pv. phaseoli (Smith) Dye] disease.

Merlot was developed by the dry bean breeding team at East Lansing, Michigan consisting of Dr. G.L. Hosfield of the U.S. Department of Agriculture, Agriculture Research Service, Sugarbeet and Bean Research Unit; Dr. J.D. Kelly and Mr. Jerry Taylor of Michigan State University, Department of Crop and Soil Sciences, and Mr. G.V. Varner of the Michigan Dry Bean Production Research Advisory Board.

Seed of Merlot for experimental purposes may be obtained from Dr. George L. Hosfield, USDA, Agricultural Research Service, Department of Crop and Soil Sciences, Michigan State University, East Lansing, MI 48824. Michigan State University has no seed for distribution. Merlot Small-red dry bean is being released as an exclusive variety. Plant variety protection (PVP) has been applied for.

Genetic material of Merlot will be deposited in the National Plant Germplasm System where it will be available for research purposes.

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AGRICULTURAL RESEARCH SERVICE
WASHINGTON, D.C. 20250

# NOTICE OF NAMING AND RELEASE OF SEAHAWK, A NEW MID SEASON, UPRIGHT, WHITE MOLD TOLERANT NAVY BEAN CULTIVAR FOR MICHIGAN AND THE GREAT LAKES REGION

The Michigan Agricultural Experiment Station and the Agricultural Research Service, United States Department of Agriculture, announce the joint release of 'Seahawk', a new navy bean (Phaseolus vulgaris L.) cultivar developed to improve the tolerance to white mold [Sclerotinia sclerotiorum (Lib.) De Bary] and canning quality of the market class.

Seahawk, breeding line N97774, was derived from a cross made in 1994 between 'Bunsi' and 'Huron' navy beans. Bunsi (Canada synonym, 'Ex-Rico 23') is a white mold tolerant, mid-season cultivar with a semi prostrate indeterminant growth habit. Bunsi exhibits the persistent green stem trait at maturity. Huron is a white mold tolerant, early-season, short stature and erect indeterminant commercial cultivar. The $F_1$ plants from the cross were grown and self-fertilized in the greenhouse in the spring of 1995. The $F_2$ was space-planted in a nursery at the Saginaw Valley Bean and Beet Farm near Saginaw, MI. A single-plant $F_2$ selection was made that possessed mid-season maturity, straw-yellow stems, erect plant stature, and navy bean seed traits. Seed from this selection was planted in an $F_3$ progeny row at the University of Puerto Rico Research Station at Isabela and mass selected on the basis of desirable agronomic and seed traits. A single plant selection was made in a space-planted $F_{4:5}$ nursery in Michigan on the basis of agronomic and navy bean seed traits. The $F_5$ progeny was advanced in a nursery at Isabela, PR. Remnant $F_5$ seed was screened for Bean Common Mosaic Necrosis Virus (BCMNV) by inoculating plants with the NL3 strain of the virus. The F5 row was mass selected and seed returned to Michigan to be grown in field trials. The $F_{4:6}$ breeding line coded N97774 entered replicated yield trials in 1997.

Breeding line N97774 was extensively tested for yield and agronomic traits at 31 locations in Michigan over six seasons (1997-2002). Breeding line N97774 averaged 3,100 kilograms per hectare (2,768 pounds per acre) and had equivalent yields compared to navy bean cultivars, 'Vista', 'Schooner', and 'Crestwood' over 23 and 12 locations, respectively. Breeding line N97774 outyielded the commercial navy bean cultivars 'Mayflower', 'Mackinac', 'Avanti', and 'Navigator' by a margin of 5-11% over 9 to 18 locations. In 2002, N97774 was grown at 8 locations in North America in the National Cooperative Dry Bean Nursery (NCDBN) trials. For those cultivars that N97774 outyielded in the Michigan trial, N97774 maintained the relative yield superiority to these cultivars in the NCGBN trials. Based on yield comparisons with other navy bean cultivars in the respective trials, appearance in the field, overall agronomic importance, and canning characteristics, N97774 was proposed for release and named 'Seahawk'.

Seahawk averages 46 centimeters in height and exhibits a Type IIb, indeterminate growth habit, with moderate tolerance to lodging. Seahawk has white flowers and blooms 45 days after planting. Seahawk is a mid-season bean, maturing 97 days after planting and ranging in maturity from 90-101 days, depending on the season and location. Seahawk matures similar to Schooner, 3 days earlier than Vista and Mayflower. Plants of Seahawk mature uniformly and show excellent "dry-down" across a broad range of environments.

Seahawk carries the single dominant hypersensitive *I* gene for resistance to bean common mosaic virus (BCMV) but is sensitive to the temperature-insensitive necrosis-inducing strains of BCMNV such as NL3 and NL8. Seahawk carries the *Co-2* gene, which conditions resistance to Races 7 and 65 of bean anthracnose [Colletotrichum lindemuthianum (Sacc. & Magnus) Lams. - Scrib.]. Seahawk is susceptible to common bacterial blight [Xanthomonas axonopodis pv. phaseoli (Smith) Dye] and is susceptible to bean rust [Uromyces appendiculatus (Pers.:Pers.) Unger] races 38, 39, 40, 41, 43, and 53 that occur occasionally in Michigan. Seahawk is tolerant to white mold and has demonstrated the highest level of tolerance to white mold among commercial navy bean cultivars grown in Michigan. In four years of comparative field-testing, Seahawk exhibited significantly more tolerance (33%) to white mold than Vista (48%).

Seahawk has large white navy bean seed, which averages 24.6 grams per 100 seed (range: 23-27 grams per 100 seed). In canning trials, Seahawk was subjectively rated by a team of panelists as having acceptable canning quality for navy beans. In 12 canning trials, Seahawk scored 3.8 on a seven-point hedonic scale (where 7 is most desirable, 1 is least desirable, and 4 is neither desirable or undesirable), and was equivalent to Schoner but had significantly better canning quality than Vista and Mayflower, which scored 3.1 and 2.8, respectively. The canning quality evaluation is based on whole-bean integrity (no splitting or clumping), uniformity of size (uniform water uptake), color (color retention), and brine free from starch extrusion into the canning liquid. The thermally processed (cooked) beans are slightly larger in size and lighter in color compared to other navy bean cultivars. Seahawk did not differ significantly from other commercial navy bean cultivars for the processing traits, hydration and washed drained weight ratios. Seahawk exhibited a firmer cooked bean texture (72 kilograms per 100 grams of beans) than the soft textured, Vista (45 kilograms per 100 grams of beans), which contributed to Vista's undesirable canning quality.

Seahawk was developed by the dry bean breeding team at East Lansing, Michigan consisting of J.D. Kelly (Team Leader), Mr. M. Ender and Mr. Jerry Taylor of Michigan State University, Department of Crop and Soil Sciences, Drs. G.L. Hosfield of the U.S. Department of Agriculture, Agricultural Research Service, Sugarbeet and Bean Research, and M.A. Uebersax of Michigan State University, Department of Food Science and Human Nutrition, and Mr. Gregory V. Varner of the Michigan Dry Bean Production Research Advisory Board.

Small quantities of seed of Seahawk for experimental purposes may be obtained from Dr. J.D. Kelly, Department of Crop & Soil Sciences, Michigan State University, East Lansing, MI 48824 (kellyj@msu.edu). The USDA, Agricultural Research Service has no seed for distribution. Seahawk navy bean is being released as a public nonexclusive variety with the option that Seahawk must be sold for seed by name only under the certified class. A research fee will be assessed on each hundred weight of foundation seed sold. Breeder seed is maintained by the Michigan Agricultural Experiment Station, East Lansing, MI 48824, in cooperation with the Michigan Crop Improvement Association (MCIA). The MCIA agrees to produce and distribute Breeder and/or Foundation seed classes of Seahawk. Genetic material of this release will be deposited in the National Plant Germplasm System where it will be available for research purposes, including development and commercialization of new cultivars.

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**BEAN IMPROVEMENT COOPERATIVE - Financial Statement**

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|---|---|---|
| BALANCE ON HAND JANUARY 1, 2002 | | $6,673.30 |
| **INCOME CATEGORIES** | | |
| Back Issues | 95.00 | |
| Dues | 4,194.00 | |
| BIC Meeting | 3,171.13 | |
| Interest | 150.29 | |
| Total Income Categories | 8,035.42 | |
| **EXPENSE CATEGORIES** | | |
| Office Supplies | 722.04 | |
| Postage | 1,632.65 | |
| Printing | 3,356.75 | |
| Total Expense Categories | 5,711.44 | |
| **GRAND TOTAL** | $2,323.98 | |
| BALANCE ON HAND DECEMBER 31, 2002 | | $8,997.28 |