ANNUAL REPORT OF THE

BEAN IMPROVEMENT COOPERATIVE

a voluntary and informal organization
to effect the exchange of information and materials

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BEAN IMPROVEMENT COOPERATIVE

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Coordinating Committee

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SITE RECENTLY UPDATED

Note: It will be assumed that interested individuals may freely cite (including author credit) any report or note in this BIC report, unless the author indicates to the contrary. As a professional courtesy, individuals citing BIC notes should notify the authors of his or her intentions. The BIC Coordinating Committee approved this statement on November 5, 1975.

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THE 48th ANNUAL REPORT OF THE BEAN IMPROVEMENT COOPERATIVE

The Bean Improvement Cooperative (BIC) invites all members and other interested parties to join us at the Eighteenth Biennial Meeting from October 28 through November 3, 2005 at the John M. Clayton Hall Conference Facility on the campus of the University of Delaware, Newark, Delaware. 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 organizer is Ed Kee. 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 Mark Bassett for his years of dedicated service as Chair of the BIC Genetics Committee and his effort to update the "List of Genes" published in 2004 BIC. I wish to welcome Tim Porch TARS, Mayaguez who joined the Genetics Committee in 2005. 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, 2005. 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 2003, Dr. F.A. Bliss was the second 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 2005. The Lectureship will be awarded at the meeting in Delaware and nominations are requested from members.

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 current edition Dr. Maurice Bennink summarizes information on the nutritional and health benefits of beans. The topic is very appropriate as health conscience consumers are showing an increased interest in beans and new funding initiatives through the Bean Health Alliance are focusing on the nutritional benefits of beans not only in affluent communities but in countries where HIV/AIDs is rampant.

Dr. James D. Kelly, BIC President

BIC COMMITTEE MEMBERSHIP - 1957 to 2005

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
2005 Beaver, **Kelly**, Kmiecik, Kurowski, Miklas, Myers, Park, Riley, de Ron, Schwartz (ex officio), Vandenberg

Awards Committee:

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

Genetics Committee

2004: Mark J. Bassett (Chair), James S. Beaver, Matthew W. Blair, Paul Gepts, Phil McClean, Phil Miklas, Molly Welsh (ex officio).

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

Coordination of Genes and Gene Symbol Nomenclature - BIC Genetics Committee

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

Questions or comments should be addressed to the chairman of the committee: **Dr. James S. Beaver, Dept. of Agronomy & Soils, University of Puerto Rico, P.O. Box 9030, Mayaguez PR 00681-9030: ph. (787) 832-4040, ext. 2566; fax. (787) 265-0220; and e-mail; j_beaver@hotmail.com**

BIC MERITORIOUS SERVICE & ACHIEVEMENT AWARD RECIPIENTS

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

2003 Fredrick A. Bliss – Seminis Seeds, Plant Breeder [Frazier - Zaumeyer Distinguished Lectureship]
Steve Beebe – CIAT, Colombia, Plant Geneticist
Paul Gepts – University of California, Plant Geneticist
Marcial A. ‘Talo’ Pastor-Corrales – USDA-ARS, Beltsville, Plant Pathologist

BIC 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 48-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 Newark, DE from October 28 to November 3, 2005. Please help us select worthy recipients.

BIC AWARD NOMINATION

Return by August 11, 2005 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. Shree Singh

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]

2005 BIC/NAPIA MEETINGS

NEWARK, DELAWARE

The BIC/NAPIA biennial meeting and associated meetings will be held Oct. 28 through November 3, 2005 at the John M. Clayton Hall Conference Facility on the campus of the University of Delaware, Newark, Delaware. Lodging will be available at a reduced rate at the Courtyard by Marriott – University of Delaware which is adjacent to Clayton Hall. On Sunday October 30 there will be an optional trip to the Hagley Museum. The museum is located at site of the first du Pont family enterprise where they manufactured their original product – gunpowder.

Registration information, fees, final meeting agenda and travel arrangements will be made available to members and other interested individuals in mid-May. General information on the meeting location and facilities is available online.
University of Delaware: http://www.udel.edu/
Courtyard by Marriott: http://marriott.com/property/propertypage.mi?marshaCode=ILGUD
Hagley Museum: http://www.hagley.lib.de.us/

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 Ed Kee of the local organizing committee (phone: (302) 856-7303; email: kee@udel.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 2005 Biennial Meeting of the BIC and associated meetings. The deadline for receiving abstracts is **Friday September 30, 2005.** Abstracts 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. **Please consider nominating** your colleagues for the BIC Awards and identifying a suitable individual to deliver the Frazier Zaumeyer Lecture. Details on nominating colleagues are provided elsewhere in this report. Dr. Jim Beaver will be the Acting Chair of the Genetics Committee for 2005 so all business for discussion at the Annual meeting should be directed to Dr. Beaver at J_Beaver@hotmail.com. A new Chair of the BIC Genetics Committee will be appointed at the meeting in November.

Tentative Agenda for the 2005 BIC/NAPIA Meeting

Friday October 28, 2005 NAPIA Meeting		**Saturday October 29, 2005** NAPIA Meeting		**Sunday October 30, 2005** BIC/NAPIA Tours	
NAPIA Registration		Oral Session (3)	9:00 – 10:00	Hagley Museum	
Introductions	8:30 – 8:40	Break	10:00 – 10:30		
Featured Speaker (1)	8:40 – 9:20	Oral Session (4)	10:30 – 11:50	BIC Registration	6:00 – 8:00pm
Featured(1)/Oral (2)	9:20 – 10:00				
Break	10:00 – 10:30				
Oral Session(4)	10:30 – 11:50				
Awards Luncheon	noon – 1:30				
Oral Session (6)	1:30 – 3:30				
Poster Session	3:30 – 5:30				

Monday October 31, 2005 BIC Meeting		**Tuesday November 1, 2005** BIC Meeting		**Wednesday November 2, 2005** BIC Meeting/BIC Genetics Committee	
BIC Registration	7:00 – 9:00	Oral Session (3)	9:00 – 10:00	Oral Session (3)	9:00 – 10:00
Introductions	8:30 – 8:40	Break	10:00 – 10:30	Break	10:00 – 10:30
Lectureship (1)	8:40 – 9:30	Oral Session (4)	10:30 – 11:50	Oral Session (4)	10:30 – 11:50
Symposium (1)	9:30 – 9:55	Lunch	noon – 1:00	Lunch	noon – 1:00
Break	9:55 – 10:10	Oral Session (6)	1:00 – 3:00	BIC Genetics Committee	1:30 – 4:30
Symposium (4)	10:10 – 11:50	Poster Session II	3:30 – 5:30		
Lunch	noon – 1:00	BIC Awards Banquet	6:00 – 9:00		
Oral Session (6)	1:00 – 3:00				
Poster Session I	3:30 – 5:30				

Thursday November 3, 2005 W150 Meeting/Phaseolus CGC	
W150 Meeting	9:00 – noon
Phaseolus CGC	2:00 – 5:30

This Schedule Accommodates

BIC Meeting:
1 Lectureship (50 min)
5 Symposium Presentations (25 min)
26 Oral Presentations (20 min)
Posters

NAPIA Meeting:
1 or 2 Featured Speakers (40 min)
17 or 19 Oral Presentations (20 min)
Posters

UPDATED BEAN PUBLICATION

The long-awaited update of the popular regional publication, *Dry Bean Production and Pest Management*, written by Colorado State University, University of Nebraska and University of Wyoming specialists is now available for distribution from.

The Cooperative Extension Resource Center
115 General Services Building
Colorado State University
Fort Collins, CO 80523-4061

(970) 491-6198
FAX: (970) 491-2961
email: CERC1@ur.colostate.edu
www.cerc.colostate.edu

Dry Bean Production and Pest Management, $19.50 [$14.50 each for 10 or more]

by H.F. Schwartz and M. A. Brick, Colorado State University, R.M. Harverson, University of Nebraska, G.D. Franc, University of Wyoming; and the Central High Plains Dry Bean and Beet Group; Bulletin 562A, 2004, 8 x 11" spiral bound, 167 pages

Dry Beans are produced in 17 states, as well as five provinces in Canada, resulting in nearly 38 million cwt from approximately 2.25 million acres in 2002. This publication is a comprehensive guide to dry bean production and pest management including economics and marketing, classification and performance, seed certification, climate effects, crop rotation, planting, nutrient management, irrigation, tillage, harvest and post harvest; and weed, insect and disease management. With more than 200 color photos, a field key, diagnostic checklist, glossary, and additional references, this publication is the industry's guide to growing beans in the Colorado and the Central Plains.

New Bean Breeder at the University of Nebraska

Dr. Carlos Urrea was recently hired as a dry bean breeding specialist/Assistant Professor stationed at the Panhandle Research & Extension Center in Scottsbluff, Nebraska. Carlos comes to Nebraska from the CIMMYT Maize Program in Nepal. Carlos received his Ph. D. in breeding from North Dakota State University and Masters at the University of Puerto Rico. Prior to that he was employed by CIAT in the bean program. Carlos plans to begin his75% research /25% extension appointment in April 2005.

Professor Clibas Vieira

The BIC mourns the passing of a friend and colleague Clibas Vieira, Professor of the Graduate Program of Genetics and Breeding at the Federal University of Viçosa, Brazil. Clibas Vieira was awarded the BIC Meritorious Service Award in 1977 and is widely recognized for his extensive studies and publications on bean breeding and genetics. The BIC recognizes him for his significant achievements to bean research.

IN MEMORY OF CLIBAS VIEIRA

Clibas Vieira, 76, retired professor of the Graduate Program of Genetics and Breeding at the Federal University of Viçosa, in the state of Minas Gerais, Brazil, passed away on 14 October 2004. He is survived by his wife of 77 years Dona Jandira and his children Rogerio (a researcher with EMBRAPA/EPAMIG) and his daughters Milene (a Professor at the University of Viçosa) and Rosana (a researcher with EMBRAPA) and three grandchildren. Dr Vieira, better known throughout Brazil as Professor Clibas, was one of the most respected scholars and researchers in Brazil and Latin America. His contributions in the area of genetics and plant breeding, particularly of the common bean, and his standing as an academician and researcher are recognized all over Brazil and internationally. He received the BIC Meritorious Service Award in 1977. During his career at Viçosa, Professor Vieira advised 54 Master and 24 PhD students and he had a very productive career publishing 251 scientific articles and 255 scientific abstracts. He wrote 9 books among them Bean and Myself. He also received 27 distinctions. Of those distinctions he was particularly fond of his election to the Academy of Science of the Third World with the invitation from Dr. Johanna Dobereiner.

Dr. Vieira was born in Sao Paulo, Brazil in December 27 1927. In 1951 he was appointed to a teaching position in geography, physics and drawing at the Viçosa High School. That year he also met Jandira and married her in 1954, the same year that he obtained his Agronomy degree from the University of Viçosa. He then started his professional life in agronomy in 1953 as an extensionist based in a small city in the state of Sao Paulo. The same year received an invitation to become an Assistant professor of the Department of Agronomy at the University of Viçosa. Later, he went to the California, where he pursued his Master graduate studies at the University of California-Davis from September 1957 to October 1958. He then returned to Vicosa. In 1963 he received the title of Doctor in Agronomy (University of Vicosa) and 1965 the title of Doctor in General Agronomy and Plant Breeding (University of Vicosa).

One of Dr. Vieira's passions was editing scientific journals. In 1969 he started his participation on the Editorial Committee of two journals, Revista Ceres and Experientiae, and in 1972 he assumed the presidency of these Editorials Committees. Only with the exception of a short period in 1974, when he was appointed as the Director of Research Department of Embrapa Headquarters, he did the editing activity until the day he passed away. During 40 years he also taught several disciplines at Viçosa. Almost all of them had some relationship with common bean and plant breeding which were his favorite subjects. Professor Clibas has defined his university career as:" Rich in experience, diversified, fascinating and some times let me upset but never make loose the enthusiasm". He retired in 1994. After his retirement he continued his dedication to research and his involvement with graduated students. In 2002 he was the president of the organizing committee of the VII National Congress on Common Bean Research were he received several distinctions. In 2004 he was invited to be a member of the Academy of Literature of Vicosa and he continued to have the responsibility as the main editor of the Ceres scientific journal and he finished writing the book entitled "Memories of half decade on studies of Common Bean". Professor Clibas Vieira was a great example for the entire bean research community. We will miss his guidance and his wisdom.

EAT BEANS FOR GOOD HEALTH

Maurice Bennink
Food Science and Human Nutrition
Michigan State University

There are two major areas of health problem that could be significantly reduced by simply eating more beans. One area is chronic diseases and the other is malnutrition. The potential for beans to mitigate chronic diseases will be addressed first.

Beans and chronic diseases Chronic diseases (certain types of cancer, Type II diabetes, heart disease, and other diseases of the blood system) typically take many years (10 to 30 years) to develop. Chronic diseases are the most common causes of death in industrialized countries and they significantly lower the quality of life for millions. The single most important factor in the etiology of chronic diseases is the perpetual over-consumption of food (energy). Excess consumption coupled with inadequate physical activity results in a positive energy balance and eventually obesity. Obesity is a common etiologic factor in the development of chronic diseases. Other central components that lead to the development of chronic diseases are chronic elevated concentrations of blood glucose (hyperglycemia) and blood insulin (hyperinsulinemia). Excess body fat leads to hyperglycemia and hyperinsulinemia and vice versa. Hyperglycemia and hyperinsulinemia are hallmark features of Type II diabetes and Type II diabetes is a major contributor to the development of heart disease and other diseases of the blood system (cardiovascular diseases). In addition, recent epidemiological studies suggest that hyperglycemia and hyperinsulinemia contribute to the development of certain cancers.

The type of carbohydrate we eat has a strong influence on food intake, maintenance of normal blood glucose and insulin concentrations, and the occurrence of chronic diseases. Foods with a high glycemic index cause a more rapid and greater rise in blood glucose and insulin than foods with a low glycemic index even though the amount of carbohydrate consumed is equal. Eating foods that have a high glycemic index for a long period of time can lead to hyperinsulinemia, insulin resistance and Type II diabetes mellitus. Also, eating high glycemic index foods stimulates people to eat sooner after their last meal than if they ate low glycemic index foods (1,2). Moreover, eating a high glycemic index meal produces the tendency to select high glycemic foods for a snack or for the next meal. This sets up a vicious cycle that leads to a greater caloric intake and greater blood glucose and insulin concentrations (3). With time, obesity and Type II diabetes develop. On the other hand when low glycemic foods are consumed, there is greater satiety and people don't feel hungry as quickly. Also the tendency to select high glycemic index foods for snacks or the next meal is reduced. Therefore, the likelihood of excessive calorie consumption is reduced and so is the likelihood of becoming obese and a Type II diabetic. Compared to other carbohydrate sources, beans have a low glycemic index, varying from 26-42 % relative to glucose (4). Beans are also high in fiber (typically 18% dietary fiber) and low in fat. While eating beans will not magically make you thin or make you loose weight, substituting beans for highly-refined cereal products, foods or beverages with a high sugar content, or any high glycemic index food will help curb caloric intake and help maintain a leaner physique.

Excess body fat increases the risk of developing heart disease, strokes, Type II diabetes mellitus, and some types of cancer (5). There has been a steady increase in the percentage of

overweight and obese individuals in North America and Western Europe. The increase in obesity is considered to be of epidemic proportions in the U.S. (6) and in most developed countries (5-9). For example, on a worldwide basis, more than one billion adults are overweight and more than 300 million are obese (5,9). In the U.S. more than 60% of the adult population is overweight or obese (7). Obesity and overweight account for approximately 300,000 deaths per year in North America (10,11) and the cost associated with excess fatness is estimated to be greater than 117 billion dollars per year (12). Most of the costs associated with excess fatness are related to Type II diabetes, heart disease, and high blood pressure (13). Perhaps even more disturbing is the great increase in overweight and obese children and adolescents (8). Accompanying the rise in excess fatness is the increased incidence of Type II diabetics in children and adolescents. Soon we will experience a tremendous increase in morbidity and mortality resulting from complications of diabetes and obesity in children and adolescents.

The exact reason why consumption of high glycemic index foods leads to an increased risk for Type II diabetes is not known but may be due to an increase in insulin demand (3,14-16). High glycemic index foods are known to cause rapid elevations in blood glucose and insulin following a meal. Chronic consumption of high glycemic index diets may in turn lead to down-regulation or desensitization of receptors for insulin, eventually contributing to insulin resistance (15). The body initially adjusts to higher circulating glucose by increasing insulin secretion from the pancreas. However, in susceptible individuals over time insulin resistance combined with exhaustion of insulin producing cells will eventually lead to Type II diabetes (3,16). Current research (17,18) also suggests that hyperglycemia and hyperinsulinemia stimulate fat cells and possibly cells that line blood vessels (endothelial cells) to secrete pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-) and interleukin-6 (IL-6). These cytokines promote insulin resistance and other clinical and biochemical symptoms associated with Type II diabetes. In addition, these cytokines are predictive of risk for cardiovascular disease.

Of the chronic diseases, CVD is generally the leading cause of death in North America and Western Europe. Factors that increase one's risk of developing CVD include high levels of total cholesterol and LDL cholesterol ("bad cholesterol"), low levels of HDL cholesterol ("good cholesterol"), obesity, diabetes, smoking, and high blood pressure. Only one epidemiological study has directly examined the frequency of legume consumption and risk of coronary heart disease in US men and women. After adjusting for confounding risk factors, individuals consuming legumes at least 4 times per week were found to have a 22% lower risk of heart disease than individuals consuming legumes less than once per week (19). In epidemiological studies where legumes are consumed as part of a healthier diet plan, consistent reductions in heart disease risk have also been observed. In the Health Professionals Follow-up Study, men that adhered to a more "prudent diet" which included greater consumption of whole grains, legumes, fish, and poultry had a 30% lower risk of having heart disease. Conversely, individuals following a more "Western" diet, characterized by increased consumption of red meat, refined grains, sweets, French fries, and high fat desserts had a higher risk of heart disease (20). Similar trends were seen in the Nurses Health Study (21). The relative risk of coronary heart disease in the 20% of women that followed the "prudent" dietary pattern more closely was 0.76 compared to 1.46 for women eating a "Western" type pattern (21). Thus, those that most consistently ate the "prudent" type of diet had one half the risk of developing heart disease compared to those that most often ate the "Western" type of diet.

Data from several human intervention trials indicate that consumption of canned (22-24) and dry beans (22,25-28) reduce serum cholesterol. Generally, in carefully controlled clinical studies where the macronutrient intake was matched and the fiber content in the bean fed group was at least twice that of the control diet, significant reductions in both total and LDL cholesterol occurred (22,29). A 1% reduction in total cholesterol corresponds to about a 2% decrease in the risk of developing heart disease (30). Beans are a good source of soluble dietary fiber, containing approximately 4 g per cup of cooked beans (31). Soluble fiber has been shown to reduce blood cholesterol in epidemiological (32), clinical (21,22,25,33), and animal (34,35) studies. The consumption of dietary fiber in the US is only 12-13 g/day, well below the recommended 25-35 g/day. Incorporating one cup of cooked beans into the diet would add 12 g of total fiber and 4 g of soluble fiber per day. This increase in fiber intake would be expected to modestly lower serum cholesterol and risk of heart disease, especially in hyperlipidemic individuals.

Correa (36) examined data from 41 countries and found a significant inverse relationship between bean consumption and morbidity due to breast, prostate, and colon cancer. Two animal studies have shown that bean consumption reduces colon cancer (37,38). Hughes et al. (37) fed rats either pinto beans or casein (milk protein) and found that feeding pinto beans reduced the number of rats with colon cancer by 50% compared to casein-fed rats. Moreover, in rats that did develop tumors, rats fed pinto beans had only 1 tumor while rats fed milk protein had and average of 2.5 tumors. In a similar study, Hangen and Bennink (38) fed rats a casein-based diet, a diet containing black beans, or a diet containing navy beans. They reported that feeding either black beans or navy beans reduced the number of animals that had colon cancer by over 50%. Similar to Hughes et al. (37), the number of tumors per rat was 50% less in bean fed rats. Hangen and Bennink (38) noted that rats fed beans were significantly leaner compared to control animals. These two animal studies corraborate the study by Correa (36) showing that bean consumption reduces colon cancer.

How beans slow cancer growth and which component(s) of beans have anticarcinogenic properties are not yet known. One potential mechanism whereby beans could inhibit cancer is related to regulation of blood glucose and insulin. Recent research findings suggest that high levels of blood insulin (39,40) and/or high levels of blood glucose (41) promote colon cancer. The Cancer Prevention Study by the American Cancer Society found that subjects with Type II diabetes have a higher propensity of developing colon cancer than individuals without diabetes (42). Type II diabetics typically have elevated blood glucose and insulin concentrations. Data from other large prospective studies also suggest that subjects with Type II diabetes have an increased risk of colon cancer (43,44). Additional evidence supporting the relationship between hyperinsulinemia and promotion of colon cancer was provided by two studies that utilized animals exposed to a colon carcinogen and subsequent injections with insulin. Insulin injections promoted both the early stages of colon cancer (45) and growth of colon tumors (46). As discussed above, eating beans produce low blood glucose and insulin concentrations compared to most other sources of dietary carbohydrates. Taken together, these studies suggest that eating beans to keep blood insulin and glucose low may be one mechanism that slows colon carcinogenesis.

There are at least two other possible mechanisms whereby bean constituents may inhibit colon carcinogenesis. In experimentally induced colon cancer, feeding fiber often does not reduce colon cancer. However, if resistant starch is added along with fiber, fermentation in the colon is altered and colon cancer is reduced. Beans contain high amounts of resistant starch (38) as well as high amounts of soluble and insoluble fibers which leads to favorable fermentation

and possibly explains why feeding beans inhibits colon cancer. Another possible mechanism of cancer inhibition is by phytonutrients. Beans contain phytonutrients such as anthocyanins, a variety of phenolic compounds, protease inhibitors, phytic acid, and saponins. Phytonutrients are not considered to be essential nutrients. However, research over the past 15 years clearly demonstrates that some phytonutrients do provide health benefits. Purified protease inhibitors, phytic acid, and saponins inhibit various aspects of carcinogenesis (47-49). But direct evidence that these phytonutrients in foods inhibit cancer is lacking. Therefore, how much of the anticancer activity associated with beans is due to phytonutrients remains to be determined.

Beans and malnutrition Protein energy undernutrition (PEU) remains a common problem in much of the developing world. More than one third of children less than five years of age in developing countries suffer from PEU and the proportion of children who are undernourished has changed very little during the past 20 years (50). Apart from PEU, deficiencies of iron and vitamin A are widespread and often severe. PEU and deficiences of vitamin A and iron account for more than 75% of the deaths of infants and young children in some developing countries (51). Greater consumption of beans by children in developing countries would significantly reduce morbidity and mortality in this age group.

PEU and micronutrient deficiencies begin during weaning and/or immediately thereafter as most food used for weaning do not provide adequate amounts of energy, protein and micronutrients. Traditional weaning foods are based on starchy staples such as maize, sorghum, finger millet, and rice or non-cereals such as cassava, potato, and plantains and these foods have been widely associated with nutrient deficiencies among pre-school age children (52,53). Beans are not typically fed to small children. However, appropriate combinations of beans and cereals, consumed in adequate amounts, will prevent PEU.

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DIVERSITY IN SIZE AND SHAPE OF THE SEEDS OF BULGARIAN COMMON BEAN GENOTYPES (Phaseolus vulgaris L.)

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INTRODUCTION

Seeds of common bean (*Phaseolus vulgaris* L.) possessed different shape, size and color. Ecological factors influenced very much those characteristics of the seeds (Genchev, 1989). Shape of the seeds is important systematic trait (Gradinaroff, 1939; Vishnevski, 1940; Hristoforov, 1973) for description of the diversity and market realization of the common bean. The main goal of that investigation was to establish the genetic diversity of 33 Bulgarian common bean genotypes.

MATERIAL AND METHODS

Three-years investigations (2002-2004) on 33 Bulgarian common bean genotypes were conducted. Size - length, width and thickness of 50 seeds per each genotype were measured. The shape of common bean seeds was determined by the methods of Hristoforov (1973) and Genchev (1989).

RESULTS AND DISCUSSIONS

As a result of conducted investigations was found (table 1), that genotypes Garmen and No 338 were with the biggest seed length and width for the three-years period of investigations, while the biggest thickness of the seeds possessed varieties Bisser and Samoranovo. Abritus, IZK-DK-4 and Prelom were genotypes with the smallest length of the seeds, while seeds of the genotypes Ludogorie, Oreol GP and 80-7-11-12 were with the smallest width and thickness. Seeds of the varieties Medkovetz 1 and Abritus were with the nearest to the sphere shape seeds and the most distant to that shape were the seeds of the genotypes No 338, DG 84-34-1, Garmen and DG 80-7-11-12. Coincidence between biometric methods of Hristoforov (1973) and Genchev (1989) for determination of the seed shape, in our study, was 67% and it is possible to maintain that suggested by Genchev (1989) method can be suitable for application in genetic investigations**.**

CONCLUSION

Investigated Bulgarian common bean genotypes represented great genetic diversity by the seed shape and size. They are interesting by breeding point of view and can be used for hybridization and creation of new common bean varieties. Genotypes with contrast traits are suitable for application of genetic analysis where determination of the shape can be done by the method of Genchev (1989).

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Table 1. Size and shape of the seeds from investigated Bulgarian common bean genotypes

Genotypes		Size:			Ratio L/ t/ w	V.c.	Shape of the seeds by Genchev (1989)
		Length (L)	Width (t)	Thick-ness (w)			
1.	*Medkovetz 98-1*	10,26	7,51	5,81	1,37/1/0,77	9,25	Sphaericus-ellipticus
2.	*Plovdiv 15M*	11,64	7,51	5,58	1,55/1/0,74	12,52	Ellipticus-ubcompressus
3.	*No 1028*	14,16	8,92	5,82	1,59/1/0,65	14,59	Subcompressus
4.	*Plovdiv 10*	11,86	7,99	5,65	1,48/1/0,71	12,30	Ellipticus-subcompressus
5.	*Plovdiv 11M*	11,61	7,67	5,52	1,51/1/0,72	12,45	Ellipticus-subcompressus
6.	*Добруджански 2*	11,97	8,20	5,03	1,46/1/0,61	13,81	Subcompressus
7.	*Dounav 1*	12,41	6,30	5,12	1,97/1/0,81	16,24	Subcompressus-compressus
8.	*A 195*	15,15	7,75	5,46	1,95/1/0,70	17,57	Compressus
9.	*Kristal 137*	12,41	8,43	5,93	1,47/1/0,70	12,17	Ellipticus-subcompressus
10.	*Bisser*	10,83	7,76	7,11	1,40/1/0,92	7,73	Sphaericus-ellipticus
11.	*Abritus*	9,12	6,18	5,34	1,48/1/0,86	9,62	Ellipticus
12.	*Dobroudjanski ran*	14,55	8,54	5,25	1,70/1/0,61	16,64	Subcompressus-compressus
13.	*No 1026*	14,24	8,63	5,46	1,65/1/0,63	16,09	Subcompressus-compressus
14.	*No 338*	15,94	9,04	4,67	1,76/1/0,52	19,16	Compressus
15.	*DG 84-34-1*	15,16	7,95	4,85	1,91/1/0,61	18,64	Compressus
16.	*IIRR-1426*	12,68	7,66	5,86	1,66/1/0,77	13,83	Subcompressus
17.	*Loudogorie*	10,91	5,99	4,55	1,82/1/0,76	15,55	Subcompressus
18.	*Troudovetz*	14,26	7,66	5,71	1,86/1/0,75	16,48	Subcompressus-compressus
19.	*Dobroudjanski7*	12,80	7,82	5,08	1,64/1/0,65	15,23	Subcompressus
20.	*IIRR-7585*	15,02	7,63	6,43	1,97/1/0,84	16,00	Subcompressus-compressus
21.	*Prissad*	13,81	8,49	5,84	1,63/1/0,69	14,42	Subcompressus
22.	*Bulgari*	14,17	8,82	5,67	1,61/1/0,64	14,99	Subcompressus
23.	*Hitovo 1*	11,33	7,27	6,25	1,56/1/0,86	10,81	Ellipticus
24.	*IZK-DK-4*	9,46	6,27	4,61	1,51/1/0,74	12,21	Ellipticus-subcompressus
25.	*Pokrovnik*	10,45	6,69	5,46	1,56/1/0,82	11,50	Ellipticus-subcompressus
26.	*Oreol GP*	10,01	5,57	4,38	1,80/1/0,79	14,87	Subcompressus
27.	*Garmen*	16,70	8,65	4,97	1,93/1/0,57	19,79	Compressus
28.	*Padesh 1*	11,21	7,58	5,73	1,48/1/0,76	11,37	Ellipticus
29.	*Prelom*	9,36	6,05	5,15	1,55/1/0,85	10,78	Ellipticus
30.	*Samoranovo*	12,21	8,21	7,08	1,49/1/0,86	9,80	Ellipticus
31.	*Dessislava*	14,76	8,34	5,43	1,77/1/0,65	16,73	Subcompressus-compressus
32.	*DG 80-7-11-12*	11,45	5,57	4,20	2,06/1/0,75	18,15	Compressus
33.	*Trakiiski*	11,55	8,16	5,72	1,42/1/0,70	11,82	Ellipticus-subcompressus

INFLUENCE OF METEOROLOGICAL CONDITIONS ON SEED CHARACTERISTICS OF SOME BULGARIAN COMMON BEAN GENOTYPES

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INTRODUCTION

The change of agro-climatic resources, in the frame of global climatic changes, will impose new criteria in development of agricultural crops with important characteristics in special districts of the country (Hershkovich, 1984). Characteristics of common bean seeds are important for Bulgarian consummators. That also related to the breeding goals. White large-scaled seeds become more preferable in the last few years and the breeding purposes have to be point at that direction.
The goal of that study was to investigate the influence of mean air temperature and amount of rainfall in plant vegetation period on seeds' length, thickness and width of some Bulgarian common bean genotypes.

MATERIAL AND METHODS

Investigations were conducted in the field on the area of city Plovdiv in Bulgaria for three-years period (2002-2004). Thirty-three Bulgarian common bean genotypes were included in that study. Length, thickness and width of 50 seeds from each genotype were measured. Hydrothermal coefficient by Seleminov was calculated and the relation between studied parameters was established statistically by special complex of programs constructed for processing of biometrical and meteorological data, integrated in Excel (Kouzmova, 2002).

RESULTS AND DISCUSSIONS

Data for meteorological conditions in the time of plant vegetation period (2002-2004) are shown on table 1.

Table 1. Plant vegetation period and meteorological conditions

Year	Vegetation period (days)	Rainfall sum (mm)	(*) $\sum t^o > 10^o$	Mean air temperature (^{o}C)	(**) HTC
2002	88	124,0	1855,5	21,1	0,67
2003	72	124,0	1498,6	20,8	0,83
2004	89	129,7	1627,3	18,3	0,80
Average	83	125,9	1660,5	20,1	0,76
Std. Dev.	7,8	2,7	148,3	1,26	0,07

(*) Sum of mean day-night air temperatures higher than 10 ^{o}C; (**) Hydrothermal coefficient calculated by Seleminov

Vegetation period for all genotypes varied from 72 to 89 days for the three-years period of study. The most varied parameter that characterized the meteorological conditions was sum of mean day-night air temperatures higher than 10 ^{o}C. Hydrothermal coefficient calculated by Seleminov is a complex indicator for characterization of humidity in outside conditions and that is why the most dried, for the three-years period of study, was 2002 (table 1).

Concerning the investigations of Gurova (1967), the optimal air temperature in the period germination – flowering of common bean plants for the area of Plovdiv is 20-22 ^{o}C, and the extension of that period decrease with increasing of temperatures.

It can be seen from table 2 that the studied meteorological conditions (table 1) influenced lesser the length, thickness and width of the seeds of the genotypes 80-7-11-12, 1028 and A-195. Length and

thickness of varieties Plovdiv 10, Plovdiv 11M and Prissad, as well as width and thickness of varieties Abritus, Garmen, Prelom and 338 were also lesser influenced by meteorological conditions. Under the influence of meteorological conditions the highest variability of length, thickness and width showed seeds of old variety Samoranovo. Length and thickness of varieties 1026 and Medkovetz 98-1, as well as thickness and width of Trudovetz also varied very much under the influence of studied meteorological conditions.

Table 2. Standard deviation (*Std.Dev.*) and variation coefficient (*V.c.*) of length, width and thickness in dependence of 50 seeds measurements per genotype and meteorological conditions for the period 2002-2004

Genotypes		Length		Width		Thickness	
		Std. Dev.	V.c.	Std. Dev.	V.c.	Std. Dev.	V.c.
1	338	1,03	1,05	0,15	0,02	0,21	0,04
2	1026	1,00	1,00	0,47	0,22	0,55	0,30
3	1028	0,12	0,01	0,10	0,01	0,20	0,04
4	80-7-11-12	0,06	0,00	0,15	0,02	0,21	0,04
5	84-34-1	0,06	0,00	0,31	0,09	0,68	0,46
6	A 195	0,21	0,04	0,15	0,02	0,10	0,01
7	Abritus	0,66	0,43	0,40	0,16	0,06	0,00
8	Bisser	0,47	0,22	0,35	0,12	0,46	0,21
9	Balgari	0,45	0,20	0,29	0,08	0,35	0,12
10	Garmen	0,95	0,91	0,40	0,16	0,10	0,01
11	Dessislava	0,57	0,32	0,38	0,14	0,26	0,07
12	Dobroudjanski 2	0,93	0,86	0,50	0,25	0,57	0,32
13	Dobroudjanski 7	0,56	0,31	0,25	0,06	0,80	0,64
14	Dobroudjanski ran	0,21	0,04	0,06	0,00	0,35	0,12
15	Dounav 1	0,95	0,91	0,46	0,21	0,67	0,44
16	IZK-DK-4	0,25	0,06	0,23	0,05	0,36	0,13
17	IIRR-1426	0,72	0,52	0,31	0,09	0,60	0,36
18	IIRR -7585	0,15	0,02	0,15	0,02	0,32	0,10
19	Kristal 137	0,06	0,00	0,17	0,03	0,32	0,10
20	Loudogorie	0,80	0,64	0,26	0,07	0,47	0,22
21	Medkovetz 98-1	0,80	0,64	0,44	0,19	0,56	0,31
22	Oreol GP	0,53	0,28	0,25	0,06	0,50	0,25
23	Padesh 1	0,56	0,31	0,25	0,06	0,51	0,26
24	Plovdiv 10	0,17	0,03	0,10	0,01	0,36	0,13
25	Plovdiv 11M	0,00	0,00	0,21	0,04	0,40	0,16
26	Plovdiv 15M	0,50	0,25	0,29	0,08	0,40	0,16
27	Pokrovnik	0,55	0,30	0,44	0,19	0,40	0,16
28	Prelom	0,45	0,20	0,35	0,12	0,12	0,01
29	Prissad	0,36	0,13	0,20	0,04	0,35	0,12
30	Samoranovo	1,12	1,26	0,50	0,25	1,08	1,17
31	Trakijski	0,93	0,86	0,32	0,10	0,35	0,12
32	Troudovetz	0,50	0,25	0,45	0,20	0,70	0,49
33	Hitovo	0,58	0,33	0,06	0,00	0,72	0,52

Genotypes with the lowest variability for the three-years period of study

Genotypes with the highest variability for the three-years period of study

CONCLUSION

Characteristics of seeds - length, thickness and width are in dependence of meteorological conditions like mean air temperatures, amount of rainfalls and atmospheric dryness. Studied Bulgarian common bean genotypes showed differences of seeds' characteristics under the studied meteorological conditions. Seeds of the genotypes 80-7-11-12, 1028 and A-195 were with the most stable characteristics for the three-years period of investigations. Those genotypes can be used in breeding programs for stable transfer of the traits - shape and size of seeds independently of meteorological conditions of the region.

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Characterization of landraces of common bean (*Phaseolus vulgaris* L.) germplasm from Mato Grosso do Sul State

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Introduction

The high genetic variability present in the common bean germplasm used by the small farmers, has been recognized, this is explained because of the diverse preferences choices made by consumers and growers, and especially due to the environmental conditions where this cultures is explored. Information about diversity and genetic divergence inter species is essential for the reasonable using of genetic sources (Loarce et al., 1996). Landrace of common bean have been showing wide genetic variability in relation to color, brightness, and seed size, and also it has been verified variations to color, texture and pod size (Fonseca et al., 2002). Multivariate analysis techniques can be utilized to evaluate the genetic divergence among access of the germplasm (Pereira et al., 1992).

Common bean plantation in Brazil, especially in Mato Grosso do Sul, have shown socially and economically importance. Genetic breeding research is largely responsible for the development of this crop. The present work had the objective to evaluate the genetic divergence among 35 landraces of common bean collected in Mato Grosso do Sul State.

Material and Methods

Thirty five landraces used in this studying were collected in Mato Grosso do Sul State, from Banco Ativo de Germoplasma (BAG) of Empresa Brasileira de Pesquisa (Embrapa), Centro Nacional de Pesquisa de Arroz e Feijão (CNPAF). The experiment was carried out in a randomized block with four replications, during the period from August to December 2003.

The following characteristics were evaluated: mean number of days to emergence (EMERG), number of days to flowering, mean height of the insertion of the first pod, mean longitudinal length of the pods, total number of pods per plant, number total of seeds per plant, mean number of seeds per pod, mean seed weight**,** and cycle. The Tocher and Nearest Neighbor methods, and the multivariate analysis techniques were made using the statistics program Genes (Cruz, 2001).

Results and Discussion

The variance analysis showed significant difference at level of 1% of probability in most of the evaluated characteristics. The genetic dissimilarities measurements using Generalized Mahalanobis Distances ($D^2_{ii'}$), demonstrated that the genotypes with greater dissimilarities were the Uberabinha Preto (13) and the Manteigão (29), because both showed the maximum value $D^2_{ii'}$, which means 473.00. Uberabinha Preto access belongs to the Mesoamerican gene pool, whereas Manteigão has Andean origin. The Carioquinha sem Cipó and Roxinho Mineiro were the most similar.

The results presented by Tocher Method demonstrated that the most divergent were Uberabinha Preto (13) and Manteigão (29), which were placed in groups VI and II, respectively, and the most similar were Carioquinha sem Cipó (17) and Roxinho Mineiro (27) in the same group I. According to the Nearest Neighbor Method the landraces were clustered in nine groups. The Groups I to V, composed by the Nearest Neighbor method contained lanraces from Mesoamerican origin, whereas Groups from VII to IX only possess Andean origin. It is possible to observe a great concordance between the two clustering standard method, based on the results from grouping analysis, related to Generalize Mahanalobis Distance. These methodologies were in agreement with the landraces belonged to both Andean and Mesoamerican gene pool

According to Figure 1, the results demonstrated a graphic dispersion related to the first two Canonic Variables, in bi-dimensional space. It is possible to establish among 35 landraces evaluated there was a wide genetic divergence, which results were in agreement with the ones presented by Tocher Method and the Nearest Neighbor Method. It was also demonstrated the replacement of the landraces from Andean and Mesoamerican origin in distinguished groups found according to results obtained by Tocher Method and Nearest Neighbor Method. The first two Canonic Variable, explained 81.90% of total variation, demonstrating that among 35 landraces there is a wide genetic divergence.

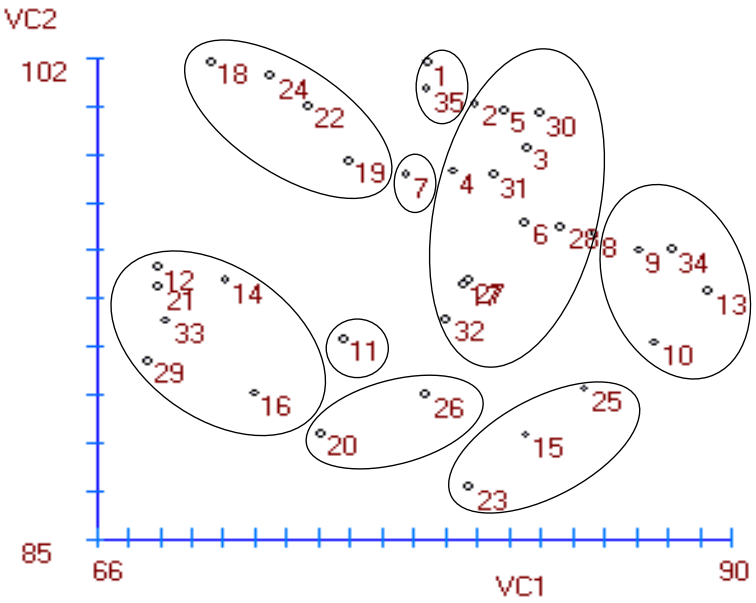

Figure 1 – Graphic dispersion of scores in relation to two representative axis of the first two Canonic Variables (VC_1 and VC_2), in nine characteristics, evaluated in 35 landraces of common bean.

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GENETIC DIVERSITY OF CARIBBEAN COMMON BEAN GERMPLASM.

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Introduction: The Caribbean basin is a secondary center of diversity for common bean where mixing of the Andean and Mesoamerican gene pools has given rise to inter gene pool introgression as shown by phenotyping as well as genotyping with RAPD and Phaseolin markers (Duran et al., Crop Sci. in press; Blair et al. 2003a, BIC 46: 63-65). The most common Andean or introgressed Andean commercial classes in the region include the red mottled Pompadour, the striped kidney Miss Kelly, the light red kidney Velasco while the most common Mesoamerican commercial classes are the small seeded black and small red beans. The objective of this study was to analyze a collection of genotypes from the region with simple sequence repeat (SSR) microsatellite markers as this class of markers is proving to be critical for germplasm categorization at CIAT.

Materials and Methods: A total of 129 entries of common bean genotypes were genotyped. These included a total of 112 traditional varieties (or selections thereof) from the Caribbean (65 from Dominican Republic; 18 from Haiti, 26 from Puerto Rico, and 1 each from Cuba, Panama and Jamaica). In addition, 16 check varieties were included of which 8 were advanced breeding lines from CIAT (DRK57, AFR735, CAL96, AFR619, AFR699, DOR364, A36, AND109), 1 was a breeding line from the University of Puerto Rico (UPR9443-4), 3 were Colombian varieties (ICA Palmar, ICA Pijao and Calima); 2 were varieties from the USA (Redhawk and Montcalm), 1 was a Peruvian variety (Blanco Laran) and another was a germplasm accession that has been extensively characterized at CIAT (G19833, Northern Peru). DNA was extracted from the young trifoliate leaf of a single seedling from each accession using a standard extraction technique (Afanador et al., 1993: BIC 35:10-11). Microsatellite PCR reactions and polyacrylamide gel electrophoresis were as described in Blair et al. 2003b (Theor Appl Genet 107: 1362-1374). Silver-stained gels were dried overnight and scanned for data analysis.

Results and Discussion: A total of 27 microsatellite markers (19 genomic and 8 gene-based markers) were selected to evaluate the genotypes. A minimum of one marker was selected per chromosome and the rest were chosen based on genomic representation and good amplification. Only single copy microsatellites were evaluated and in all cases single bands were called. Band sizing and allele calling was done based on comparisons to 25 bp size standard ladders that were used two times per gel. The total number of alleles evaluated across all 27 markers was 118 and on average each marker revealed 4.4 alleles each. The number of alleles and discrimination power (D) values were significantly higher for genomic microsatellites (6.1 alleles, 0.622 D value) than for gene-based microsatellites (4.3 alleles, 0.513 D value) based on unpaired t-test (at $P<0.05$). Microsatellite analysis uncovered two major groups among the Caribbean germplasm: an Andean group that was similar to the controls G19833 and Calima; and a Mesoamerican group that was similar to the controls ICA Pijao and DOR364 (Figure 1).

The Andean and Mesoamerican genepools were related at a 0.18 Dice similarity value with a greater number of accessions and a higher overall diversity within the Andean group (79 accessions, 0.47 to 0.99 Dice similarity values) than within the Mesoamerican group (49 genotypes, 0.65 to 0.99 Dice similarity values). Microsatellite markers were ideal markers to distinguish the fine-scale relationships within these genepools. In the case of the Caribbean germplasm with an Andean affinity, all the landraces and breeding lines were more similar to the Colombian control genotype Calima (red mottled cream), than to the Peruvian control genotype G19833 (red mottled yellow). This was expected since many of the Caribbean Andean genotypes are red mottled like Calima and probably derived from the Northern Andes rather than from Central Andean areas of South America such as Peru. It was also noteworthy that the Caribbean genotypes with a Mesoamerican affinity were more closely related to each other than they were to the black seeded and small red seeded control genotypes DOR364 and ICA Pijao. This also might be expected given the Caribbean Mesoamerican genotypes represent distinct germplasm probably derived from inter genepool hybridization but isolated from the Central American germplasm from which they arose. Such inter genepool hybridization may explain how the red mottled seed coat color was transferred from the Andean genepool into Mesoamerican-like germplasm. We can conclude that the microsatellite analysis uncovered relationships between the Caribbean landraces that may reveal the history of bean introduction into the Caribbean.

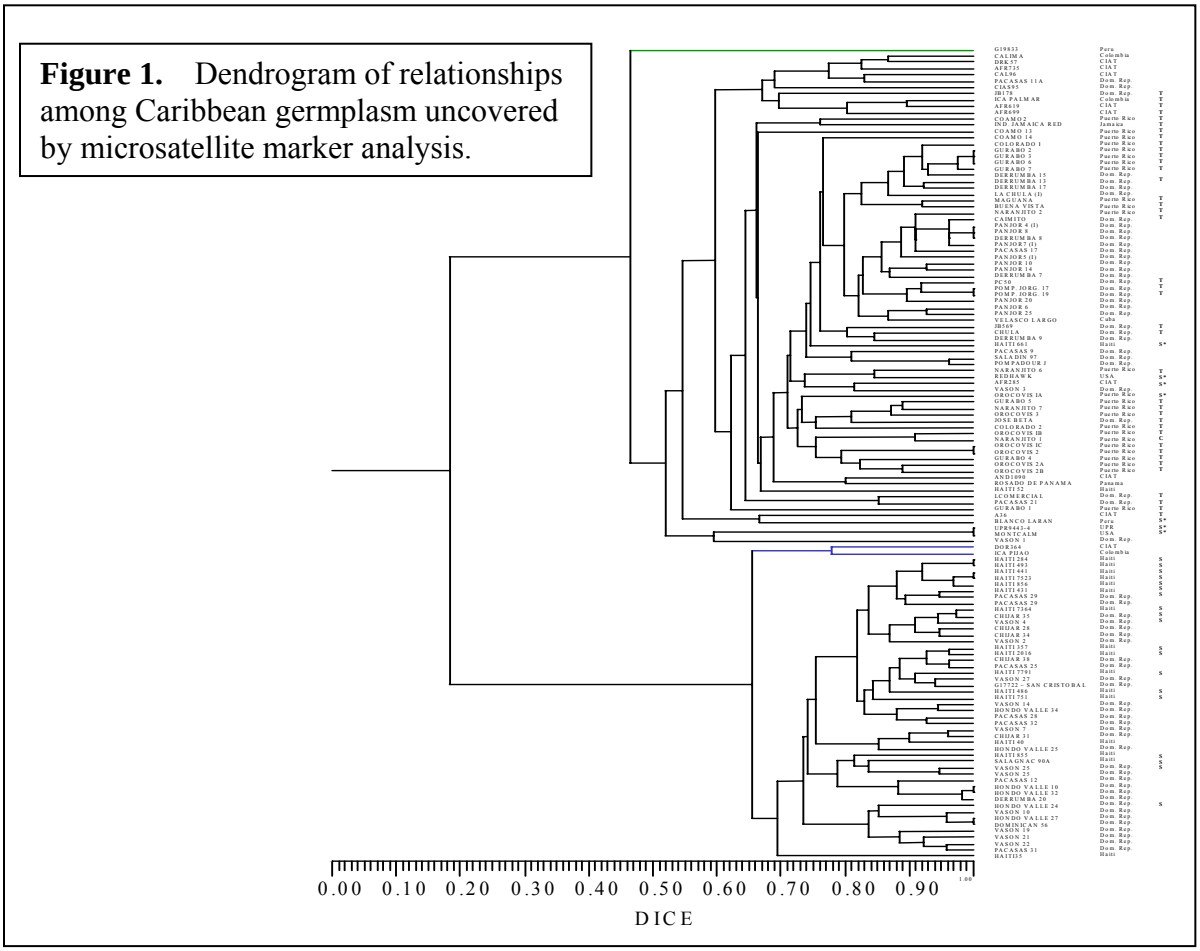

Figure 1. Dendrogram of relationships among Caribbean germplasm uncovered by microsatellite marker analysis.

ANALYSIS OF DIVERSITY IN WILD AND CULTIVATED COMMON BEANS FROM ARGENTINA

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Northwestern Argentina is the southernmost limit of the Andean domestication area of common bean hosting, like other areas in South America, wild relatives of beans, which are the main source of variability of beans. In addition, different cultivars developed at CIAT that are adapted to the environmental conditions of the country have been introduced and incidentally turn out to be important commercial cultivars.

Argentina is a major producer and ranked third among countries exporting common beans. Therefore the genetic characterization of commercial cultivars and of wild relatives of common bean by molecular markers is particularly important to link markers to genetic traits of interest. Therefore the purpose of our work was to characterize and identify sources of bean diversity by means of molecular markers in commercial and wild cultivars.

The ten most important commercial cultivars of common beans were characterize base on RAPD and ISSR markers (Table 1). Out of 16 RAPD primers, 4 showed polymorphisms among beans. Furthermore, cluster analysis and principal coordinates analysis associated beans either as members of the Andean or the Mesoamerican gene pool. These results were later confirmed by means of ISSR, which turn out to be better tools than RAPD markers to identify beans by their gene pool of origin though not to show differences between individuals.

Burkart and Bruchner (1953) describe the existence of wild populations of beans in the provinces of Jujuy, Salta, Tucuman, San Luis and Córdoba. Because these places have different environmental conditions and are geographically distant the development of landraces and wild populations might have been favored suggesting the existence of either genomic plasticity or genetically diverse beans. Therefore, we analyzed diversity of 10 wild populations of common bean based on morphological and molecular markers such as RAPD and ISSR. Even though molecular markers unlike morphoagronomic traits are neutral, they grouped bean populations, same as agronomic traits, based on their site of collection. The fenogram showed low levels of diversity suggesting also a common ancestor. Partitioning of the genetic variation between wild populations and agroecological areas by molecular markers (RAPD and ISSR) indicated that a considerable part of it was attributable to differences within populations. These results showed a less geographical structure of the Andean wild beans compared to the Mesoamerican ones.

Table 1. RAPD and ISSR polymorphic primer sequences used for analysis of ten cultivars of *Phaseolus vulgaris* L. with primer annealing temperatures and number of polymorphic bands amplified.

Primer name	Primer sequence (5'-3')	Annealing temp. (°C)	Number of polymorphic bands
RAPD			
OPA 02	TGCCGAGCTG	36	3
OPA 09	GGGTAACGCC	36	4
OPA 20	GTTGCGATCC	36	6
OPE 20	AACGGTGACC	36	4
ISSR			
1	$GAG(CAA)_5$	52	5
4	$CTC(GT)_8$	54	3
6	$(AG)_8CG$	54	2
7	$(AG)_8TG$	52	5
11	$(AG)_8$	49	2
13	$(CCA)_5$	55	4
16	$(AC)_8$	57	5
19	$(GCC)_5$	66	5
23	$(GAA)_5$	54	2

Reference

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Acknowledgements

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Principal Component Analysis (PCA) of Dry Bean Collection

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Collections of bean genotypes have been established at the research centers dealing with bean breeding. The collection was put together in such a way that it comprised genotypes with all grain colors of interest for the market. Simultaneously, such organization of the collection allows breeding for important market categories. The collection has been formed with the objective to study the variability of the genotypes in order to select those that are most suitable for breeding, both for hybridization and selection of lines from populations.

We studied the divergence of our beans collection for a period of three years. They represent a part of the working collection of Institute of Field and Vegetable Crops in Novi Sad. The study included two qualitative traits, grain color and shape, and 13 quantitative traits, namely three components of plant height, five direct yield components and five chemical properties of grain. For calculation of correlations among traits and differentiation of genotypes by multivariate analysis, each color and shape of grain were assigned a numerical code according to the idea of Hussani *et al*. (1977). The principal component analysis (PCA) showed which of the traits were decisive in genotype differentiation. The principal components analysis was based on Pearson correlation matrix and Euclidean distances. The Warimax method was used for the rotation of principal components. The percentage contribution of particular main components to total variability was shown, as was the accumulation of variability. The variability of the collection in was interpreted based on the seven principal components.

The first main component could be named component of productivity since it contains pronounced traits which determine the yield level. In the highest correlation with this trait are pod number, grain mass and grain number per plant. From hight components there is productive plant hight assissted by smaller portion of plant hight. Apart from that in lower correlation with this component are grain color and grain oil content. These traits have the largest participation in the divergence of the researched collection and carry the largest portion of its variability (Tab.1). Using this main component for genotype differentiation, we have distinguished between yielding genotypes with large number of pods and grain per plant, large productive height and high grain oil content. It is specific for the tested beans that black and white grain genotapes with the highest numeric values had the highest yield, eventhough they were included only due to grain color multivariate analysis. Such correlation is not necessarily expected in different samples and genotypes, as is neither connection of grain color in this manner with the first main component.

The second main component could be named grain shape and it shows large variability for the tested genotapes. Grain shape is one of the most obvious grain quality indicators (Kelly *et al*., 1998), grain market characteristics which sells bean as a species and foodstuff (Rosić *et al*., 1970). Selection route is based on grain shape (Acquaah *et al*., 1992; Vasic *et al*., 2001), which is why genetic collections are based on the presence of all grain shapes, with other traits different within one shape. The genotypes differentiated using it would take their place similarly within previously performed grain size distribution (Krusteva, L., 1997).

The third main component comprises of direct grain-related yield components. It combines explanation of productivity and grain quality. The traits which are in the strongest correlation with it are grain number per pod and grain number per plant as important productivity factors, as well as 1,000-grain mass which is grain quality determining trait. Considering the fact that kernel size is in negative correlation with this component, it could be used to distinguish

between genotypes with high 1,000-grain mass and small number of grains per pod which would have small number of grains per plant in large percentage, and genotypes with small kernel size with large number of grains per pod.

The fourth component would best describe genotype harvestability since it comprises of the highest portion of first pod height with plant hight influence. **In the following three main components** seed chemical composition content influence is dominant. Correlation of starch component with the fifth main component is high, as well as correlation of cellulose with the sixth and protein with the seventh component. Since these components are independent, the portion of other traits in the last three main components is low.

Table. 1. Principal components analysis traits of common bean collection

Traits	**Principal components: Rotated component loadings**						
	Y1	**Y2**	**Y3**	**Y4**	**Y5**	**Y6**	**Y7**
Grain color	0,63	-0,29	-0,06	-0,20	-0,23	0,46	0,24
Grain shape	-0,11	0,88	-0,28	0,11	0,05	0,05	-0,05
Total plant height	0,62	0,06	0,37	0,41	0,08	0,40	0,26
Height of the first pod	-0,30	0,12	0,01	0,86	-0,03	-0,11	0,10
Productive height	0,71	0,01	0,36	0,06	0,08	0,42	0,21
N^{o} of pods per plants	0,89	-0,06	0,25	-0,20	0,05	-0,01	0,16
N^{o} of grains per plants	0,79	-0,06	0,54	-0,13	0,07	0,01	0,14
N^{o} of grains per pod	0,12	-0,09	0,92	0,03	0,04	0,07	-0,01
1000 grains mass	-0,36	0,24	-0,78	0,05	-013	-0,11	-0,26
Grain mass per plant	0,87	0,01	0,05	-0,28	-0,12	-0,08	-0,05
Proteins	0,05	0,01	0,12	0,11	0,21	0,10	0,90
Ashes	0,13	0,47	0,16	-0,49	0,03	0,24	0,47
Starch	-0,07	-0,06	-0,11	0,02	-0,92	-0,15	-0,23
Fat	0,72	-0,07	0,02	-0,01	0,33	0,14	-0,260
Cellulose	0,05	0,11	0,10	-0,11	0,18	0,89	0,073
Latent roots	4,23	1,19	2,20	1,36	1,14	1,50	1,455
% of total variance explained	28,21	7,92	14,66	9,035	7,60	9,98	9,70
Summary communality	28,21	36,13	50,79	59.83	67,43	77,41	87,11

Acquaah et al. (1992) have found some of these traits (grain weight, number of grains per pod) important in research associated with architecture and seed size in breeding genotypes of middle kernel size dry bean.Combining usage of the set seven main components could yield a sucessful selection of genotypes suitable for donors of one or more important traits in breeding.

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Correlations in Dry Bean Breeding Collection

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Knowledge of correlations among bean traits is important for several reasons - it is possible to fully perceive the diversity of breeding material, to identify traits needed by a bean genotype to grow successfully under certain ecological conditions, to define breeding target and cultivar model and to recognize impediments and benefits of a breeding process well in advance.

We studied the divergence of our dry bean breeding collection (domestic populations, cultivars and selected lines) for a period of three years. They represent a part of the working collection of the Institute of Field and Vegetable Crops in Novi Sad. The study included seed color and shape, three components of plant height (TPH-total plant height, FP-height of the first pod, PH-productive height=portion of the stem where pods are formed), five direct yield components (P/Pl-number of pods per plant, S/Pl-the number of seeds per plant, S/P-the number of seeds per pod, AT-1000-seed mass or absolute mass and M/Pl-seed mass per plant or yield per plant) and five chemical properties of grain (Pr-proteins, Sta-starch, Cell-cellulose, ash and fats).

Seed shape, height of the first pod and the contents of proteins, ashes, starch and cellulose in grain had lowest correlations with the other traits. The highest correlations were found between plant height and productive plant height and between the number of pods and the number of seeds per plant. Negative correlations existed between seed size on one side and the number of seeds per plant, number of pods per plant, number of seeds per pod, plant height and productive height on the other. Seed mass per plant was correlated with seed color, productive height, number of pods per plant and the number of seeds per plant. Of the chemical substances in seed, only fat content was correlated with the number of pods per plant, number of seeds per plant and seed mass per plant (Tab.1).

It was expected that seed color and shape, basic market characteristics, will be the least dependent on the other characteristics. This is why bean genotypes differ so much in seed color and shape and yet they are invariably high yielding, adapted for growing in pure stand and with good culinary properties. In the studied collection, seed color was found to be associated with most traits. The occurrence of these correlations was due to the selection of materials included in the collection, which is not necessarily expected in different samples and genotypes.

There existed a high correlation between plant height and productive height, but neither of these was highly correlated with the height of the first pod. The same pattern was observed between these three traits on one side and the yield components on the other. In the study of currently grown SCG bean cultivars correlations of plant height and productive height with yield were established via the number of pods per plant and the number of seeds per plant. (Vasic *et al*., 1997). These results give a clear indication that the yield components are mutually very closely associated. Mitranov (1981) and Vasic *et al.* (1997) concluded that productivity was more dependent on the number of pods per plant than on the number of seeds per pod because the latter characteristic was quite stable in the climatic region, of the Balkan Peninsula, in which the study was conducted.

Correlations between 1000-seed mass and seed size on one side and other plant traits on the other are interesting for both breeding work and the understanding of bean ontogeny. In this study, 1000-seed mass had high and very high negative correlations with the other yield components and plant height. However, seed size had only a low negative correlation with seed mass per plant. According to Vasic *et al.* (1997) the Yugoslav assortment exhibited even a

positive direct correlation between seed size and yield, which was masked by the negative correlation between seed size and the number of pods per plant. Contrary to this, most authors that worked with the American bean gene pool mentioned a negative effect of seed size on yield level (Quinones, 1965; Duarte and Adams, 1972; Nienhuis and Singh, 1986; White and Gonzales, 1990). On the Balkan Peninsula, demands of the local markets dictated the development of cultivars that combined high yield performance with large seed size, so that the negative correlation between these two traits, if it existed, had been overcome by breeding. Bulgarian authors (Mitranov, 1981; Dimova and Svetleva, 1992; Stoilova, 1995) studied bean genotypes similar to those from Yugoslavia and their results were in full conformity with ours.

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Table. 1. Correlation coefficients (below the diagonal) and percentages of probability for lack of correlation among traits (above the diagonal)

	Col	Sha	TPH	FPH	PPH	P/PL	S/Pl	S/P	AT	M/Pl	Pr	Ash	Sta	Fts	Cell
Col	1,0						9,9				1,1		85,9		
Sha	-0,27	1,0	25,0	7,2	6,9					7,9	59,3	7,3	51,7	9,8	24,1
TPH	0,45	-0,10	1,0	22,5								1,4			
FPH	-0,38	0,16	0,11	1,0				96,7	4,8		16,2		69,2	0,8	10,5
PPH	0,52	-0,16	0,92	-0,29	1,0										
P/Pl	0,58	-0,24	0,58	-0,40	0,71	1,0					2,7		5,1		4,9
S/Pl	0,52	-0,30	0,65	-0,31	0,75	0,93	1,0						2,0		3,2
S/P	0,15	-0,31	0,42	-0,0	0,40	0,27	0,57	1,0		2,7	3,1	20,5	8,3	2,1	11,6
AT	-0,34	0,42	-0,58	0,18	-0,60	-0,63	-0,78	-0,71	1,0			2,6			1,0
M/Pl	0,57	-0,16	0,36	-0,44	0,52	0,83	0,74	0,20	-0,27	1,0	69,4	4,6	75,6		44,3
Pr	0,22	-0,05	0,37	0,12	0,30	0,19	0,23	0,19	-0,38	-0,04	1,0			61,4	1,2
Ash	0,25	0,16	0,22	-0,27	0,31	0,25	0,25	0,11	-0,20	0,18	0,33	1,0	1,3	11,6	
Sta	0,02	-0,06	-0,27	0,04	-0,28	-0,17	-0,21	-0,15	0,27	0,03	-0,38	-0,22	1,0	1,3	
Fats	0,43	-0,15	0,42	-0,23	0,50	0,54	0,52	0,20	-0,27	0,52	-0,05	0,14	-0,22	1,0	8,0
Cell	0,35	0,10	0,36	-0,14	0,41	0,17	0,19	0,14	-0,23	0,07	0,22	0,33	-0,33	0,16	1,0

Limit value for: 0,05 - r ,<0,174 – nonsign.; 0,01 - 0,174 > r < 0,225 - sign.; r > 0,225 – very sign. corr.
to 0.10 – no corr.; 0.11 - 0.25 - very low; 0.26 - 0.40 – low; 0.41 - 0.50 - middle;
0.51 - 0.75 strong; 0.76 - 0.90 - very strong; 0.91 - 1.00 - absolute

Assessing Heirloom Dry Bean Varieties as a Niche-Market Crop For Small-Scale Farmers

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Introduction

Consumers select heirloom varieties for their taste as well as the glimpse into history that they provide. A story connected with an heirloom variety may be passed down from generation to generation, or a variety may have cultural significance from centuries past. Farmers can save their own seed of heirloom varieties and the maintenance of genetic diversity may be one of the most significant reasons to grow heirlooms. Gene pools will continue to decrease if old varieties are not maintained.

The purpose of this research project is to gain a better understanding of heirloom dry bean varieties. Information such as emergence, stand, yield, and additional uses (such as green beans) could be useful for small-scale farmers. Additionally, understanding genetic similarities and differences among heirloom varieties will help us to maintain genetic diversity, will enable plant breeders to provide small-scale farmers with new niche-market varieties, and can provide consumers with an historical connection when they purchase the "old" varieties.

Methods

Replicated field trials were conducted in 2004 and will be repeated in 2005 at Washington State University Vancouver Research and Extension Unit. We planted 36 dry bean entries on May 20 in a randomized complete block design with four replications. Of the 36 entries, 9 were heirloom varieties and 27 were selected as representatives from each market class and also included breeding lines. Plots were two rows wide and 10 feet long. The field was certified organic and was maintained accordingly. Data was collected for days after planting (DAP) to 50% emergence and first flower. Plants were harvested from the center of each plot, for a total harvest area of ten feet per plot. Whole plants were harvested, dried, threshed and cleaned by hand. Total marketable bean yield (g) was measured. One hundred beans were randomly selected and weighed from each plot, length and width of 25 beans (cm) were measured. Five pods were randomly selected and the number of beans per pod was measured.

Results and Discussion

Heirloom varieties Calypso (68), Brown Dutch (67), Magpie (64), Pinto (62), Red Mexican (56), and Peregion (49) all exceeded the overall average plant stand of 47. The overall average total bean yield was 459 g. Mean yields of Nagel (691 g), CELRK (653 g), H9673-87 (639 g), Othello (616 g), and Burke (604 g) were all greater than 600 g. Mean yield of Pinto was the highest among the heirloom varieties with 538 g, Magpie (499 g) and Brown Dutch (480 g) were slightly above average, while Calypso (320 g) was the lowest. The overall average of beans per pod was 4.6. Of the heirloom varieties, Peregion had the greatest number of beans per pod (5.8) while Navy Pea (5.4) and Pinto (5.2) were both above average. The results from this study indicate that heirloom varieties are generally not low yielding as compared to newer varieties, however there may be room for selection within heirlooms to improve productivity.

Table 1. Results from heirloom trial at WSU Vancouver REU in 2004.

Entry	DAP 50% emergence	DAP to 1st flower	Pl. Stand # at Hrv	Yield (g)	Wth 25 beans (cm)	Lth 25 beans (cm)	Wt 100 beans (g)	Beans/ pod
Dark Red Kidney								
H9659-37-2	12.5	52.5	51.8	452.5	19.0	37.8	45.0	4.3
Montcalm[y]	14.5	44.8	34.3	396.0	20.3	40.0	57.8	4.2
Fiero	14.3	45.0	56.8	470.8	19.5	39.0	60.5	4.6
Light Red Kidney								
CELRK	13.5	42.5	50.8	652.5	19.8	42.5	63.3	3.8
Blush	13.8	45.5	45.8	507.5	20.3	39.8	35.5	4.1
W6 14733	-[z]	55.0	5.0	148.0	18.0	34.3	53.5	4.2
W6 14737	16.5	47.0	33.3	512.0	18.3	33.5	49.0	4.3
Great Northern								
BEL/NEB-RR-1	26.5	54.3	21.5	465.0	17.5	30.3	40.5	5.3
Small White/Navy								
Navy Pea[y]	17.3	54.8	38.0	402.5	14.0	21.3	18.8	5.4
Small Red/Mexican								
LeBaron	12.5	48.0	53.8	459.8	19.5	29.3	41.0	4.4
Red Mexican	13.0	45.5	55.8	428.8	18.3	27.5	32.0	4.6
UI-239	12.0	50.5	63.5	519.5	18.3	27.5	43.0	4.8
USRM-20	21.5	53.8	25.3	263.5	19.8	33.0	37.0	4.3
Black								
H9673-87	12.0	53.8	69.0	639.0	16.0	23.0	21.8	5.9
ICB-10-5	13.0	55.0	52.8	563.8	16.0	27.0	26.8	5.7
UI-911	13.5	46.8	45.3	488.0	15.8	24.5	31.0	6.2
Cranberry								
95:8186C	14.3	46.3	46.8	522.0	21.0	33.8	56.8	4.9
Cardinal	12.8	42.8	50.8	510.0	22.0	33.5	60.5	4.8
USCR-14	-	45.0	24.3	271.0	20.0	33.0	22.8	5.0
USCR-15	13.3	46.3	47.0	408.8	23.5	37.0	50.7	4.7
Pinto								
Burke	12.0	46.8	68.0	603.5	19.5	30.5	38.0	5.0
Othello	16.5	46.0	44.5	616.3	20.0	29.5	40.8	4.9
Pinto[y]	12.8	46.8	61.5	538.3	18.5	28.3	35.0	5.2
Nagel	13.0	47.3	64.8	690.8	20.0	33.0	66.8	5.6
USPT-CBB-1	15.0	46.8	34.8	420.5	19.3	30.0	42.5	4.5
Yellow Eye/Partially colored								
Main Yellow Eye	25.0	43.8	22.0	234.0	19.7	28.3	46.3	3.6
Molasses Face	13.8	51.0	50.5	436.0	20.0	39.5	45.8	4.3
Red Soldier	13.3	43.0	44.8	353.8	21.0	38.0	50.3	3.8
Soldier[y]	14.0	46.0	42.8	419.8	18.5	37.0	52.3	4.3
Brown or Yellow								
Brown Dutch[y]	12.0	42.3	66.5	479.5	21.0	30.8	43.3	4.4
PI 353479	13.3	45.8	54.7	437.7	18.7	31.7	48.7	3.6
PI 549776	12.3	45.3	57.8	483.3	20.0	34.3	54.0	3.7
Other								
Calypso[y]	15.5	42.8	68.0	380.0	20.5	30.5	55.0	3.4
Magpie[y]	12.0	44.3	63.5	498.8	14.8	35.8	35.8	4.7
Orca	14.5	54.0	41.0	449.5	18.0	29.5	34.5	4.6
Peregion[y]	12.0	52.0	48.8	398.8	16.5	27.3	27.3	5.8
Average	14.5	47.7	47.4	458.9	19.0	32.3	43.4	4.6
p value	0.0000	0.0000	0.0000	0.0031	0.0000	0.0000	0.0000	0.0000

y = heirloom
z '-' indicates 50% of the seeds for this entry did not emerge by 25 DAP.

New sources of phaseolin variation found in populations of *Phaseolus vulgaris* L. collected in its primary center of diversity

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Phaseolin (Phs), the major seed storage protein of common bean, has proved to be an excellent –cheap and polymorphic – marker in evolutionary studies (Gepts, 1988). Although this globulin has narrow range of molecular weights (45-52 kD) and isoelectric points (5.6-5.8) (Brown et al. 1981), up to the present a great number of types and sub variants of phaseolin have been found in wild, landraces and improved bean genotypes (Gepts and Bliss, 1986; Gepts et al., 1986; Koenig et al., 1990; Debouck et al., 1993; Tohme et al., 1995; Beebe et al., 1997). The present paper informs of several Phs types not reported previously and establishes standards for Phs morphotypes which are available internationally as genetic stocks.

Materials and Methods

The new phaseolin types were found in sampled seeds of several wild, weedy populations and landraces of *Phaseolus vulgaris* L. collected in its primary center of diversity. These Phs were analyzed as "selfed materials" of phaseolin type found for each analyzed seed. The accessions wich are reported here, were obtained from the world-wide collection held in CIAT (Table 1). The samples were analyzed in ID-SDS-PAGE (Brown et al. 1981) and confirmed later in 2D-IEF-SDS-PAGE (O'Farrel, 1975).

Results and Discussion

New phaseolin diversity found in beans from Mesoamerica. Using 1D/2D-IEF-SDS-PAGE, two new phaseolins were identified in wild and cultivated accessions from Mesoamerica (Figure 1). The first phaseolin was found only in the G11027 wild population from México, in the state of Durango. We suggest that this pattern be designated as "Durango" pattern ("Dur": as it brief name) making reference to the name of the Mexican state where it was found. This phseolin displays a simple type, similar to the "I" and "A" patterns, described previously among Andean wild beans (Gepts et al., 1986; Koenig et al., 1990). This pattern is constituted only by two bands with high molecular-weight, especially among the phaseolin bands (Table 1). The second new phaseolin type was first observed among wild accessions from Honduras (G50722B) and Costa Rica (G51062A). However, later it was found in several cultivated accessions (for example the G18970) from the Costa Rican locality of Telire (Table 1). This is the first time that the Mesoamerican cultivars have been observed to have a phaseolin type other than the "S", "CH" and "B" types (Gepts and Bliss, 1986; Koenig et al., 1990). According with the previous result, we suggested that this phaseolin be designated as "Telire" ("Tel").

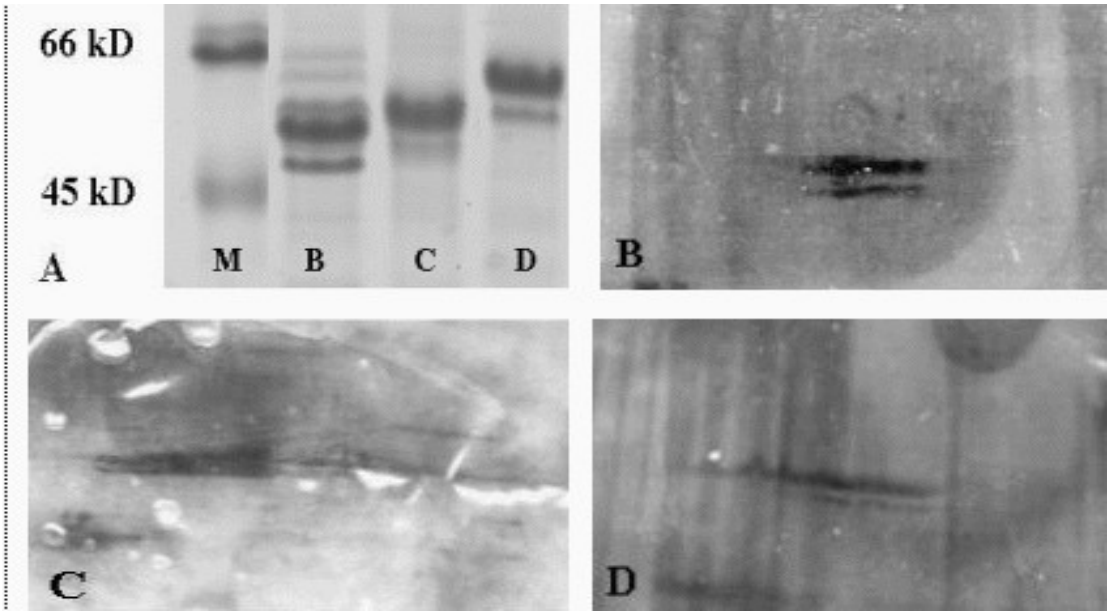
A novel simple phaseolin found in beans from Colombia. using 1D/2D-IEF-SDS-PAGE, a simple phaseolin type was observed for first time and exclusively in a Colombian cultivar (G24674) (Figure 1). According with the previous result, we suggested that this phaseolin be designated as "Quincho" ("Qui") making reference to the name of the cultivar where it was found. This phaseolin reveals a simple type (two bands in 1D-SDS-PAGE, see Table 1) similar to the simple patterns previously identified only in wild. This study confirms again that germplasm from Middle America and Colombia has the greatest amount of genetic variability of phseolin (Gepts and Bliss, 1986; Gepts et al., 1986; Koenig et al., 1990; Beebe et al., 1997).

Table 1. The molecular weight (MW) of bands in 1D-SDS-PAGE and isoelectric focusing (IEF) of peptides within each band are described for the new phaseolin types.

Phaseolin Types	Biological population			1D-SDS-PAGE (bands)		2D-IEF-SDS-PAGE (peptides)	
	Identification	Status	Gen. St[A]	Number	MW (kD)	Number	Total
Durango (Dur)	G11027	Wild	Mexdu-01	2	58.94	5	10
					56.00	5	
Quincho (Qui)	G24674	Cult.	Fi-4421	2	56.00	6	11
					53.20	5	
Telire (Tel)	G18970 G51062A G50722B	Cult. Wild Wild	Fi-5791 Fi-5765 Fi-3925	2	54.12	5	9
					50.55	4	

A: The accessions which are reported here as the source of each Phs morphotype will be maintained in CIAT as Genetic Stocks.

Figure 1. 1D-SDS-PAGE (A) and 2D-IEF-SDS-PAGE (B-D) electrophoresis of newly found phseolins. A: molecular weight marker (M) with its value of kilo-Daltons (kD), B: "Tel" phaseolin type, C: "Qui" phaseolin type, D: "Dur" phaseolin type.



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Molecular Characterization and Phylogeny of Thirty Common Bean Varieties

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Molecular characterization of varieties of potential use in research programs involving gene mapping, marker search or plant breeding can facilitate the choice of the most appropriate crosses. It can also be used to develop a phylogenetic analysis that can more accurately establish the centre of origin of such varieties.
In the present study the results of a molecular characterization of thirty common bean varieties are reported.

The following varieties were studied: the anthracnose differential cultivars, Michelite, MDRK, Perry Marrow, Cornell49-242, Widusa, Kaboon, Mexico222, PI207262, TO, TU, AB136 and G2333; nine breed lines or varieties carrying anthracnose resistance genes, A493, A321, A252, SEL1360, SEL1308, Sanilac, Catrachita, V204, and V225; seven bean varieties currently grown in Spain, Andecha, Xana, Cimera, Canela, Tolosana, Riñón and Ganxet; and the parent lines of a RIL mapping population taking part of the integrated genetic map, BAT93 and jaloEPP558.

Eighty-one molecular markers were analyzed, including 4 CAPs (Geffroy et al. 1998, Murray et al. 2002), 2 ISSRs (Hamann et al. 1995), 1 RAPD (Gonçalves-Vidigal and Kelly 2003), 19 SCARs previously described by different authors, 25 SCARs recently obtained in our laboratory (Pañeda et al. in preparation) and 30 microsatellites (Yu et al. 2000, Blair et al. 2003). Most of the SCARs were linked to disease resistance genes or other genes of interest. The markers were distributed among the eleven linkage groups (fig. 1).

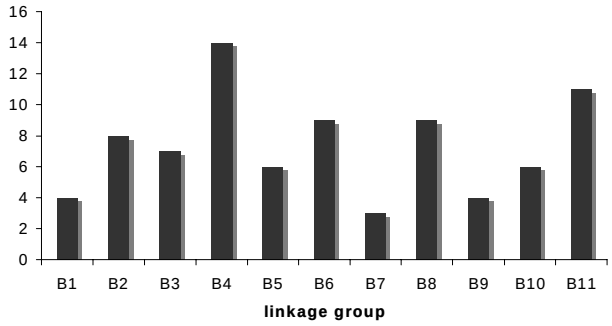

Figure 1. Number of markers located in each linkage group.

In summary, the 81 primer pairs used amplified a total number of 219 different polymorphic DNA fragments, microsatellites being the most efficient in this respect.

Figure 2 shows the phylogenetic tree resulting from the data obtained. It was built using the Wagner parsimony method for discrete traits (Eck and Dayhoff 1966, Kluge and Farris 1969). This was carried out using the PARS program from the PHYLIP package (Felsenstein 1989), using the default options (unrooted trees, ordinary parsimony and no weighted sites).

The varieties analyzed are distributed in two main groups, most probably related to their centers of origin, Andean (left) and Mesoamerican (right). In addition, when considered in some detail, the results are consistent with the previously known pedigrees. For example, Xana proceed from Andecha, and this variety, together with Cimera are very similar landraces from Asturias, Spain; BAT93 and AB136 proceed from PI207262 and Catrachita, respectively; Sanilac was obtained after X-irradiation from Michelite; and A493 proceed from a cross between Alubia (Andean) and BAT93. Finally, the relative positions of the twelve anthracnose differential cultivars are consistent with their previously known geographical origins.

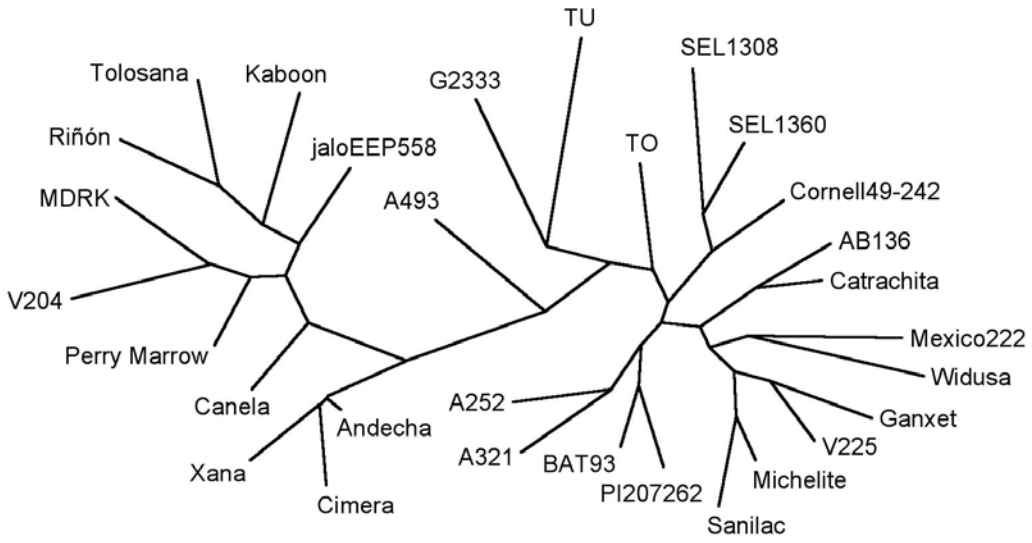

Figure 2. Phylogenetic tree of thirty common bean varieties. The length of the different lines in the drawing is proportional to genetic distances.

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GROWTH ANALYSIS OF *Phaseolus vulgaris* L. and *Phaseolus coccineus* L. IN A SALINE SOIL

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INTRODUCTION

Salinity is a major factor limiting crop growth and yield in the soils. In Mexico, the case of Montecillo Méx., sodium and other salts limit crop growth and seed yield. In particular, common bean which is salt sensitive. The yield of bean in saline soils has been reported by Escalante *et al.* (2003); however, it is required more knowledge of how the yield is limited. The aim of this study was to determine the relation between growth analysis index and biomass and seed yield of *Phaseolus vulgaris* L. and *Phaseolus coccineus* L cultivars.

MATERIALS AND METHODS

The study was carried out in Montecillo, Méx (19º N, 98º W and 2250 m of altitude) with dry climate (Bs) during the rainy season. Three cultivars of *Phaseolus vulgaris* L. (Bayomex, Criollo [indeterminate type], and Canario 107 [determinate type]) and one cultivar of *P. coccineus* L. ("Ayocote" with indeterminate type) were sown at a plant density of 6.25 (80*25 cm) plants m^{-2} on June 19, 2000 in a dry clay soil (pH 8-8.7, electric conductivity (EC) of 7-14 dS m^{-1}, and exchange sodium of 9.73-37%). When the soil is wet during the raining season, the EC is reduced to 1-2 dS m^{-1}. The bean species are commonly cultivated by farmers in no saline regions. All experiments were fertilized with 100-100-00 NPK. The experimental design was randomized block with four replicates. The biomass (dry weight) and leaf area of plants were sampled at 24, 53 and 83 days after the sowing (das) to determine the specific leaf area (SLA, leaf area/leaf dry weight, dm^2 g^{-1}), leaf area duration (LAD, days), crop growth rate (CGR, g m^{-2}

day^{-1}), and net assimilation rate (NAR, g dm^{-2} day^{-1}) according to Hunt (1981). The phenologycal stages were determined according to Escalante and Kohashi (1993).

RESULTS AND DISCUSSION

The cultivars of both species showed differences in days to reach flowering (FW) and physiological maturity (PM). The FW occurred at 51,42,42, and 73 das; and PM at 118,132,90 and 132 das for Bayomex, Ayocote, Canario 107 and Criollo, respectively. The SLA was different among cultivars and during the crop cycle. The highest value of SLA was 132 das except for Canario 107, and there were not a clear relationship between SLA and biomass. The highest LAD and CGR values gave high biomass and seed yield (Ayocote>Bayomex>Criollo>Canario-107), but it was not associated with the NAR. At PM the biomass was 371, 353, 260 and 206 gm^{-2}, and seed yield of 132, 179,86 and 41 gm^{-2} for Ayocote, Bayomex, Criollo and Canario 107, respectively. These results of the growth analysis indicate that LAD and CGR are the indices more related with biomass and seed yield in bean species. A high NAR is not associated with the biomass, and biomass production is more depend with LAD.

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Interspecific hybridization between *P. vulgaris* and *P. acutifolius* to transfer bruchid resistance

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While successful interspecific hybridization between *P. vulgaris* and *P. acutifolius* has been accomplished, only common bacterial blight resistance has been successfully transferred (5). Challenges include need for embryo rescue, and low fertility of generated hybrids caused by sterility and meiotic abnormalities. Abnormal chromosome recombination inhibits gene transfer (2,3,4) and can interfere with transfer of quantitatively inherited traits (1). Successful interspecific hybridization also depends on the choice of parents and the direction of the cross (3).

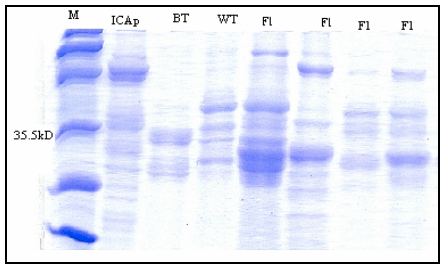

Figure 1. Seed storage protein profiles for ICA Pijao (ICAp), cultivated tepary (BT), G40199 (WT) and interspecific hybrids (F1).

Three *P. vulgaris* lines 'ICA Pijao', 'Rojo', and 5-593 were crossed with two *P. acutifolius* accessions (G40199 and an F_2 selection from G40199 x a cultivated brown-seeded tepary accession). G40199 is reported to be to be highly resistant to bruchids, and we have found that it has a novel 37 kd seed storage protein that may be an arcelin (Fig.1).

Flowers on *P. vulgaris* plants were emasculated and pollinated with *P. acutifolius* pollen. Developing pods were harvested 22 to 28 days after pollination and embryos were excised for *in-vitro* culture. Developing plantlets were transferred to potting soil and maintained until flowering and seed set.

Table 1. Comparative efficiency of three *P. vulgaris* female parents in generating interspecific hybrids with *P. acutifolius*.

	Cross combination			
	ICA Pijao x G40199	Rojo x G40199	5-593 x G40199	ICA Pijao x BTF_2
	Number			
Regenerated plants	127	143	118	8
Surviving in soil	56	12	36	4
F_1's generated	33	0	4	4
BC_1F_1	43	0	1	4

Putative interspecific hybrids were compared to the parents for leaf shape and size, and flower bud size. Days to flower, male fertility and relative pod set and parthenocarpy and percent backcross seed set was also determined. In addition to morphological traits, the 37 kd seed storage protein was evaluated using SDS-PAGE.

Of the three female parent lines, ICA Pijao had the highest overall crossing efficiency (Table 1). Rojo and 5-593 initially had good pod set with vigorous developing embryos that had rapid *in-vitro* growth. However, growth slowed upon transfer into soil, with plants showing dwarfing and severe chlorosis, and most subsequently dying. Some 5-593 hybrids flowered despite dwarf growth habit. F_1 hybrids from ICA Pijao produced smaller, slower growing embryos under *in vitro* conditions, but plants grew vigorously following transfer to soil. F_1

hybrids from ICA Pijao expressed intermediate morphological characters for leaf shape and size and flower bud size (Table 2).

Early flowering was observed from interspecific hybrids with the tepary F_2 selection. This line carried the novel 37 kd seed storage protein band observed in G40199 and was easier to manipulate because of its photoperiod insensitivity and earliness to flowering. All F_1 lines were male sterile and produced parthenocarpic pods. The level of sterility diminished with backcrossing; BC_1F_1 lines had reduced fertility, but some produced selfed seeds of poor quality. Percent seed set was increased with the second backcross. A higher percentage of seed were set in BC_1F_1 using the F_2 tepary selection as compared to backcrosses using G40199.

Table 2. Fertility and the morphological characteristics of rescued interspecific hybrids from tepary x common bean (ICA Pijao) crosses.

Generation	Percent		Hybrid characteristics			
	Parthenocarpic pods	Aborted (selfs)	Crossed seed set	Leaf W/D ratio	Floral bud (mm)	37 kd seed storage protein
F_1	many	100	5.1	0.61	10.3	Present
BC_1F_1	52.6	25	high	0.72	10.3	Present
G40199	-		-	0.53	7.4	Present
ICA Pijao	-		-	0.76	10.4	Absent

We screened some BC_1F_1 seeds for the 37 kd seed storage protein, and found some interspecific lines segregating for the protein. In an F_2 population of tepary x tepary lines that segregated for the seed storage protein, it behaves as a single dominant gene, but segregation in the interspecific crosses has not been determined. There apparently are few barriers to interspecific transfer of the novel seed storage protein. Next steps include demonstrating that the novel seed storage protein is associated with bruchid resistance, and that this resistance is retained in a *P. vulgaris* background. If so, further crosses will incorporate this trait into improved dry bean varieties for Africa.

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Two novel globulin variants found in the wild teparies beans (*Phaseolus acutifolius* A. Gray)

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We are reporting two novel globulin types found in wild populations of the tepary bean. In addition we present here a brief review of the reported patterns in the previous works. In teparies, the seed major storage protein is called globulin and has similar chemical characteristics (molecular weight, isoelectric points, serological cross-reactivity, etc.) as the phaseolin of common bean (*Phaseolus vulgaris* L.) (Sathe et al., 1994). However the sequence homology between its coding genes has not yet been verified. The globulins have provided an excellent model to study the domestication process and the geographic patterns of genetic diversity of tepary bean. Schinkel and Gepts (1988) report fourteen different patterns among wild forms, whereas only one pattern was identified in the cultivars. This first study suggests a single domestication in this species leading to a strong reduction in diversity. Another study contributed by Toro and Debouck (1989) increases the number of reported patterns to 25: 23 patterns were identified among wild teparies and two patterns in the domesticated form. Later Florez (1996) confirms these results of the previous studies and increases the number of patterns found to 27 (25 patterns among wild teparies and two patterns among the domesticated form). Therefore, we made a further screening of the seed storage protein (globulin) variability, focused on the material that was not worked by the previous investigators: we analyzed 68 accessions (50 wild and 18 cultivated) of *P. acutifolius* from the world-wide collection held in CIAT. This variation was first analyzed using one-dimensional SDS/PAGE electrophoresis (Brown et al. 1981) and confirmed later in two-dimensional IEF-SDS-PAGE electrophoresis (O'Farrel, 1975).

Results and Discussion

A survey of the globulin variability. The clasification of all the globulin types found in tepary bean with ID-SDS/PAGE electrophoresis was similar to those obtained previously (Schinkel and Gepts, 1988; Toro and Debouck, 1989; Florez, 1996). Figure 1 shows all these patterns [25 patterns among wild populations (patterns I to X, XII to XVIII, XX to XXVII) and two patterns in the cultivars (patterns XI and XIX)] with an excellent visualization of peptides over the globulin range, including the new reported patterns in the present study (patterns XXVIII and XXIX). These patterns consists of a series of similar polypetides whose molecular weight ranges between 41, 000 and 54, 000 daltons.

Finding the new globulin variants. Two new patterns were observed among wild populations of *var. acutifolius* and collected in Mexico. The first pattern (XXVIII) was found in the G40103 population collected in Sinaloa by D. G. Debouck, which showed previously the pattern XVII (Schinkel and Gepts, 1988) and the second pattern (XXIX) was found in the G40298 population (collected in Guerrero by Robert Reid). These patterns can be identified by one-dimensional electrophoresis parameters (Table 1): the pattern XXVIII by the presence of a pair of equally strained bands and with an average molecular weight (42.52 and 46.22 kD). The second pattern (XXIX) reveals a stronger band with a molecular of 44.70 kD. These patterns also can be observed in two-dimensional electrophoresis (Figure 2), using the isoelectric focus parameter. A single band in 1D-SDS-PAGE is often composed of peptides with slight differences in molecular weight but which overlap, creating a smear of peptides, which can be observed in 2D-IEF-SDS/PAGE. The IEF peptides within each band are presented for both patterns in Table 1. These results confirm the presence of two new globulin types for the teparies beans. In addition the fact that these new globulin patterns have been found only in wild material confirms the previously described trend of strong reduction in genetic diversity in the cultivated form of *P. acutifolius* Gray.

Table 1. Description of new patterns: the molecular weight (MW) of bands in 1D-SDS-PAGE and isoelectric focusing (IEF) of peptides within each band are presented for the globulins XXVIII and XXIX.

Pattern	Populations	1D-SDS-PAGE (bands)		2D-IEF-SDS-PAGE (peptides)	
		Number	MW (kD)	Number	Total
XXVIII	G40103	2	42.52	4	8
			46.22	4	
XXIX	G40298	1	44.70	4	4

Figure 1. One-dimensional SDS/PAGE gel of globulin types found in the tepary bean accessions (indicated under the pattern with its G number). First lane shows the molecular weight marker (MW) in kilo-Daltons (kD). The patterns are shown in the time order of publication: Patterns of I to XV (Schinkel and Gepts, 1988) and Patterns of XVI to XXVII (Toro and Debouck, 1989; Florez, 1996). The two last patterns (XXVIII and XXIX) are reported in the present study.

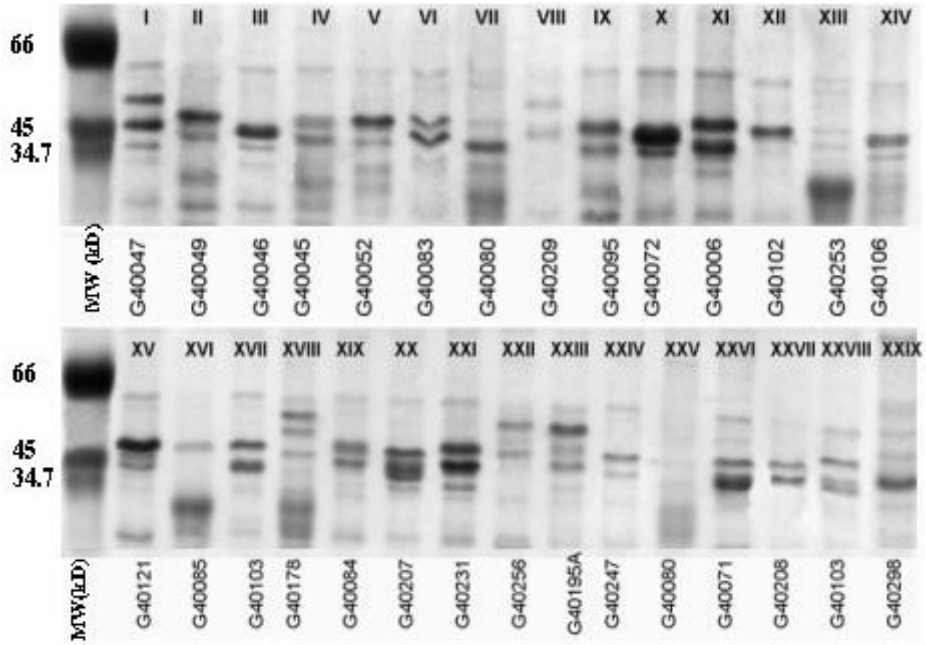
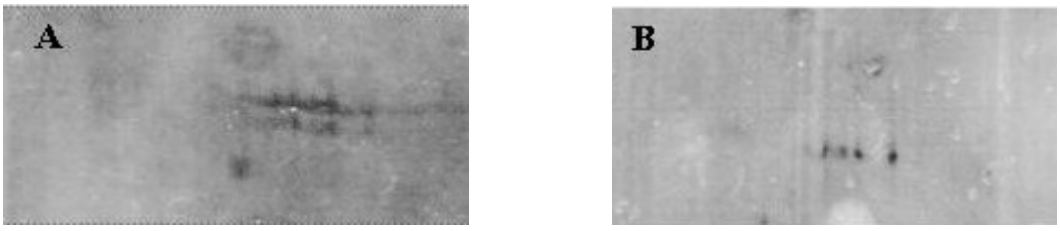


Figure 2. Two-dimensional IEF-SDS-PAGE electrophoresis of newly found globulins. A, pattern XXVIII (G40103) and B, pattern XXIX (G40298).



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Different expression levels of Lipid Transfer Protein gene during early stages of *Phaseolus coccineus* embryogenesis

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Introduction. In the genus *Phaseolus*, *Phaseolus vulgaris* L. is the most important species. It occupies more than 90% of production areas sown to *Phaseolus* species. In many regions, the common bean is susceptible to numerous pests and diseases, and for this reason, seed yield is low and instable. Other constraint limiting the yield is the lack of improved varieties tolerant to abiotic stress. In order to overcome these production problems, interspecific hybridizations between *P. vulgaris* and the two donor species, *P. coccineus* and *P. polyanthus,* are carried out to introgress desired traits into the recurrent species. Crosses between *P.vulgaris,* and the two other donor species lead to abortion of immature embryos, particularly when the donor parents are used as female [1]. These abortions can be caused by the disruption of major genes involved in embryogenesis process, as it is shown in studies using model plants embryogenesis. Significant genes in the embryogenesis process of these plants such as *Arabidopsis thaliana, Zea mays, Oryza sativa,* etc., have been studied. Among the genes analyzed, Lipid Transfer Protein (LTP), Monopteros, Twn1, Knotted-like, Empty Pericarp 2, etc. genes play also a significant role in the normal development of the embryo. LTP genes are involved in the polar transfer of the lipids towards the peripheral layers of the cells. Transcripts of these genes are confined to the outermost cell layer at the late proembryo stage but are absent in the suspensor, that distinguish the basal pole to the apical one of the proembryon. LTP genes are used as molecular markers to detect defects which occur during maize embryogenesis. In addition, they were found to be secreted and located in the cell wall. Thus, different roles were suggested for plant LTPs: participation in cutin formation, embryogenesis, defence reaction against phytopathogens, symbiosis and adaptation of plants to various environmental conditions [2, 3]. In this report, we show different expression levels of LTPs gene during *Phaseolus coccineus* embryogenesis by RT-PCR.

Materials and Methods. Lipid Transfer Protein gene was revealed in different genotypes of *P. vulgaris* (NI 637 and G 21245), *P. polyanthus* (G 35348) and *P. coccineus* (NI 16), using the following specific primers: GAGTTGTTTCCATGGCCACC (forward) and GAGTAGTTTTCAGTGCCTTC (reverse), and 'Titan One Tube RT-PCR' kit. Total RNA was extracted from young leaves using TRIZOL Reagent (Invitrogen) and the protocol provided. RT-PCR reaction was carried out using the following profile: 50°C for 30 min, 94°C for 2 min, 35 cycles of (94°C for 30 sec, 45°C for 30 sec, 68°C for 45 sec), 68°C for 7 min and 20°C for 5 min. The cDNA amplified fragments were sub-cloned into pCR 2.1 vectors using TA cloning kit (Invitrogen) and sequenced with LICOR System (IR^2, DNA Analyzer). The obtained DNA sequences were *in silico* translated into protein sequences, which were compared with some other LTPs protein sequences. Total RNA was extracted from *P. coccineus* (NI 16) ovules at different stages from 0 to 12 days after pollination (DAP) and semiquantitative RT-PCR was performed as described above. RT-PCR products were separated on 1% of agarose gels.

Results and discussion. The fragments revealed in the different tested genotypes have the same size (400 bp). Comparisons between amino acid sequences in NI 16 and LTPs amino acid sequences in other species show 92%, 78%, 76% and 67% identities respectively with

Phaseolus vulgaris (AAC49860), *Vigna radiata* (AAQ74627), *Helianthus annuus* (CAA63340) and *Davidia involucrata* (AAL27855) LTPs. The figure 1 shows the phylogenetic tree (A) and the amino acid sequences alignment (B) from these five sepecies.

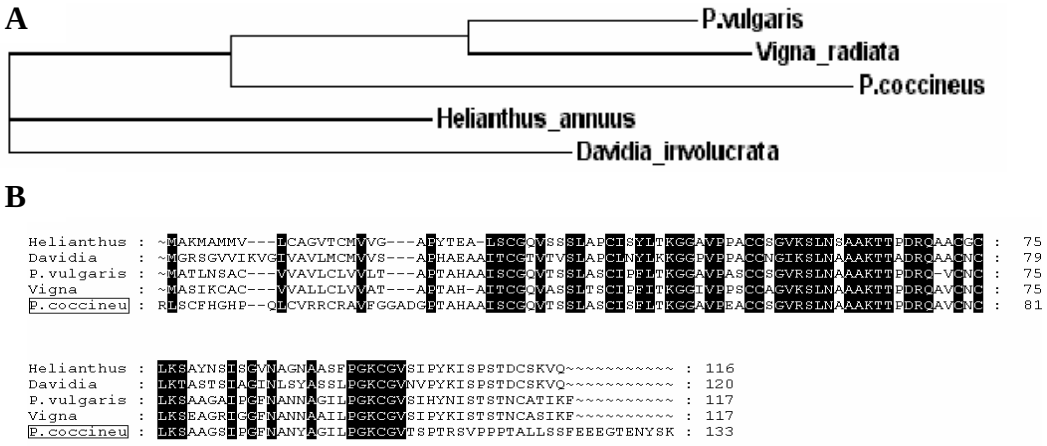

Figure 1. Relationships between *Phaseolus coccineus* LTP protein and other LTP protein sequences (*ClustalW*).

Expression of LTPs in NI 16 ovules at different stages from 0 to 12 DAP was analysed by RT-PCR (Fig. 2) using LTP specific primers. An amplification product was obtained in ovules from 0 to 10 DAP, but not at 12 DAP. Low transcript accumulation is detected at the earliest stages around pollination (0-1 DAP). Maximum transcript accumulation is reached between 2 and 5 DAP. Around 7 DAP, LTPs transcripts start to decrease and is absent 12 DAP. Different expression levels of LTP genes during *Phaseolus* embryogenesis suggest an important role played by the target genes during this process.

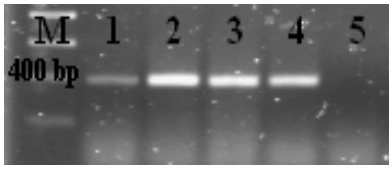

Figure 2. Expression pattern of LTP during *P. coccineus* embryogenesis in ovules at different stages. RT-PCR reactions were carried out on total RNA samples from ovules 0-1 DAP (**1**), 2-5 DAP (**2**), 6-8 DAP (**3**), 10 DAP (**4**) and 12 DAP (**5**), **M**: Molecular weight.

Prospects. We sub-cloned LTP cDNA fragments into pBluescript vectors, in order to synthetize labelled probes for *in situ hybridization.* This technique will be performed at different evolution stages of ovules resulting both from self pollinations and from degenerated ovules from interspecific hybridisations. This will enable us to locate the spatial pattern of this gene inside the ovule.

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Comparison of stigma areas among wild and cultivated forms (landraces and modern cultivars) of *Phaseolus vulgaris* L.

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Common bean is reported as a preferentially autogamous crop but high percentages of outcrossing have been reported (Ibarra et al. 1997). Natural crossing in this species depends on biotic factors (pollinators activity) associated with ecological conditions, and genotypic differences in the reproductive organs (Wells et al. 1988). The shape and spatial orientation of stigma play an important role in the process of pollination (Webster et al. 1977). We analyzed here the differences in the area of stigma among the cultivated (landraces and modern cultivars) and wild forms (representing thus the three biological states) of the Mesoamerican and Andean gene pools as currently known (Gepts 1988). The objective was to compare the proportions corresponding to the total, terminal and internal areas of the stigma. A preliminary analysis of 30 flowers for one cultivated accession and another wild showed that it was not necessary to analyze a high number of stigmas per accession to get enough precision in the statistical treatment.

Materials and methods

Four completely colored flower buds were collected for each of 39 accessions for each biological state in the two gene pools. These flower buds were immersed in a FAA solution (Bridson & Forman 1992). A cut of the style in the part proximal to the stigma allows diagrams of the stigma zone with a lucid camera adapted to a stereomicroscope (40x increase). Diagrams were black-inked (Figure 1) to make a scan of these images. Using the program WinRhizo.Pro.V2002c we obtained the exact data area (with a transformation of 1mm =3 cm).

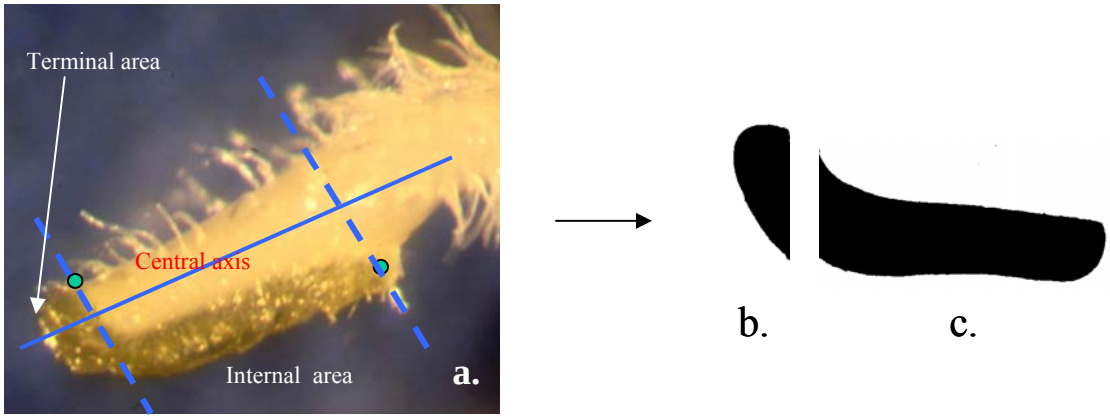

Figure1**. a.** Stigma of *Phaseolus vulgaris* showing terminal and internal area. **b,c.** Images of each zone.

Results and Discussion

A variance analysis indicates significant statistical differences at 95% probability in three major aspects:

1. Comparing the total areas there was a difference in the size of stigmas for the two gene pools, with a bigger size for the material belonging to the Andean pool irrespective of the biological state (0.35 mm^2 vs 0.3 mm^2).

2. Comparing each biological form, a bigger size of stigmatic area has been found in the cultivated materials as compared to the wild. For the Andean gene pool the average values of total area in modern and traditional varieties were of 0.35 mm^2 and 0.39 mm^2, respectively, in contrast with 0.30 mm^2 in the wild materials. For the Mesoamerican gene pool the mean values were of 0.31 mm^2 and 0.30 mm^2 in contrast with 0.27 mm^2.

3. Very significant statistical differences were observed about the proportions between the terminal and internal areas among the biological forms (Figure 2). This might be due to a bigger proportion of

stigmatic area towards the style tip in the wild material for both gene pools. In the Andean gene pool the terminal area occupies 46.03% of the stigma and in the Mesoamerican one 49.76%, which contrasts with an average proportion of 22.23% for the cultivated materials in both gene pools. These differences in the proportions of the stigma showed a bigger area towards the internal part of the flower in the cultivated material, possibly associated with gigantism resulting from the domestication process (Smartt 1988). This spatial balance of stigmatic areas promotes self-pollination, and by this way wanted characteristics in the cultivated materials can be conserved. The terminal area of the stigma – possibly an ancestral trait shared with related species of the genus (Freytag & Debouck 2002) - is probably associated with processes of cross-pollination. Proportionally bigger in the wild it could act as a precursor event leading to increased remanent genetic diversity, as shown by isoenzyme studies (e.g. Koenig & Gepts 1989) and gene flow as reported by Beebe et al. (1997) and Papa & Gepts (2003).

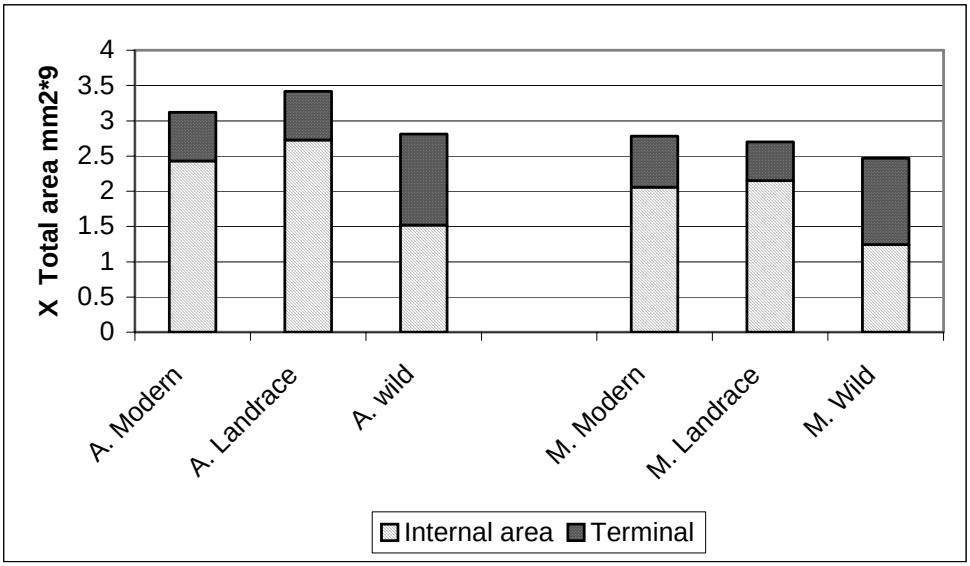

Figure 2. Comparison of proportions of stigma areas in Andean (left sided bars) and Mesoamerican (right sided bars) gene pools.

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Linkage Relationship Between a Common Bean Seed Protein and *Fin/fin*, a Gene Involved in the Genetic Control of Growth Habit

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Introduction

Plant growth habit is an important trait in common bean related to aspects such as yield or severity of different diseases. For this trait, four main phenotypes have been described in this specie (Singh, 1982; Debouck & Hidalgo, 1985), and different loci have been described as being involved in its genetic control (Bassett 1996; Tar´an *et al*., 2002). The first described locus was the *Fin/fin* gene (Norton, 1915) whose recessive genotypes (*finfin*) show determinate growth habit. This gene was located in B1 linkage group (Gepts *et al*., 1993; Koinange *et al*., 1996; Johnson & Gepts, 2002), close to *Ppd/ppd*, a gene involved in the genetic control of photoperiod sensitivity (Coyne & Schuster, 1974; Gu *et al.,* 1998). At present, not many efficient molecular markers linked to *Fin/fin* have been reported.

In this work a linkage relationship between a seed protein and the *Fin/fin* gene is described.

Materials and Methods

In order to investigate the inheritance of seed proteins and growth habit (determinate/indeterminate), two segregations were analysed. The first population was constituted by 104 $F_{2:7}$ recombinant inbred lines (RILs) derived from a cross between Xana (*finfin*) and Cornell 49 242 (*FinFin*). The second progeny included 226 F_2 seeds obtained from a cross between Sanilac (*finfin*) and G12587 (*FinFin*). The G12587 parent is an indeterminate landrace with photoperiod sensitivity. In addition, 193 accessions preserved in the S.E.R.I.D.A. collection, showing different growth habit phenotype, were analyzed for seed proteins.

The seed protein analysis and the designation of the different polypeptide bands were carried out according to Ferreira *et al*. (2000). Figure 1 presents a SDS polyacrylamide gel in which the *Sp9* (42 Kd) and *Sp10* (38Kd) polypeptides are indicated. The co-dominant nature of these two polypeptides have been previously described (Ferreira *et al*., 2000). The genetic distance between the loci were determined with the aid of MAPMAKER (Lander *et a*l., 1987), using a LOD score minimum of 3.0.

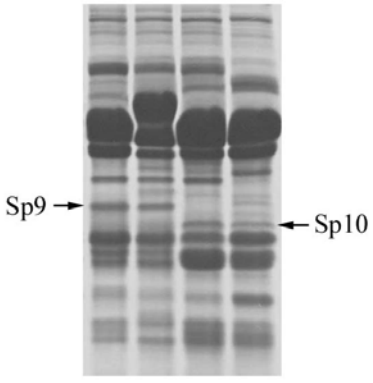

Figure 1.- SDS polyacrylamide gel (17% w/v) showing the allelic seed proteins *Sp9/Sp10* in four $F_{2:7}$ RILs derived from the cross between Xana (*Sp9Sp9*) and Cornell 49 242 (*Sp10Sp10*).

Results and Discussion

In the RILs Xana/Cornell 49 242 population, the observed phenotypic ratio for growth habit was 40 determinate and 59 indeterminate ($\chi^2_{1:1}$=3.65; p>0.05), indicating that this trait is determined by a single gene (*Fin/fin*). On the other hand, a segregation of 43 *Sp9* : 57 *Sp10* ($\chi^2_{1:1}$=1.96; p>0.05) was obtained. The *Sp9/Sp10* seed protein loci was linked to *Fin/fin* at a recombination fraction of 0.039 (LOD= 18.28), corresponding to 4.0 cM.

The efficiency of this molecular marker in the selection of determinate habit was successfully tested in the F2 progeny derived from a cross between Sanilac (*Sp10*, photoperiod insensitive) and

G12587 (*Sp9*, photoperiod sensitive). A total of 246 seeds were analyzed, and a segregation of 52 *Sp9 :* 120 *Sp9/Sp10* : 48 *Sp10* ($\chi^2_{1:2:1}$ =2.92; p>0.05) was obtained. The 48 seeds with the *Sp10* phenotype were selected and grown in the greenhouse, and 39 of them survived. Among these plants, 6 had determinate growth habit and were photoperiod sensitive, 32 were determinate and photoperiod insensitive, and only one exhibited indeterminate growth habit and photoperiod insensitivity. These results support the close linkage between the *Sp9/Sp10* locus, the *Fin/fin* gene and the *Ppd/ppd* gene, and their location in the B1 linkage group.

Finally, the polymorphism for the *Sp9/Sp10* seed protein was investigated in 193 accessions conserved in the S.E.R.I.D.A. collection (Table 1). The *Sp9* polypeptide was the most common in determinate accessions while the *Sp10* polypeptide was the most common in indeterminate materials. This association can be attributed to the linkage between the molecular marker and the phenotypic trait previously described.

Table 1. Identification of *Sp9/Sp10* proteins in 193 accessions conserved in the S.E.R.I.D.A. collection

Material	Determinate Sp9	Determinate Sp10	Indeterminate Sp9	Indeterminate Sp10
Accessions	21	3	64	105

Several RAPD markers linked to the *Fin/fin* gene have been previously identified (Park *et al.*, 1999; Pañeda *et al.*, 2004**),** although only four of them ($OF16_{1400}$, $OQ3_{450}$, $OA17_{950}$, $OA17_{600}$) were closer than 5 cM. These molecular markers are dominant and mostly showed coupling linkage with the dominant allele of *Fin/fin*. In this work, evidence of a new maker tightly linked to the *Fin/fin* gene is presented. Due to its co-dominant inheritance and to its probable linkage to the *Ppd/ppd* gene, the *Sp9/Sp10* protein can be very efficient in marker assisted selection for growth habit and photoperiod insensitivity.

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Flavanone 3-Hydroxylase: a Candidate Gene Product for the *P* Color Gene

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Introduction

Over the last decade there has been a heightened effort to educate the public on the benefits of a healthy diet, with an emphasis on the consumption of fruits, vegetables, and whole grains. The current USDA/CNPP- Proposed Daily Food Intake Pattern suggests consuming 7 servings of fruits and vegetables a day (based on a 2000 calorie diet), including 6 servings of legumes a week (3 cups). A healthy diet alone can prevent 30-40 percent of certain types of cancer, 60-75 percent of colon cancer (Doll and Peto, 1981). Consuming fruits and vegetables also reduces the risk of ischemic stroke, heart disease and type II diabetes, while increasing bone health. The increased media coverage on nutrition and health research has generated an increased consumer interest in diet and nutrition. The science of nutrition and health is focused not only on the essential nutrients (proteins, carbohydrates, fats and oils, minerals, vitamins, and water) present in fruits and vegetables, but also on the potential health benefits of plant phytonutrients. Phytonutrients include all plant compounds that are non-essential for human biological function.

Interest in plant phytonutrient research has steadily increased as researchers strive to understand how phytonutrients function in disease prevention. The flavonoids are a group of phythonutrients that have garnered a great amount of interest due to their potential health benefits. Flavonoids are present in all fruits and vegetables, however the amounts and types of flavonoids can vary greatly even between different varieties of the same species. The red wine and soybean industries have been very active in publicizing the potential health benefits of the naturally occurring flavonoids- flavonols and isoflavones, respectively- found in their products. Research shows that plant flavonoids, including the ones in red wine, soy, and dry beans, possess high levels of antioxidant activity. Researchers use antioxidant activity to estimate the disease-fighting potential of different phytonutrients. Research has shown that dry bean flavonoids impart color in bean seed coats. These same color compounds possess antioxidant activity. Three bean flavonoids- two anthocyanins from black beans (3-*O*-glucoside and petunidin 3-*O*-glucosode) and a flavonol glycoside from dark and light red kidney beans (quercitin 3-*O*-glucoside)- were shown to have high levels of antioxidant activity (Beninger and Hosfield, 2003).

Seed coat color in dry bean, *Phaseolus vulgaris* L., is largely determined by the eight principal color genes: *P, C, Z, J, G, B, V* and *Rk* (Prakken 1970) and their interactions, which lead to the expression of the colored flavonoids: flavonol glycosides, anthocyanins, and proanthocyanidins (flavonoid polymers)(Beninger and Hosfield, 2003). The ground factor gene, *P* (Prakken, 1934), is essential for color production in bean seed coat. When *P* is present in the homozygous recessive form, *pp*, the seed coat is always white. When dominant *P* is present, flavonoid pigments can accumulate in the seed coat. The identity of the gene product encoded by *P* is unknown. This research uses a candidate gene approach to determine if *P* could encode the enzyme flavanone 3-hydroxylase (F3H). F3H is the enzyme in the flavonoid pathway that hydroxylates flavanones at the 3 position of the C-ring and leads to the formation of dihydroflavonols (the precursors of both flavonols and anthocyanins). In several plant species F3H has been shown to have a pivotal position in this pathway. In *Fragaria vesca* (diploid strawberry), *F3H* maps without recombination to the known color locus (Deng and Davis, 1997).

In *Perilla frutescens* (Japanese basil), F3H is the first enzyme in the flavonoid biosynthetic pathway to be expressed only in red forma, anthocyanin-containing leaves (Gong et al., 1997). In *Zea mays* (maize) there is a direct correlation between *F3H* RNA levels and anthocyanin content (Deboo et al., 1995). This research focuses on the comparison of *F3H* expression and the coordinate expression of flavonoid compounds in white versus colored beans.

Materials and Methods

Crosses were made between black beans (5-593, San Fernando, Shiny Crow) and navy beans (Sanilac, Voyager and Nep-2). These crosses were propagated in the greenhouse to generate F_2 plants and seed. DNA extraction and PCR amplification were performed on selected F_2 progeny that represented both colored and non-colored seed. DNA extraction was performed using Edwards, et al. (1991) and DNeasy Plant Mini Handbook (Qiagen, Jan. 2004). PCR amplification was performed using a.) degenerate consensus primers from literature (Gong et al., 1997) and b.) primers designed using the program Primer Select, based on the published F3H sequences of *Arabidopsis*, *Fragaria*, and a partial sequence of *Phaseolus vulgaris* L. PCR products were run on a 0.8% agarose gel with <1% Ethidium bromide. A DNA molecular weight marker (100-1500 bp), 100 bp ladder was run on the gel (Roche) to determine approximate size of PCR products.

Results and Discussion

PCR amplification using the Gong consensus primers produced 2 bands of approximately 1000 and 500 bp. The primers designed from published sequences failed to amplify any product. The two amplified PCR products are being prepared for sequencing to determine if either sequence shows homology to known F3H sequences. RNA expression of the F3H gene and its association with color expression is forthcoming. Resolution of the functions of the genes responsible for flavonoid formation in bean may aid in the selection of varieties with higher antioxidant and nutritional values.

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Identification and sequencing of a BAC clone belonging to the *Phaseolus vulgaris* (L.) insecticidial Arc4 lectin locus

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Lectins are sugar-binding proteins that accumulate in high amounts in storage tissues such as seed cotyledons. In the *Phaseolus* genus, the ancestor lectin locus has extensively evolved into a single complex genetic locus, called APA locus, that contains a multigene family coding for up to three major components: arcelins (ARC), phytohemagglutinins (PHA) and α-amylase inhibitors (α-AI). It has been shown that these proteins, which are accumulated as seed storage proteins, are also involved in protection of the seeds against insect attacks.

Gene duplication with subsequent diversification has probably played a very important role in the evolution of this locus. Molecular evolutionary analysis of lectin and lectin related genes in common bean (*P. vulgaris*) demonstrated that a lectin ancestor gene underwent a paralogous duplication event, which gave rise to the progenitor of the true lectin (PHA) and to the progenitor of the lectin-related genes (Sparvoli et al. 2001, Lioi et al. 2003). The latter evolved originating the active form of α-AI, and in some wild genotypes underwent a second duplication event, giving rise to arcelin genes. Therefore, most likely arcelin and α-AI originated from a common ancestor and arcelin does not represent a precursor of α-AI.

To better understand the molecular evolution of the APA locus, the isolation and comparison of the entire locus from genotypes with different sets of APA members is under way in different laboratories, using BAC libraries. The wild accession G12949 (CIAT) containing the arcelin 4 variant was chosen as template for our BAC library construction. This accession contains the entire multigene lectin family (Arc/PHA/α-AI) and shows high resistance against some major storage insect pests.

The BAC library, consisting of 30,720 clones, was screened by PCR and filter hybridization. Thirty-nine positive clones were selected and analysed in order to identify overlapping BAC clones necessary to represent the entire APA locus. Preliminary results revealed that the complete locus is covered by at least three or four overlapping BAC clones. One of these clones (P58-F18) was chosen and sequenced. Putative coding sequences were predicted using FGENESH (http://www.softberry.com) and TWINSCAN (http://genes.cs.wustl.edu/) while repeated DNA sequences and microsatellites were identified using Tandem Repeats Finder Program (http://tandem.bu.edu/) and Sputnik Program (http://espressosoftware.com/pages/sputnik.jsp) respectively.

The BAC clone P58-F18 has an insert of about 130 Kb and sequencing data show that, besides many repetitive DNA sequences, microsatellites and retrotrasposons, it contains three full CDS for PHA-L, ARC4-I, ARL4 (Lioi et al. 2003) and a new CDS sharing 87% of similarity with ARL4 (called ARL4-I). In addition, several interrupted lectin and arcelin releted sequences were also found. Interestingly, the new gene ARL4-I (708bp long) shows 97% of identity in a stretch of 668 ungapped nucleotides with ARL5-IV pseudogene (EMBL acc. AF255723), suggesting that a close relationship between arc4 and arc5 genotypes might exist.

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Transcriptional analysis of the unfolded protein response (UPR) in lima bean cotyledons shows the regulation of many aspects of the secretory pathway

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Because of their abundance, storage proteins represent a good model system to study secretory protein synthesis and trafficking in plant cells. We are currently investigating the role of a particular kind of stress, the inhibition of N-glycosylation, on storage protein folding in the endoplasmic reticulum (ER) of developing lima bean (*Phaseolus lunatus*) cotyledons. This subcellular compartment is extremely important in developing cotyledons, whose major function is the accumulation and compartmentalisation of secretory storage proteins. Correct folding of newly synthesized proteins in the lumen of the ER is a fundamental prerequisite for their transport to other cellular compartments. Misfolded proteins, if not refolded by resident chaperones, are destined to degradation or might accumulate as aggregates in the ER (1). In both cases these events detrimentally affect the function, localization and, in the case of storage proteins, eventually the amount of proteins that are accumulated in the seed. The way cells monitor the physiological load placed on their ER and respond to perturbations in ER functions is known as unfolded protein response (UPR) (2). The UPR has been extensively studied and characterized in mammalian cells and in model organisms, such as yeast and the nematode *Caenorabditis elegans* (2), but very few data are available for plant cells (3).

We are interested to identify the function of genes/proteins involved in the mechanisms that lima bean cotyledonary cells activate when the UPR is triggered. The UPR is induced by treating developing cotyledons with tunicamycin (Tm), an inhibitor of protein N-glycosylation that causes accumulation of malfolded proteins in the ER (1). In order to identify genes which expression is induced by this treatment, we used the suppression subtractive hybridization (SSH) method to generate a subtracted cDNA library. About 30.000 recombinant clones were been obtained and about 1500 were double spotted onto a 3x3 array. Four replicas of each array were prepared and hybridized both with subtracted and unsubtracted control and treated mRNA probes in order to identify also poorly expressed genes. The screening analysis allowed us to identify 67 unique sequences that appear to be upregulated by Tm treatment. Among these, besides genes coding for chaperones (such as BiP and calnexin), we found genes involved in: protein translocation (*SEC61* and *SLS1* yeast homologues), ER-associated degradation (*HRD1* and *HRD3* yeast homologues), vescicle trafficking/transport (such as *VTI11* and *SYP5* Arabidospis homologues), glycosylation/modification (such as the UDP-galactose transporter). The upregulation of some of these genes was already reported in other organisms (*Saccharomyces cerevisiae* and Arabidopsis) (3, 4). In addition to the above cited genes many other genes (such as putative zinc finger proteins, CBF/NFY putative transcription factors, etc.) with a potentially regulative role have been identified and their true involvement in the UPR will be validated by qRT-PCR.

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Variation within the binding loops of Bowman-Birk inhibitors in common bean (*Phaseolus vulgaris* L.).

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Naturally occurring proteinase inhibitors are abundant in many food plants. Bowman-Birk inhibitors (BBI) are an important class of this inhibitor family and are typically found in legume species. They are present in all plant tissues but are particularly abundant in seeds. BBI are small proteins (6-9 kDa) able to form a stoichiometric enzyme-inhibitor complex with trypsin and chymotrypsin (Rachis et al 1986). The high interest towards this inhibitor class is due to the multiple roles that have been attributed to them. BBI decrease the protein digestibility and adsorption of dietary proteins, are a source of sulphur amino acids in plant tissues and play an active role in the mechanisms of defence towards insects and fungi. Recently, they have attracted the attention of researchers being effective in preventing or suppressing carcinogenic processes in a wide variety of *in vitro* and *in vivo* models (Kennedy, 1998). Some studies on soybean have shown that the trypsin inhibitory site is involved in bioavailability of dietary proteins (Liener, 1994), while the chymotrypsin inhibitory site is related to the anti-carcinogenic activity (Kennedy, 1998).

BBI isoforms have been isolated and characterised in many legume species. The potential exploitation of these inhibitors in plant breeding and human health-promotion programmes is strictly related to the elucidation of the molecular bases of the variation in biological activity among the different isoforms. The presence of several types of BBI in common bean, slightly different for both binding loops and/or single residue, has been recently described (Piergiovanni and Galasso, 2004). To understand better the binding loop polymorphism existing within and between the two common bean gene pools, we have isolated and sequenced the BBI obtained from 16 samples belonging to both Mesoamerican and Andean gene pool (6 and 10 samples, respectively).

Genomic DNA was extracted from young leaves and BBI sequences were amplified by PCR using two specific primers designed on the partial gene sequence encoding for the Bowman-Birk type inhibitor (Piergiovanni and Galasso, 2004). The amplified product of about 300 bp was gel purified and cloned in pGEM®T plasmid (Promega, USA). Several clones for each sample were sequenced with an automated sequencer.

By comparing the inserts of 104 clones, it were found 37 BBI types sharing 91 to 99% of similarity. The two types more frequent were found in more than 50% of tested common beans. The substitutions detectable by comparing the 37 BBI types generally involved more than one residue located outside as well as within the trypsin and the chymotrypsin binding loops.

On the bases of data available in literature it is difficult to assign significance of variation in inhibitory activity to particular amino acid residues outside the two binding loops. On the contrary, studies carried out on synthetic peptides, based on both active site loops, showed a correlation between specific residues and corresponding inhibitory activity. The binding loops relative to both the enzymes are constituted by a nine residue disulphide-linked motif. It is

known that differences of the inhibition activity as well as of BBI rate of hydrolysis are related to the residues in specific position of binding loops (Gariani et al 1999).

By analysing the deduced amino acid sequences we found that two residues, Ile or Arg, were present at the P'_2 position of binding loop towards trypsin. Moreover, Leu or Phe were detected at P_1 position of binding loop towards chymotrypsin. The calculation of the frequency of each type of binding loop within the common bean gene pools provided interesting information. In fact, the two variants observed for trypsin binding loop (Ile or Arg at P'_2 position) were always present in all tested Mesoamerican samples, while only four common bean samples belonging to Andean gene pool showed both types.

The variation observed for chymotrypsin binding loop involved the P_1 position. Fourteen investigated samples contain both Leu or Phe at this position of binding loop. The accession G 12569, belonging to the Andean gene pool, and the Mesoamerican landrace 'Fagiolo a pisello peligni' showed BBI types with only P_1-Phe or P_1-Leu respectively.

The existence of distinct active-site variants that can influence the protein inhibitory activity is very important for a nutritional point of view. In fact, selection for low BBI content is considered a desirable goal in breeding programmes aimed to increase the nutritional value of animal feed. Further studies on the BBI polymorphism in wild common bean material, and on the promoter effect on the quantitative gene expression are necessary. Very interesting information about the pattern of expression of BBI genes with active-site variants could be obtained from the common bean EST sequences that during next months will be released in GenBank (Hernandez , pers. comm.; Melotto pers. comm.).

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ENVIRONMENTAL INFLUENCE ON COOKING TIME

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A bean breeding program is carried out at the Instituto Tecnológico Agrario de Castilla y León (ITACyL), Valladolid, Spain. The main objective of this program is to introduce resistance to halo blight [caused by *Pseudomonas syringae* pv. *phaseolicola* (Burkh.) Young et al.], common blight [caused by *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye] and *Bean common mosaic virus* (BCMV) and *Bean common mosaic necrosis virus* (BCMNV), into high quality landraces. Maintaining culinary quality of landraces, in addition to improve yield and other agronomic features, is necessary since a bean cultivar with poor culinary quality may be rejected by consumers regardless of how agronomically superior it is. A large number of traits are measured at ITACyL to characterize culinary quality of genotypes (100-seed weight, water absorption, number of hard shell seeds, tegument content and cooking time, among them).
Dry bean culinary quality is influenced by cultivar, unpredictable environmental factors and storage conditions. The objective of this study was to determine the influence of the genotype and the environment in some physical traits (100-seed weight, water absorption, number of hard shell seeds, tegument content and cooking time), and correlation between them, that could aid to reduce the number of characteristics to measure and could simplify the selection method of culinary quality.

Materials and Methods

Twenty-five bean strains were grown in three locations in 2002 (Valladolid in experimental field of ITACyL, and León and Arévalo in grower field) and four in 2003 (same 2002, and Palencia in grower field) in Spain. Twenty-five genotypes were tested, including landraces from Spain and Portugal, selections from local landraces, new Spanish cultivars and varieties from USA and CIAT. Most of them are large or medium white-seeded Andean genotypes, but small and colored-seeded genotypes are also present. The entries were planted in 4-row plots, each 5 m long, in a row and column latinized alpha design with 3 replications. Distance between rows was 50 cm apart in Valladolid, Arévalo and Palencia, and 55 cm in León. Plants were spaced 8 cm in the row for all genotypes. Standard cultural practices were employed. At maturity, plants from the 2-central rows of the plot were harvested. One sample of dry beans from each replication was assessed for 100-seed weight, number of hard shell seeds and cooking time, and two samples for water absorption and testa content. Beans with a fresh weight equivalent to 10 g were soaking for 10-20 hours at 12ºC, to obtain water absorption, testa content and number of hard shell seeds. Cooking time was determined with a 30-seed Mattson pin-drop cooker (Mattson 1946). Cooking time was calculated as a time from initial cooking until the time when 80% of pins penetrate seeds in cooker. Data were subjected to analysis of variance (proc GLM) using as environment a combination of year and location. Also, data were used to calculate the correlation matrix for the traits. All data were analyzed using a SAS statistical package (SAS Institute, 1985).

Results and Discussion

Differences among cultivars, environments and cultivar by environment interactions, were highly significant for all the traits, as reported earlier by Balausbramanian et al. (1999) (Table 1). The location effect was larger than the genotype effect for almost all the evaluated quality traits, as has been reported earlier (Ghaderi et al. 1984, Hosfield et al. 1984), except for the number of hard shell seeds, which seems a cultivar characteristic.

Landraces, represented in the experiment by typical large and white-seeded cultivars from Iberian Peninsula, showed small number of hard shell seeds and low testa content. Also, landraces showed lower cooking time and higher water absorption.

2003 growing season was extremely hot, whereas 2002 was an atypical cool season. Results for number of hard shell seeds, testa content and seed size recorded in 2002 were more desirables than in 2003, although the harvest dates were extremely delayed and part of the harvested seed had to be discarded because of lack of commercial value. The data of this experiment indicate that temperature at seed filling stage influences on the quality traits measured.

Correlation between several traits was found, indicating that a relationship existed among some characteristics (Table 2). The number of hard shell seeds was not correlated with cooking time, because of hard shell seeds were removed before cooking. That is the reason because the correlation between cooking time and water absorption was positive, contrary to previous reports (Castellanos et al. 1995).

Table 1. Mean squares and means for quality traits of 25 genotypes grown in seven environments in Spain.

	Mean Squares			
Trait	Cultivar	Environment	Cultivar*Environment	Mean
Number of hard shell seeds	764.10 ***	141.46 **	105.90 ***	6.32
Cooking time	625.83 ***	3546.62 ***	140.29 **	56.93
Water absorption	309.29 ***	972.19 ***	183.79 ***	114.40
Testa content	2.43 ***	28.71 ***	0.68 ***	8.68
Seed size	1616.65 ***	1839.38 ***	53.52 ***	40.90

*, ** , *** Significant at 5% (*), 1% (**) and 0,1% (***) levels

Table 2. Correlation coefficients indicating the relationship among 5 quality traits of 25 genotypes tested in seven environments in Spain.

	Cooking time	Water absorption	Seed size	Testa content
Number of hard shell seeds	0.0143	-0.1578 **	-0.1663 ***	0.1217
Cooking time		0.2378 ***	0.0301	0.3798 ***
Water absorption			0.0552	0.1521 **
Seed size				-0.3481 ***

*, ** , *** Significant at 5% (*), 1% (**) and 0,1% (***) levels

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Canning quality and Common Bean Preference in Brazil.

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In the early years large seeded beans (LSB) were planted in cool climate and preferred by the Brazilian consumers. Due to limited land for expansion for these LSB in the cool regions, bean production moved to warmer regions and small seeded beans (SSB) dominated the production. SSB have better adaptation to the warm climate and possess higher and more stable yield. Several large seeded beans are still being grown and consumed locally and command a higher price than the small seeded ones. Cv Pérola has been well accepted and dominates the market due to 40% larger seed size by the consumers and has become a commercial standard for SSB. This marked the quality demand by the urban consumers, who wants slightly larger bean size among the commercial available in SSB, thus the appearance is the first step, before cooking time and taste, to make the decision of purchasing the product. More than 80% of Brazilian population live in the cities and bean consumption is diminishing due to long cooking time. The bean industry want to capture the market by offering canned bean, but the commercial cultivars crack and split during the processing giving the impression of second quality bean.

The Grain Quality Laboratory at CNPAF-Embrapa in Goiania tested nine large seeded and two commercial beans (Pérola and BRS Valente) for their quality parameters, e.g.,: broth thickness, fiber and protein contents, cooking time with and without 0.5% NaCl, seed coat percentage, water absorption before and after cooking, cracking percentage, appearance, and broth, using the standard methods utilized by the CNPAF. Darkening coefficient is calculated from measured reflecting light at 9 and 15 days of storage in the dark and exposed to sunlight, to accelerate the darkening process.

The results are shown in two Tables below. Differences exist between LSB and SSB groups and within the group in several parameters e.g., water absorption before and after cooking; cooking time with and without salt addition, soluble solutes, split after cooking. Hectoliter weight is the specific weight of the bean grain and on average both groups have similar density. Diacol Calima has the highest density and the BRS Radiante has the lowest. Seed coat percentages in LSB are lower than SSB. Percentages of water absorption after 16 hours was higher in LSB but variation among the cultivars were also great. WAF 69 and Hooter in LSB group had lower water absorption and also the longest cooking time without NaCl. In general LSB needed longer cooking time than SSB. This may be the reason that in the future LSB will remain in the market for culinary purposes and SSB will dominate the daily bean consumption in Brazil due faster to cook. Cooking time with additive such as NaCl lower the cooking time for all cultivars tested. Soluble solutes in LSB are lower than SSB and consumers like thick broth. Fiber content did not differ either within or between the groups. The protein content is almost the same between the two groups but variation within LSB group is large. The DRK 18, WAF 69, Etna and Hooter had the lowest protein content while SUG 33 had the highest one.

Almost all cultivars except the white seeded turned darker over time, but in some cases the darkening process stabilized after 9 days.

Conclusion:

Small seeded beans will still dominate the market in Brazil but need urgently new bean cultivars that attend the canning criteria. Large seeded bean will be used as culinary commodity and

command a higher price. Large seeded bean production will take place for exportation because of the low cost of production, provided there are suitable cultivars that meet the international market standard.

Table 1. Canning quality parameters for large and small seeded beans.

Identification	100 seed wt (g)	Hectoliter wt (Kg)	Seed Coat (%)	Water Absorption Before Cooking (%)	Water Absorption After Cooking (%)	Seed Darkening Coefficient after harvest*	
						9 days	15 days
						Treated Seed	
SUG-33	72,53	69,8	9,41	108,05	138,82	4,3	6,1
DRK-18	61,31	74,3	7,87	111,26	139,96	4,8	3,3
DIACOL-CALIMA	60,30	78,4	9,31	109,16	132,49	3,0	2,1
ETNA	49,51	76,2	9,18	109,96	135,48	8,5	6,6
HOOTER	49,08	74,0	9,95	99,89	126,71	11,4	4,3
WAF-69 (white)	44,31	71,2	11,29	81,18	113,85	0,0	0,0
BRS-RADIANTE	44,21	69,2	8,43	101,94	129,22	5,7	5,7
JALO EEP	41,19	68,6	9,49	109,86	120,00	2,0	5,9
JALO PRECOCE	36,97	68,0	9,27	105,59	155,09	1,9	3,5
Mean	**51,05**	**72,2**	**9,36**	**104,10**	**132,40**	**4,6**	**4,2**
PÉROLA (carioca)	28,36	71,6	9,78	104,12	125,87	8,1	5,7
VALENTE (black)	23,51	74,3	10,23	94,40	111,55	-	-
Mean	**25,94**	**73,0**	**10,01**	**99,26**	**118,71**	**8,1**	**5,7**

*Darkening coefficient is calculated with the following formula for treated seeds: [(LC - LTd)/LC]*100, where LC is the luminosity value of control seeds; LTd is the luminosity value of treated seeds after 9 or 15 days harvest. Control refers to seeds kept in the dark and treated means that seeds were exposed to sunlight for 9 and 15 days.

Table 2. Canning quality parameters for large and small seeded beans (cont.).

Identification	Cooking Time (min.)		Soluble Solutes (%)	Fiber Content (%)	Split (%)	APEAR	Broth Thickness	Slime	Protein (%)
	w.o. NaCl	w. 0,5% NaCl							
SUG-33	37,0	28,0	6,32	12,82	3,5	Great	thick/dark	-	23,87
DRK-18	21,0	15,5	7,44	11,35	13,0	Good	thick/dark	+	15,55
DIACOL-CALIMA	16,5	16,5	7,64	12,18	6,0	Good	thick/dark	-	20,28
ETNA	36,5	35,5	7,07	10,22	8,0	Great	thick	++	14,70
HOOTER	38,0	33,0	6,25	11,55	2,5	Great	thick	-	15,73
WAF-69 (white)	39,5	37,5	4,98	12,71	4,5	Good	very thin/light	-	15,77
BRS-RADIANTE	19,5	18,0	8,74	12,67	2,0	Great	ralo/turbid	+	20,65
JALO EEP	24,0	19,0	8,91	12,53	4,0	Good	thin/light	+++	19,99
JALO PRECOCE	22,5	17,5	9,67	13,04	0,5	Great	thin/light	+	21,66
Mean	**28,3**	**24,5**	**7,45**	**12,12**	**4,9**				**18,69**
PÉROLA (carioca)	23,5	20,5	10,19	12,33	9,5	Regular	thin/light	+++	17,41
VALENTE (black)	22,5	18,0	10,51	12,37	13,5	Good	thick	+	18,13
Mean	**23,0**	**19,3**	**10,35**	**12,35**	**11,5**				**18,13**

EVALUATION OF CANNING QUALITY OF DRY BEANS GROWN IN DIFFERENT REGIONS IN CANADA.

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Introduction: Recently dry bean production in Manitoba has increased, especially navy beans. Further expansion of bean production of various market classes are realized to the western Prairie provinces. The processing quality of dry beans has become a concern for the bean industry because bean cultivars might interact with various production conditions (environments). This prompted an evaluation of the canning quality of beans grown in all provinces. Thus an initial study on canning quality of navy beans grown in Ontario and prairie province was conducted. Four early maturing white bean cv. AC Mariner, OAC Seaforth, Envoy and AC Skipper were grown at St. Thomas, ON, Morden, MB, Saskatoon, SK and Lethbridge, AB during 1999-2001. Bean samples from these sites were processed and canned in tomato sauce, and then examined for organoleptic and instrumental attributes.

Materials and Methods

Four early maturing navy bean cultivars, AC Mariner, OAC Seaforth, Envoy and AC Skipper were grown at St. Thomas, ON, Morden, MB, Saskatoon, SK, and Lethbridge, AB. The four cultivars were seeded at an appropriate planting time at each site and the trials were laid out in RCBD with three replications at each site during 2000-2001. Upon harvesting the trials, 500 g of bean samples from each site were collected.

Processing of beans at Harrow, 500 g of beans are tested for moisture and dry bean colour. Beans were blanched in a static blancher for 40 min at 88 C, rinsed in cold flowing water for one min and allowed to drain for one min. The total volume of beans was calculated and beans were placed in 8 oz cans using 180 ml container and potential yield of canned beans were estimated. The can was topped off with hot tomato sauce leaving approximately ½ cm headspace. The can was seamed and sterilized in a static retort at 118 C for one hr and then cooled. All cans were then stored for at least 2 weeks prior to evaluation.

Evaluation of canned beans was conducted two weeks after canning and shelving for organoleptic and instrumental tests at Harrow as follows:

Organoleptic test: Eight panellists gave a sensory evaluation for appearance, flavour, and texture and these attributes were scored as 1=poor, 3=good and 5=excellent. Total score was obtained as an average of the summed scores (max. 15) of the three attributes.

Instrumental test: Canned bean yield was estimated by number of 8 oz. cans filled with blanched beans of 1,000g of dry beans. Colour of dry and canned beans was measured using a Hunter Labscan colorimeter (Hunter model D25) on three reflectance scales, L(white to black), a (red to green), and b (yellow to blue). Packing (1-5) in can was scored visually as a degree of clumping of beans as 1= no clumping and 5=at least half of beans clumped, and drain weight (% solid) and hydration coefficients were measured as percent of water absorption of a 500 g sample. Texture of washed-drained canned beans were estimated on an Instron texture measurement system using wire extrusion cells, and recorded for plateau force as an average of force during 4 cm of travel of the plunger measured by N, and firmness as a slope of the line leading to plateau expressed in N/mm.

Analysis of data: Combined analysis of the location and years were conducted using SAS.

Results and Discussion

Beans supplied from all four sites in 2000 and 2001were processed at Harrow and combined analysis of the data collected from evaluation of the canned beans is presented in Table 1. Sensory evaluation showed significant total scores for yearly variation and year*location interaction. This suggests that sensory scores at each site over two years were not consistent. Dry bean colour (L) showed significant difference for years (Y) and locations (L). Overall seed colour of beans produced in Saskatoon and Lethbridge appears to be whiter than those grown in Morden and St. Thomas probably because of dry weather. Also a significant Y*L interaction suggests that colour differs at different locations over the 2 years.

Instrumental test results showed significant differences for seed colour, packing (clumping), hydration coefficient, washed-drain solid weight and texture measured as firmness and plateau force between years, and among locations (Table 1). In addition, these attributes also showed significant Y*L interactions. Beans grown in Saskatoon and Lethbridge showed slightly higher can yield than beans produced in the eastern provinces. Beans grown in St. Thomas exhibited less packing than those from Morden. Beans grown at Lethbridge showed higher water uptake and those from St. Thomas had lowest hydration coefficient. Washed-drain weight (% solid) is required by regulation to be over 60%. All the cultivars met the requirement, ranging from 63.7 % at Saskatoon to 67.9% at Morden. Beans produced at St. Thomas showed firmer texture than beans produced in the western provinces but it fluctuated among the locations over 2 years as detected by a significant Y*L interaction. Cultivars reacted differently at different locations as indicated by significant location by cultivar for hydration coefficient and washed-drain weight.

Overall quality attributes varied by Y*L interaction in general in addition to most attributes differing among locations and years. There was no single attribute rated poor at any site. Generally beans produced in Ontario and the Prairie are acceptable for processing of canned beans.

Table 1. Combined analysis of variance of canned bean quality attributes of navy bean cultivars grown in Ontario, Manitoba, Saskatchewan and Alberta during 2000-2001

Source of variation	Organoleptic test				Dry bean colour[4]			Can [1]	Packing [2]	Hcoef [3]	WDWt [5]	Texture [6]	
	appr	flav	text	total	L	a	b	yield	(1-5)		(%)	firm	platf
Year (Y)	**	**	**	**	**	*			*	**	**		*
Loc (L)					**	*	**	**	*	**	**	**	**
Y * L	**		**	**	**	**	**	**	*	**	**		**
E (a)													
Entry (E)	**			**		**	**		**	**	**	**	**
Y * E											*		
L * E						**	*			**	*		
Y*L*E						**				*			

*, ** significant at p=.05 and .01

1. Yield number of 8oz. Cans filled from 1.0 Kg of blanched beans.
2. Packing: Degree of clumping of canned beans is visually scored as 1=no clumping and 5=at least half beans clumped.
3. HC = Hydration Coefficient (water uptake); percent water absorption of 500g dry beans.
4. Seed and bean colour recorded by Hunter colorimetric readings of 'L' (white to black), 'a' (red to green) and 'b' (yellow to black).
5. Washed drain weight (%), requires over 60%.
6. Texture: PF = plateau force: average force during 4cm of travel of the plunger, measured in N. F= Firmness; slope of line leading to plateau expressed in N/mm.

PHENOLIC ACIDS PROFILES OF THREE CULTIVARS OF BLACK BEANS

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Introduction

Dry beans are a staple food for many Latin American and African countries and are largely recognized as a beneficial source of proteins. There are several publications linking bean consumption to reduced risk of diabetes, obesity, cancer and cardiovascular diseases (1). These potential health benefits of beans have been attributed to the presence of micronutrients, such as phenolic compounds, that possess antioxidant properties (2,3). There are several reports in recent literature on extraction and analyses of polyphenols from fruits, vegetables, herbs and soybeans. However, isolation extraction, structural elucidation and analyses of phenolics from beans have received limited attention. The research publications on beans have primarily been focused on identification of anthocyanins, flavonol glycosides, and isoflavones and their influence on the seed coat coloration (4-6).

In our research on phenolic acids, we have observed that black beans contained significant amounts of phenolic acids. A systematic study was undertaken to isolate, identify and quantify phenolic acids in three varieties of Black bean.

Materials and Methods

Dr. M.A. Pastor-Corrales of the vegetable Laboratory at USDA (Beltsville, Maryland) provided three black bean cultivars, namely, T-39, Jaguar and Eclipse. Standards of phenolic acids (caffeic, ferulic, para-coumaric and sinapic) were purchased from Sigma (St. Louis, MO, USA).

Saponification and extraction of free phenolic acids

All bean samples were ground in a coffee grinder and stored under nitrogen at -60oC until analyzed. Approximately, 200 mg of powdered bean sample (particle size < 0.825 mm) was saponified by stirring sample with 5 mL of a basic solution (2 N NaOH, 10mM EDTA and 1% ascorbic acid) for 30 min at 40-45 (C. The reaction mixture was acidified with 1.4 mL of 7.2 N HCl. The mixture was vortexed for 5-10 sec. Free phenolic acids were extracted with ethyl acetate (2 x 6.4 mL) by vortexing the mixture twice for 1 min. The extraction tubes were placed on a low speed centrifuge for 15 min and the upper organic layer was transferred to a separate vial. The combined organic layer was evaporated to dryness under a steady stream of nitrogen. The residue was redissolved in 2ml 75:25 methanol:water (% v/v). The vial was placed in a sonicator for 5 min. The extract was filtered through a PVDF syringe filters (0. 20 μm) prior to HPLC analysis. All extractions were carried out in triplicate and all identified phenolic acids were quantified with external standards by using HPLC analysis (7).

Results and Discussion

The challenges associated with the determination of phenolic acids in different food matrices arises from their structural complexity, as these compounds may exist in multiple forms (free, esterified, glycosylated or as a polymer) (8). Dried bean samples were saponified, and free phenolic acids were extracted with ethyl acetate after acidifying the saponified extracts. The liberated free phenolic acids were analyzed by high performance liquid chromatography (HPLC).

Identification of phenolic acids in bean samples was carried out by comparison of UV spectra and retention times with authentic standards obtained from commercial sources. Ferulic acid, p-coumaric acid and sinapic acid were identified in all three varieties. All three phenolic acids were reported in four other bean classes (Navy, Dark Red Kidney, Pinot and Black Turtle Soup) by gas chromatography (GC) analysis (9). However, caffeic acid was identified in quantifiable amount only in two varieties namely, T-39 and Eclipse. Ferulic acid was the major phenolic acid in all three black bean cultivars. Intermediate levels of p-coumaric acid and sinapic acid were identified in all three cultivars. The average total phenolic acid content for all three black bean cultivars was determined to be 0.38 mg/g. The total phenolic acid content among all samples varied between 0.24-0.48 mg/g. Highest amount of total phenolic acid was extracted from T-39 cultivar. However, total phenolic acid content in Jaguar cultivar was 40%-50% lower as compared to the two other cultivars (T-39 and Eclipse). Further work on determination on phenolic acids content in 14 bean cultivars belonging to the other nine bean classes is in progress.

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ANALYSIS OF NUTRITIONAL QUALITY TRAITS IN AN ANDEAN RECOMBINANT INBRED LINE POPULATION.

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Introduction:

Legumes provide essential micronutrients that are found only in low amounts in the cereals or root crops (Wang et al., 2003). An ongoing project at CIAT has shown that bean seeds are variable in the amount of minerals (iron, zinc and other elements), vitamins and sulfur amino acids that they contain and that these traits are likely to be inherited quantitatively. In this study we analyzed iron and zinc content in an Andean recombinant inbred line (RIL) population derived from a cross between G21242, a Colombian cream mottled climbing bean with high seed iron/zinc content and G21078, an Argentinean cream seeded climbing bean with low seed iron/zinc content.

Materials and Methods:

The population was analyzed over two locations in Colombia (Popayán and Darién) with 100 RILs planted in the first site and a subset of 83 RILs planted in the second site. A lattice design was used for the first trial (with 3 repetitions) and a randomized complete block design was used for the second trial (with 2 repetitions). Both experiments were planted with trellis supports since the population is predominantly made up of climbing bean genotypes and agronomic management consisted in recommended practices. In both seasons, plots were bulk harvested and grain was combined across repetitions before sub-sampling for mineral analysis. Two methods of mineral analysis were implemented. The harvest from Popayán was analyzed first with Inductive Coupling Plasma (ICP) analysis at the University of Adelaide and second with Atomic Absorption (AA) Spectrophotometry at the CIAT analytical services lab. Sample preparation for the ICP technique involved grinding 10 g of seed in a coffee mill, while for the AA technique 5 g of seed was ground in aluminum chambers using a Retsch mill and aluminum grinding balls. While replicate sampling with two repetitions each was done for the AA mineral analysis it was not possible to do this for the ICP analysis due to cost considerations.

Results and Discussion:

Iron and zinc content in the RILs presented a continuous distribution, suggesting that mineral content behaved as a quantitative trait. The range and averages for iron content was higher in Darién than in Popayán, while the zinc content range and average was lower in Darién than in Popayán (Table 1). The parents of the population showed significant differences and tended to be on the edges of the population distribution. G21242, the high mineral parent, was always higher in mineral content than G21078, the low mineral parent. In the case of iron concentration, G21078 tended to have values similar to the means of the population while G21242 was closer to the upper extreme of the population distribution, while in the case of zinc concentration the parents were more intermediate but still contrasting. Given this, transgressive segregation for low iron and for both high and low zinc was evident in the population.

Results with ICP analysis had similar population distributions as AA spectrophotometry, however G21078 was lower in iron concentration in the ICP analysis than in the AA analysis, even though G21242 was similar. For zinc, ICP values were higher than those found with AA but the population distribution was similar. AA spectro-photometry provided a savings in reagent costs and required smaller amounts of ground samples so this was the preferred method. Reliability of the AA spectrophotometric method was high with low standard deviation for parental genotypes and coefficients of variation averaging 6.8% for iron and 5.6% for zinc per genotype in the analysis of variance conducted for each location.

Genotype x environment interaction was measured for the AA results for seed iron and zinc content in a combined analysis over the two locations. Location and treatment effects were all significant at P=0.0000 level, showing that both genotype and G x E effects were important for both minerals, confirming the difference in the distribution and parent means discussed above. It was notable that location effects were stronger for zinc than for iron although G x E effects were similar for the two minerals. Despite the significant G x E effects, highly significant correlations were also observed between locations for both iron (r=0.665 under AA and r=0.715 with ICP) and zinc content (r=0.439 with AA and r=0.450 with ICP) irregardless of the mineral detection method. Correlations were even higher between the AA and ICP results (r=0.849 for iron and r=0.860 for zinc). Correlations were also high between iron and zinc concentration in both Darién (r=0.301) and Popayán (r=0.653 for AA and r=0.651 for ICP).

Table 1. Average seed iron and seed zinc content in two locations (Popayán and Darién) using atomic absorption (AA) spectrophotometry and inductive coupled plasma (ICP) analysis. Standard deviations are shown in parenthesis.

	IRON CONTENT			**ZINC CONTENT**		
	$\overline{X}$ **dar03AA**	$\overline{X}$ **pop98AA**	**pop98ICP**	$\overline{X}$ **dar03AA**	$\overline{X}$ **pop98AA**	**pop98ICP**
G21242	83.13 (3.09)	88.27 (4.34)	98	32.48 (1.03)	41.20 (1.49)	49
G21078	35.46	24.14 (2.39)	33	21.81	26.71 (1.77)	27
$\overline{X}$ **progeny**	53.06 (13.48)	47.63 (12.03)	59.02 (12.33)	25.93 (4.29)	31.21 (5.41)	34.83 (5.35)
Range	23.14 - 99.06	22.45 -91.34	33 - 98	13.25 - 7.66	21.02 - 9.62	25.0 - 49.0

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Effect of Polyphenol and Sugar Content on Seed Coat Darkening of Pinto Bean Cultivars

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INTRODUCTION

In Mexico, common bean (*Phaseolus vulgaris* L.) quality is regulated by the government norm NMX-FF-038-SCFI-2002. The bean quality is determined by several characteristics: grain damage, seed disease, pests, etc. It has been suggested that grain damage is the most important factor in the control of quality of beans. (Delgado, 2004). Not only grain damage is important but other grain defects are significant, too. Grain prices are defined as a function of grain defects, the most common defects in pinto beans are seed coat darkening, presence of blister and fracture of such seed coat (Delgado, 2004). There is also a great influence of time length on the development of a number of grain defects in common beans, but unfortunately there is modest knowledge particularly on the seed coat darkening phenomenon. Pinto Saltillo was registered and released as a seed coat darkening resistant improved cultivar (Sanchez, et. al., 2004), therefore a study was carried out to assess some factors that may be playing a role on seed coat darkening of pinto bean cultivars.

MATERIALS AND METHODS

A series of breeding and commercial Mexican pinto bean cultivars were used to study the seed coat darkening phenomenon, namely: Pinto Saltillo (Sanchez-Valdez y col., 2004), Pinto Bayacora, Pinto Villa, Pinto Mestizo, Pinto PTD99099, Pinto PTD99057, Pinto PTD98012 and Pinto PTD99092. Bean cultivars were developed by the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Mexico. Grain samples were obtained by separating apart the seed coat and cotyledon from the seed using a sharp blade. Only seed coat was used and stored under controlled conditions: 60% of relative humidity (%RH) and 45°C of air temperature. Seed coat samples were exposed to light for 12 h period every 48 h during the three-month period of storage. Sugar content was determined at different times across the study: at beginning of the experiment, 1, 2 and 3 months after storage according to Dubois et al., (1956). Seed coat color assessment was done using the Hunter-Lab (Junk y col., 2003) and color parameters were estimated according to Cheng y Shewfelt (1988) and Claybon and Barringer (2002). Phenol content was also determined following the technique of Folin-Ciocalteu for phenols suggested by Waterman y Mole (1994). Data was analyzed by the appropriate ANOVA and Tuckey test ($p \geq 0.05$).

RESULTS AND DISCUSSION

Hue estimate was the only parameter that showed differences among bean cultivars and that being a function of time, suggesting that this parameter could be used as an indicator of the darkening defect in pinto bean grain. The highest Hue estimate was obtained with Pinto Saltillo (66.76±1.1), such estimate was constant over time (three-month); in contrast, the other seven pinto bean cultivars had lower Hue values and had a strong effect over time (data not shown). Results on the seed coat phenol content indicated that Pinto Saltillo cultivar had the lowest (16.35 mg of catechine/g of sample±0.35) in comparison to the rest of cultivars evaluated and PTD99012 breeding line had the highest (37.27±1.22 mg catechine/g of sample). It is important to point out that light exposure over time had a higher effect on the Hue estimate for PTD99012; in contrast, Pinto Saltillo did not have such an effect over time, suggesting a possible association between phenol content and the seed coat darkening of pinto bean.

Results also indicated that Pinto Saltillo had the lowest sugar content (6.30±0.11 mg glucose/mL of sample), while Pinto Mestizo had the highest (17.1±0.65 mg glucose/mL of sample), the rest of cultivars showed intermediate values (11.5 ±0.43 mg of glucose /mL of sample. Shiga et al., (2004) reported a time effect on seed coat sugar content in common beans, similar results were obtained in our study for all pinto bean cultivars but for Pinto Saltillo which is resistant to seed coat darkening. These results suggest that phenol and sugar content might have an effect on the darkening phenomenon in pinto beans. Data needs to be fully analyzed and more research needs to be conducted in order to verify these conclusions.

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STATE AND PROSPECTS OF DRY BEAN BREEDING AND PRODUCTION IN BULGARIA

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Bean is the most simple and the most preferred food of Bulgarians. This fruit of the Bulgarian land has grown into the mind of the common Bulgarian in such a way that he considers that its cultivation began in ancient times. Therefore many people are surprised at hearing that this symbol of the Bulgarian every day life became known to our ancestors a little more than three centuries ago. Bean originated in America and was introduced in Europe only after America was discovered, i.e. at the beginning of 16th century. It was introduced into the boundaries of the Ottoman empire a century and a half later, i.e. in the middle of the 17th century, simultaneously with maize.

Due to the favorable climatic conditions, and due to the fact, that bean is preferred by the population in the country, a large number of botanical forms and landraces have been differentiated; they differ by the type of plant, by flower, form and size of seeds, as well as by economic qualities. By its variability of forms and types, bean is a crop unmatched by any other in our country. Gradinarov (1939) established 91 landraces of common bean and two of runner bean. Vishnevsky (1940) described 124 landraces. Rachinsky (1968) collected and investigated 496 landraces, and Ganeva (1983) - 4323 forms. In IPGR–Sadovo there are 1710 accessions (Stoilova, T., 1998). At Dobroudja Agricultural Institute (DAI) - General Toshevo 400 accessions have been collected and are being investigated. Undoubtedly, there is even greater variability in Bulgaria, especially concerning the biological and physiological differences which are still insufficiently studied.

Bean reached its peak of distribution during 1941-1946 - up to 160 000 ha annually. Since then, however, the area sown with bean has been decreasing and now it is about 20 000 ha.

The climatic conditions in Bulgaria are very variable. According to the soil and climatic regions (Hristoforov *et al*., 1969), the areas, favorable for growing of bean encompass the greater part of the Danubian plane and the Dobroudja plateau, which also includes Dobroudja Agricultural Institute - General Toshevo. The sum of mean twenty-four hour air temperature from spring (10^{o} C) to autumn (15^{o} C) is 2800 - 3200^{o} C, while the temperature sum necessary for normal vegetation is 1900^{o} C. During the period of seed development, the mean air temperature is 21-22^{o} C. The years with high maximum temperatures exceeding 34^{o} C are 20-40 %. The hydro-thermal coefficient of the bean vegetation period varies from 1.10 to 1.51. In these regions 7-40 % of the obtained yield is over 2000 kg/ha, 20-47 % - from 1500 to 2000 kg/ha, 20-47 % - from 1000 to 1500 kg/ha, and 7-27 % - under 1000 kg/ha.

The biological potential of dry bean under the conditions of Bulgaria based on trials in 15 stations for State varietal testing with the most widespread varieties Dobroudjansky 2 (IIIb growth type), Dobroudjansky 7 (IIIb) and Abritus (IIa) for 10 years has been 1760 kg/ha. In these stations yields varied from 1200 to 2400 kg/ha. The traditions and experience accumulated in cultivation of dry bean under non-irrigation conditions are in north-east Bulgaria, where about 80 % of the areas and 90 % of the production were concentrated , the realization of the production potential being only 50 %. The reason for this low realization of the productivity potential is the lack of varieties and technology for direct harvesting. Variety Abritus has disposition of pods on the growth habit suitable for direct sowing, which remains stable by years and regions.The only disadvantage according to Bulgarian consumer is that its seeds are small (seed weight of 200-220 mg). DAI will soon be able to supply varieties with IIa growth type with large white seeds and the problem with this variety will be solved. The varieties with IIa growth type are also suitable for growing with irrigation and direct harvesting.

In the mountain regions, where mechanized harvesting is not possible, varieties with IVa and IVb growth type dominate. They provide more produce per unit area (3000-4000 kg/ha). They are grown on poles of *Corylus* sp. 3-4 m long. These regions with altitude from 600 to 1000 m are characterized with more abundant and more evenly distributed rainfalls. Besides, there are possibilities for irrigation. All this

is a prerequisite for high and stable yields during the years, and of better cooking properties. The most prominent peculiarity of bean growing in such places are the considerably greater labor expenses.

The diseases are a major problem for production of common bean in Bulgaria. Among the more than 20 bacterial, fungal and viral diseases, the following have economic importance: common bacterial blight (*Xanthomonas axanopodis* pv. *phaseoli*), halo blight (*Pseudomonas savastanoi* pv. *phaseolicola*), anthracnose (*Colletotrichum lindemuthianum*), bacterial wilt (*Curtobacterium flaccumfaciens* pv. *flacumfaciens*), white mold (*Sclerotinia sclerotiorum*), bean common mosaic (BCMV and BCMNV). Among the pests, bean weevil (*Acanthoscelides obtectus*) has economic importance.

The breeding efforts of DAI are directed toward breeding of varieties which can meet the various demands of the consumer. These demands are related mainly to: 1) the way of growing; 2) climatic peculiarities and 3) demand on the market. Therefore we are working for the development of varieties suitable for all types of growing that have proved their suitability in practice. The new varieties possess: 1) variable form, size and color of seeds and 2) different biology allowing production of maximum amounts and quality under certain environmental conditions. However, the main direction of breeding in DAI is developing varieties with simultaneous flowering and maturation, with erect habit (Ia, IIa, IIIb, IVa and IVb), with high disposition of the pods on the habit, suitable for growing as monoculture and direct harvesting.

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Effect of Harvest Dates on Seed Coat Color of Pinto Beans

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Introduction

Dry beans need to have a typical color of a particular market class to be free of off-type seeds and seed discoloration. Seedcoat color is an important trait in Pinto Beans. Consumers prefer pinto cultivars with bright and shiny mottle colored seedcoat and bright yellow hilum. (Muharrem et al., 2001). Several factors may cause seedcoat discoloration, including light, temperature, moisture, storage, diseases, as well as the genotype of the cultivar. Pinto seed coat color darkens while the beans are in storage. The color becomes darker with increasing lengths of storage (Huges and Sadsted, 1975). Light exposure and temperature may also interact with moisture and genotypes to cause seedcoat discoloration (Muharrem et al., 2001). Park and Maga (1999) reported that color stability of pinto beans was influenced by moisture content, physico-chemical seed characteristics and genotype.

Material and Methods

Twenty pinto bean cultivars (Apache, Aztec, Bill-Z, Burke, Buster, Chase, Elizabeth, Fargo, Focus, GTS-900, Kodiak, Maverick, Montrose, Othello, Rally, Remington, Topaz, UI-114, UI-320, and Winchester) were planted at Prosper and Fargo, North Dakota in 2003 and 2004 growing seasons. The study was harvested at four different dates with an interval of 15 days between consecutive harvest dates. The Fargo location in 2004 was lost due to excessive precipitation. The experimental design was RCBD with three reps in a split-plot arrangement. Cultivars constituted the main plot, while the harvest dates constituted the subplots. Seed color was evaluated for each sample using a Agtron Color Quality Meter (calibration: black = 0, white = 90).

Results and Discussion

Results from 2003 and 2004 growing seasons at Fargo and Prosper, ND show significant differences among harvest dates and cultivars. The interaction cultivar x harvest date was only significant in 2003 at both locations. In General beans harvested on the first and on the last harvest dates had the lightest and darkest color across all cultivars, respectively. Seed coat color was darker in 2004 than 2003, probably due to cold temperatures and excessive rainfall during the growing season. On average color score reduced 8 points between the first and the last harvest date in both seasons.

Results obtained in 2004 were not consistent with the data obtained in 2003, with the exception of Montrose, which is one of the cultivars with the lightest color in both growing seasons. Color score means for the 1st and 4th harvest dates are presented in Table 1. >Winchester=, which was one of the best from 2003 data, resulted to be one of the darkest cultivars in 2004 growing season. >Kodiak= which scored as the darkest seed coat color in 2003, is among the middle range in seed coat color in 2004 results.

Conclusions

- Harvest date had a significant effect on seed coat color of pinto beans.
- Cultivars respond differently to the environment, so genetic factors could be involved.
- Pinto beans should be harvested as soon as possible, since the longer the pinto beans are exposed to the environment the darker the color of the seed coat.

Table 1. Color score means from the first and the fourth harvest dates, at Fargo and Prosper, North Dakota, during 2003 and 2004 growing seasons.

	Harvest Dates (hd)					
	Fargo 2003		Prosper 2003		Prosper 2004	
Cultivar (cul)	1st	4th	1st	4th	1st	4th
	scu[1]					
APACHE	41.6	34.1	39.0	33.8	36.4	29.4
AZTEC	45.0	30.3	37.9	29.5	35.4	30.0
BILL-Z	45.9	37.2	41.5	34.2	37.3	31.5
BURKE	48.6	34.4	39.0	31.5	35.2	28.9
BUSTER	48.4	34.3	40.0	28.6	39.6	33.0
CHASE	44.5	36.2	39.7	33.1	37.3	31.1
ELIZABETH	42.3	32.8	39.2	33.3	34.2	30.6
FARGO	44.2	36.7	41.2	35.9	39.4	32.5
FOCUS	44.5	33.8	40.8	30.1	36.6	30.9
GTS-900	40.2	29.7	38.3	29.3	35.6	29.3
KODIAK	53.6	29.7	40.1	28.7	39.7	30.0
MAVERICK	46.9	36.2	41.2	35.6	39.4	32.3
MONTROSE	44.6	38.6	40.0	35.8	40.3	33.1
OTHELLO	49.2	36.3	39.5	33.6	36.3	29.8
RALLY	43.1	31.5	36.4	28.3	36.6	30.2
REMINGTON	52.3	35.6	42.2	33.8	38.3	32.4
TOPAZ	45.3	33.9	39.9	30.8	37.6	31.5
UI-114	43.4	36.9	38.8	34.9	37.7	29.3
UI-320	40.9	31.7	37.5	31.1	31.8	28.4
WINCHESTER	42.0	33.1	38.1	32.3	32.8	27.7
cul x hd $LSD_{0.05}$ (p-value)	2.2 (0.0001)		2.5 (0.0001)		NS (0.459)	

[1] standard color units; the higher the number, the lighter the color.

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ADOPTION OF IMPROVED BEAN VARIETIES IN THE SEMIARID HIGHLANDS OF MEXICO

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Common beans (*Phaseolus vulgaris* L.) are the second most important crop in Mexico after maize, both in terms of production and consumption. During the period 1990-2000, the total area planted to common beans averaged 2.2 million hectares, harvested area averaged 1.9 million hectares, yields averaged 632 kg/ha, and total production averaged 1.2 million mt. Approximately 85% of the country's bean crop is grown under rainfed conditions. In 2000, Chihuahua, Durango, and Zacatecas (located in the semiarid highlands of northern México)--the most important states growing beans under rainfed conditions--accounted for 62% of the total rainfed bean area (SAGAR, 2000).

During the 1990s, the National Research Institute for Forestry, Agriculture, and Livestock (INIFAP) released several improved bean varieties (e.g., Pinto Villa, Flor de Mayo M38, Pinto Mestizo, Pinto Bayacora, Negro Altiplano, and Negro Sahuatoba) that were developed for the rainfed conditions of Mexico's semiarid highlands—characterized by low rainfall (less than 450 mm a year), low fertility soils, and agronomic problems associated with monocropping beans (Acosta *et al*., 1995a; Acosta *et al*., 1995b; Acosta *et al*., 2001a; Acosta *et al*., 2001b; Acosta *et al*., 2001c; Acosta *et al*., 2001d).

In 1996, as one component of the Alliance for the Countryside, the Mexican government started the Kilo per Kilo program with the objective of increasing yields by promoting technical change (under irrigated as well as rainfed conditions) through the substitution of modern varieties for traditional varieties of beans and other important crops. The program delivered seed of the improved varieties to farmers at a price equivalent to commercial grain. This significant reduction in price made improved seed very affordable to farmers.

The objective of this study was to: 1) estimate farmer adoption (at the aggregate level) of INIFAP's improved bean varieties in the states of Chihuahua, Durango, and Zacatecas, 2) assess the impact of adoption on farmers' yields, and 3) identify factors affecting the adoption of these varieties at the farm-level**,** using cross-sectional data obtained from farmer surveys conducted in 2001.

During the period 1997-2001, the share of the bean area planted to improved varieties averaged 71% in Chihuahua (Pinto Villa 56%, Pinto Mestizo 15%), 42% in Durango (Pinto Villa 38%, Pinto Mestizo 4%), and 8% in Zacatecas (Negro Zacatecas).

A statistical analysis of yields indicated that the yields of improved pintos were 20.6 percent higher than the yields of traditional pintos. However, for the other market classes (black and light-colored beans), there were no statistically significant differences (5% level) between the yields of improved and traditional varieties.

To identify factors associated with adoption of improved varieties, the farmers surveyed in the spring-summer 2001 season were divided into two groups: adopters and non-adopters. Adopters accounted for 67 percent and non-adopters for 33 percent of the farmers surveyed in northern México. When comparing adopters versus non-adopters, statistical analysis indicated no

significant differences in production costs, credit, tied-ridger ownership, off-farm employment, farm size, bean area, soil quality, and travel time to the nearest market. In addition, there were few significant differences in age, experience, education, land tenure, soil preparation method, planting dates, cultural practices, quantities and months of bean storage, and for farm location (distance to nearest city). The analysis of selling price by market class shows that in general, light color beans commanded the highest prices, followed by pintos and black beans. However, there were few significant differences between adopters and non-adopters regarding market prices.

The most important difference between adopters and non-adopters was participation/non-participation in the government's seed distribution program, Kilo per Kilo. In 2001, program participation explained adoption in 37% of the cases and non-participation in the program explained non-adoption in 43% of the cases, totaling 80% of the cases. The remaining 20% of the farmers were adopters who did not participate in the program in 2001, but they got seed of an improved variety directly or indirectly from the program in previous years.

The surveyed adopters were asked their perceptions regarding the advantages of improved varieties, compared to traditional varieties. Analysis of the data indicated that the most salient varietal characteristics that influenced farmers' decision to adopt improved varieties were better consumption quality, market acceptance, fewer days to maturity (which is related to drought resistance and frost evasion), and higher yields.

The study has important implications for extension services, research administrators, and government officers, regarding farmer adoption of improved bean varieties. Extension services need to provide farmers training on the appropriate management practices required to preserve seed quality from season-to-season, so the Kilo per Kilo program doesn't have to provide seed to the same farmers every year and thereby benefit more farmers. Research administrators need to continue to continue to give high priority to developing improved pinto varieties and greater priority to developing improved varieties in other market classes—especially black beans. Finally, given the highly positive impact of the Kilo per Kilo program on farmer adoption, the government need to continue to support this innovative initiative in order to insure that farmers continue to have access to newly-released improved varieties.

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AN ASSESSMENT OF THE COMMON BEAN SUBSECTOR IN CENTRAL AMERICA

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Beans, which account for 12% of Central America's crop area, are the region's second most important "basic grain", the main source of vegetable protein (especially for the poor) and are an important cash crop for small farmers. In early 2000, annual consumption was highest in Nicaragua (25 kg/capita), followed by El Salvador (14 kg), Costa Rica (11kg), Honduras (10 kg) and Guatemala (7 kg). Nicaragua, Honduras and El Salvador mostly produce small reds, while Guatemala and Costa Rica mostly produce blacks. In all countries, beans are grown primarily by small-scale farmers with limited access to credit and purchased inputs.

During 1990-2001, the bean area averaged 501,360 ha. While Nicaragua accounted for the largest share of the bean area (32%)--followed by Guatemala (26%), Honduras (18%), El Salvador (15%) and Costa Rica (9%)—Guatemala accounted for the largest share of production (28%)--followed by Nicaragua (28%) and Honduras (19%). During the 1990s (1990-1992 to 1999-2001), planted area declined in Costa Rica (-56%), Honduras (-19%) and Guatemala (-8%), but increased in Nicaragua (111%) and El Salvador (6%). Bean production declined in Costa Rica (-52%), Guatemala (-18%), and Honduras (-21%), but increased in Nicaragua (+171%) and El Salvador (+12%).

During the decade (1990-1992 to 1999-2001), regional yields increased at 0.5%/year, from 694 to 727 kg/ha. Yields increased most rapidly in Nicaragua (2.8%/year), followed by Costa Rica (1.2%) and El Salvador (0.6%), but declined in Guatemala (-1.2%) and Honduras (-0.3%). In 1999-2001 yields were highest in El Salvador (877 kg/ha), followed by Nicaragua (746 kg/ha), Honduras (739 kg/ha), Guatemala (689 kg/ha) and Costa Rica (583 kg/ha).

Bean research is conducted by government institutions and universities. Breeders have focused on developing improved varieties (IVs) resistant to numerous constraints, including disease and drought. For small reds, breeders have focused on developing IVs with quality characteristics that consumers demand (light color). Since 1990, 22 improved varieties have been released, despite a substantial decline in bean research and extension funding. Seed of IVs is primarily multiplied/distributed by government agencies in cooperation with universities and/or NGOs. While few farmers plant certified seed, recycled IV seed of IVs is widely planted.

Marketing channels are similar among countries. Producers sell surplus to intermediary, who retail beans to small full-service stores (*pulperias*), central markets, small self-service stores and supermarkets. Most consumer buy beans in central markets or at small stores, but growing concentration in the retail marketing system is influencing how and where beans are sold.

Value is added to beans via cleaning/packaging and transforming beans into canned, flexible pack, or frozen products. While the packaging industry is most developed in Costa Rica, the processing industry is most developed in Guatemala. In El Salvador, Nicaragua and Honduras, the packaging industry is growing rapidly, driven by the incentive to sell to local supermarkets and export to regional markets. The region's 10 large canners are based in Guatemala (7) and Costa Rica (3) and1 Honduran firm produces a flexible pack. Most firms have their own brand, but some also can for other companies.

Supermarket chains in the region's capital cities sold 67 different brands of value-added products (bagged, processed beans). In each country, 6-15 different brands were sold in plastic bags, vs. 4-12 brands of canned beans. The demand for processed products is limited by their high price (canned beans averaged US$ 2.49/kg, vs. US$1.25/kg for bagged beans), but more affluent consumers are willing to pay a premium for processed products and high quality beans.

The region is increasingly dependent on imports. During 1994-1996 to 1999-2001, imports grew 18%/year (total increase of 127%). Guatemala's imports increased the most (28%/year), followed by Costa Rica (24%), Nicaragua (16%), El Salvador (15%) and Honduras (9%). Despite annual supply deficits, countries export beans within the region and to niche markets in developed countries. In 1999-2001, the main exporters were Nicaragua (42%) and Honduras (35%). According to exporters, exports to outside the region have increased in recent years due to growth in the number of Central Americans living abroad and the demand for ethnic food at restaurants in Canada, the U.S., and Europe.

To assess competitiveness, local wholesale prices were compared to FOB bean prices from outside the region (with/without the tariff, 30%). During 1997-2002, Guatemalan bean prices (w/tariff) were lower than U.S. prices from 1997 to mid-1998 and after mid-2001. However, without the tariff, U.S. prices were generally lower throughout the period. While no country outside the region produces a good substitute for small reds, countries import reds when local production is insufficient. From mid-1997 until mid-2000, Nicaraguan prices were higher than U.S. prices (with and w/o tariff), but after 2000 Nicaraguan prices declined to substantially below U.S. prices. During 1997-2002, Honduran prices (w/tariff) were generally lower than U.S. prices, but after early 2002 they were also lower without the tariff. While the region's products do not face tariffs, agricultural products (including beans) are protected by a common external tariff (20%-40%). As most countries depend on bean imports from outside the region, the tariff increases the price that consumers pay for beans.

This study has several policy implications. First, to be competitive in an increasingly global market, continued research is needed to breed IVs resistant to biotic/abiotic stresses. Second, given rapid structural change in the region's bean subsector (consolidation in wholesale/retail markets, expanding export markets, increased demand for value-added products, growing consumer preferences for superior quality beans) and the need to insure that future IVs meet consumers' preference, scientists need to involve wholesalers, processors, retailers and exporters in establishing quality standards and evaluating promising lines prior to their release. Third, to increase productivity, scientists and governments need to assess the strengths/weaknesses of existing seed multiplication/distribution schemes and identify ways to insure that small farmers have greater access to future releases. Forth, while countries have established projects to expand small farmer access to market information, small farmers still have limit access to information about prices, private grades & standards, and market opportunities; there is little coordination among producers, processors, and retailers; and growing consolidation of the food retail system and the packaging industry has created new challenges for bean farmers who seek to access urban markets. Thus, national programs need to help farmers develop strategic alliances with retailers/intermediaries to insure that farmers have information (color, grain appearance) required to access these markets. Finally, as bean supplies expand the potential to sell locally and export high quality beans (e.g., *Rojo de Seda*) at premium prices to niche markets in developed countries will increase. To access this potentially promising market, farmer association should explore the possibility of establishing farmers-owned brands and targeting sales towards high-income local consumers and consumers in developed countries.

GENETIC CONTROL OF MELOIDOGYNE INCOGNITA RESISTANCE IN COMMON BEAN

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One of the problems that most affects the common bean crop in Brazil is the increasing occurrence of nematodes, especially of the genus Meloidogyne. The damage done by these pathogens causes great loss, mainly where climatic conditions favor the reproduction and development of the nematodes, along with the intense exploitation same areas, without the due crop rotation.

There are several control methods, among them the chemical and management control which are however costly and little used. The principal alternative is the use of resistant cultivars. The occurrence of great variability among common bean lines in respect to damages has been documented (Moura & Regis, 1987; Mulliin et al (1991); Omwega & Roberts, 1992). Information regarding genetic control was obtained from plants in generation F_2. Since the evaluation of individual plants is of low precision, our study aimed at achieving information regarding the genetic control through progenies with a possibly greater experimental precision.

MATERIAL AND METHODS

Progenies $F_{2:3}$ of the crossing Pérola (resistant) x Batatinha (susceptible) were used. Sixty-eight $F_{2:3}$ families plus both parents were evaluated in the complete randomized block design with three replications. The plots consisted in a pot with two plants. Additionally, a (susceptible) tomato plant was grown in each pot as an indicator for the occurrence of nematodes. Each plot was inoculated after the emergence with 6000 *M. incognita* eggs. Sixty days after the emergence the incidence of the egg mass in the roots was evaluated.

The analysis of covariance was realized using the number of egg mass of the tomato plant as covariable.

RESULTS AND DISCUSSION

By means of the tomato indicator plant a variation in the nematode incidence was observed between pots, reducing the evaluation precision. However, the use of this information as covariable adjusted them all to a mean incidence, improving the precision (Steel et al 1997). Significant difference ($P \leq 0.01$) was detected among the progenies. The frequency distribution of the number of egg mass evidenced this fact (Figure 1). The contrasting performance of the parents was noteworthy. The resistance of cultivar Pérola is probably one of the reasons for the good performance in several regions of Brazil, especially in center-pivot irrigation systems.

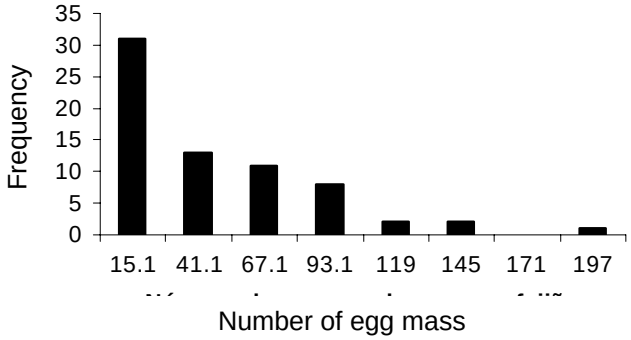

Figure 1. Frequency distribution of M. incognita egg mass number

An asymmetric frequency distribution was observed. There is a greater concentration of families in the classes of smaller occurrence of egg mass. This fact indicates the occurrence of dominance in the control trait, where the dominant allele(s) is(are) responsible for the resistance. These results are in agreement with earlier studies that evaluated F_2 generation plants. It is difficult to draw conclusions on the number of genes. The number is probably low, as reported earlier by Owmega et al 1992. However, the obtained estimates of heritability ($h^2 = 53.6\%$) are evidence that the environment effect is pronounced which may affect the success of the selection for this trait

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Virus Resistance Assessment of Plant Introductions

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Introduction

Since first identified in 2000, the soybean aphid virus complex has cost the snap bean industry millions of dollars in losses. Economic losses were greatest in 2000, 2001 and 2003 while losses in 2002 and 2004 were minimal. In 2004, soybean aphid populations were low, migrations were late and in some production areas, the soybean aphid and viruses were never detected. Cool temperatures and excessive rainfall throughout much of the growing season probably influenced aphid numbers. Extensive surveys of Wisconsin production areas in 2002 and 2003 identified Cucumber Mosaic Virus (CMV) and Alfalfa Mosaic Virus (AMV) as the major components of the complex (German, et al., 2004). However, the role of Clover Yellow Vein Virus (ClYVV) is not clearly understood and unidentified viruses, different strains of viruses and other agents may be involved. In 2004, ELISA results indicated that some of these symptoms were associated with CMV, AMV, ClYVV or a combination. In other instances, the symptoms could not be associated with the presence of any of these viruses. In these cases, with the exception of virus-like foliar symptoms, the plants appeared healthy and no apparent loss in yield was observed. Large populations of other aphid species and insects were detected in some areas and may have contributed to these symptoms.

Materials and Methods
Germplasm

Trials at Arlington, WI Agriculture Research Station (ARS) identified (2002) and reconfirmed (2003) 12 single plant accessions expressing possible tolerance to CMV and AMV (Sass, et al., 2003; 2004). In 2004, in collaboration with Drs. Walt Stevenson and Craig Grau, UW-Madison and Dr. Ben Lockhart, University of Minnesota, these 12 accessions were evaluated at West Madison ARS and in snap bean production fields near Oostburg, WI and Fox Lake, WI. In addition, three cultivars and PI 309881 were included as checks. Dr. Phillip Griffiths, Cornell University had previously identified PI 309881 as being CMV resistant. Although trials at these three locations included 38 entries, we focused our efforts on the 12 individual plant selections, PI 309881 and three cultivars chosen at random (Romano Gold, IDC IX and Redon). Additionally in 2004, 423 PI *P. vulgaris* accessions were evaluated at Arlington, WI ARS. These 423 accessions represent the USDA core *P. vulgaris* collection.

Experimental Design

423 PI accessions were planted at Arlington, WI ARS using a replication within block design. Cultivars and the individual plant selections were also included in the trial. Twelve days prior to planting the trial, spreader rows consisting of a 50:50 mix (by weight) of soybean and the snap bean 'Hystyle' were planted. The same day the trial was planted; expanding trifoliate leaves of Hystyle in the spreader rows throughout the trial were chosen at random and manually inoculated with AMV and CMV.

At all locations composite samples of 10 expanding trifoliate leaves from 10 plants with virus-like symptoms per plot were harvested for ELISA. This sampling method was chosen because our research objective was to confirm tolerance, not quantify incidence.

Results – Oostburg, WI, Fox Lake, WI and West Madison ARS trials

The Oostburg trial yielded little data. Plants appeared healthy throughout the growing season. Visual ratings were negative for virus symptoms and ELISA results were CMV and AMV negative. In the Fox Lake trial and in the surrounding commercial snap bean field, virus-like symptoms were consistently observed throughout. ELISA results from the harvested samples were CMV and AMV negative. Plots at W. Madison were visually scored as having virus-like symptoms and soybean aphids. ELISA was performed when the individual plants selections were flowering and again at the pod harvest stage. ELISA for the 12 selections, PI 309881 and the cultivars confirmed the presence of AMV (Table 1).

Results – Arlington, WI ARS trial

Visual evaluations of the 423 PI accessions and checks were positive for virus-like symptoms and soybean aphids. Data indicated a 90% infection level. However, composite leaf sample ELISA results indicated approximately 15% of the accessions tested AMV or CMV positive. These results suggest that there maybe have been other viruses, virus strains or agents involved in the virus complex in 2004.

2004 Summary of Results

- Based 2004 data, four individual plant selections (2313.9.1000, 2313.9.2000, 2313.9.3000 and 2313.10.3000) identified in 2002 and 2003 continue to demonstrate tolerance to AMV and CMV.
- Based on data from two replications at Arlington ARS in 2004, 11 of the 423 PI accessions were visually symptomless and ELISA negative. These accessions will be increased for further evaluation.

Acknowledgements

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Table 1. ELISA readings for 3 replications including 12 individual plant selections, PI 309881 and three cultivars. Readings above 0.1 are considered infected. 0.1 represents a value that is more than 3 times the standard deviation of our negative control. The selections demonstrating tolerance to AMV in all replications are noted in bold.

Accession Name	Rep #	09/09/04 –W. Madison ELISA reading (AMV)	09/30/04 – W. Madison ELISA reading (AMV)
2292.1.200	1	0.004	0.019
PI309881	1	0.002	0.008
2292.2.1000	1	0.004	OUT
2292.11.200	1	0.004	0.011
2319.1.1000	1	0.001	0.004
2313.9.3000	**1**	**0.008**	**0.014**
2313.9.1000	**1**	**0.010**	**0.001**
2313.4.3000	1	0.020	0.009
2313.9.2000	**1**	**0.006**	**0.006**
2313.10.3000	**1**	**0.003**	**0.003**
2319.1.3000	1	0.000	0.246
2319.4.2000	1	0.420	1.015
2295.5.3000	1	0.195	0.028
Romano Gold (Seminis)	1	0.699	1.614
Redon (Syngenta)	1	0.680	1.242
IDC IX (Del Monte)	1	0.520	1.283
2292.1.200	2	no plot	no plot
PI309881	2	0.769	0.023
2292.2.1000	2	0.021	-0.007
2292.11.200	2	0.066	-0.0
2319.1.1000	2	no plot	no plot
2313.9.3000	**2**	**no plot**	**no plot**
2313.9.1000	**2**	**0.011**	**0.005**
2313.4.3000	2	0.086	0.001
2313.9.2000	**2**	**0.008**	**0.003**
2313.10.3000	**2**	**0.036**	**-0.003**
2319.1.3000	2	0.085	-0.004
2319.4.2000	2	0.154	-0.005
2295.5.3000	2	0.055	0.008
Romano Gold	2	0.665	1.357
Redon	2	0.515	1.680
IDC IX	2	0.796	not sampled
2292.1.200	3	no plot	no plot
PI309881	3	0.199	0.023
2292.2.1000	3	0.022	0.005
2292.11.200	3	0.025	0.005
2319.1.1000	3	0.825	2.634
2313.9.3000	**3**	**0.005**	**-0.0**
2313.9.1000	**3**	**0.005**	**0.004**
2313.4.3000	3	0.009	-0.003
2313.9.2000	**3**	**0.026**	**0.006**
2313.10.3000	**3**	**0.034**	**0.002**
2319.1.3000	3	0.649	2.064
2319.4.2000	3	0.007	-0.001
2295.5.3000	3	no plot	no plot
Romano Gold	3	0.176	0.104
Redon	3	0.695	1.334
IDC IX	3	0.480	1.827

The Bean Anthracnose Resistance Gene *Co-5*, is Located in Linkage Group B7

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Introduction

Anthracnose, caused by fungus *Colletotrichum lindemuthianum* (Sacc.&Magn.) Scrib., is one of the most important diseases of common bean (*Phaseolus vulgaris* L.), causing economic losses in many parts of the world. At least, nine independent genes involved in the genetic control of the resistance to this pathogen have been described in common bean (Kelly&Vallejos, 2004). However, only six anthracnose resistance genes have been mapped: *Co-1* in linkage group B1; *Co-2* in B11; *Co-3/Co-9* in B4; *Co-4* in B8; *Co-6* in B7. In addiction, the existence of other anthracnose resistance genes in the genetic map of common bean has been suggested: *Co-x* and *Co-w* in linkage group B1, *Co-u* in B2*, Co-y* and *Co-z* in B4 and *Co-v* in B7 (Kelly *et al*., 2003).

The *Co-5* resistance gene was originally described in the bean cultivar TU. This is one of the twelve differential cultivars used for the identification of pathogenic variants of the fungus, containing resistance to numerous races (Mahuku & Riascos, 2004). The results of various allelism tests using specific races showed that the resistance present in TU is independent of genes *Co-1*, *Co-2*, *Co-3/Co-9* and *Co-4* (Kelly & Vallejos, 2004). The objective of this study was to map the *Co-5* anthracnose resistance gene using molecular markers previously included in genetic maps of common bean.

Material and Methods

The analysis was developed on a F_2 population obtained from the cross between the differential cultivars TU and MDRK. The TU cultivar is resistant to race 38 while the MDRK cultivar is susceptible to this race. The evaluation for the reaction against race 38 was carried out on a total of 86 $F_{2:3}$ families. At least 16 F_3 seedlings derived from each F_2 plant were inoculated using race 38 according to standard methods (Pastor Corrales *et al*., 1994).

In order to position the *Co-5* gene, the segregation of different SCAR or RAPD markers linked to anthracnose resistance genes (including the SCAR SAB3, previously linked to *Co-5*; Vallejos & Kelly, 2001), and SSR markers previously included in the genetic map of common bean (Blair *et al*., 2003), were used. The different DNA markers were analyzed according to the instructions of the respective authors. On the other hand, the phaseolin segregation and other seed proteins were examined. The seed proteins analysis and the designation of the different bands were carried out according to Ferreira *et al*. (2000).

The genetic distance between the loci were determined with the aid of MAPMAKER using a LOD score minimum of 3.0 (Lander *et al*., 1987).

Results and Discussion

The $F_{2:3}$ population was evaluated for resistance to race 38, and the observed segregation showed a fit to a 1:2:1 ratio, agreeing with the hypothesis of one dominant resistance gene being involved in the resistance ($\chi^2_{1:2:1}$= 0.34; p>0.05). Table 1 shows the analysis of linkage between the resistance gene and several markers. The resistance locus was linked to marker SAB3 (*Co-5*), and showed an independent segregation of the molecular markers linked to other anthracnose resistance genes: $OF10^{500}$, linked to *Co-1* (Young & Kelly, 1997); SCAReoli, linked to *Co-2* (Geffroy *et al*., 1998); SW12, linked to *Co-3/Co-9* (Méndez-Vigo *et al*., 2005); $OI16^{850}$, linked to *Co-4* (Cardoso *et al*., 2000); $OAK20^{890}$ linked to Co-6 gene (Young & Kelly, 1997); and $OF10^{1000}$, linked to *Co-10* gene (Alzate-Marín *et al*., 2003). This indicates that the gene involved in this resistant reaction is *Co-5*.

On the other hand, the resistance was linked to the seed proteins, phaseolin (Phs) and Sp4/Sp5. Phaseolin is the main seed protein and it has been mapped on linkage group B7. In order to confirm the localization of *Co-5* in this linkage group, molecular markers BM183 and SAS8, mapping on B7 (Blair *et*

al., 2003; Larsen &Miklas, 2004), were also analyzed (Table 1). Phs, Sp4/Sp5, SAS8, BM183, SAB3 and the resistance gene were located in the same linkage group, and a genetic map was developed (Figure 1).

In linkage group B7, the presence of two anthracnose resistance genes (*Co-v* and *Co-6*), has been described (Kelly *et al*., 2003). The absence of linkage found between $OAK20_{890}$ and the resistance gene studied in this work confirms the independence between *Co-6* and *Co-5*. However, the proximity between *Co-v* and the phaseolin (Kelly *et al*., 2003), suggests a possible allelism or close linkage between *Co-v* and *Co-5*. A cluster including resistances to several diseases, closely linked to marker SAS8, in linkage group B7, has been also described (Larsen & Miklas, 2004). The results obtained here concerning the relative positions of SAS8 and *Co-5* with respect to the phaseolin (Figure 1), suggest that *Co-5* is not included in such cluster.

Table 1. Observed ratios, recombination frequencies (RF) and LOD obtained in the population derived from the cross TU x MDRK for the resistance to race 38 of *C. lindemuthianum* and different markers situated in the genetic map of common bean. +/- = presence/ absence of the corresponding marker; R/r = resistant/ susceptible.

				F_2 plants										
				RR			Rr			rr				
Markers	Linked gen or linkage group	TU (RR)	MDRK (rr)	+/+	+/-	-/-	+/+	+/-	-/-	+/+	+/-	-/-	RF	LOD
Phs	B7	S (-)	T (+)	1	3	18	5	25	4	13	2	1	0.13	12.49
Sp4/Sp5	B7	-	+	9		13	26		8	15		1	0.25	3.23
SAS8	B7	+	-	16		3	30		8	8		11	0.30	1.96
BM183	B7	+	-	16		6	27		9	11		8	0.43	0.20
SAB3	*Co-5*	+	-	22		0	32		6	3		15	0.12	8.61
SCAReoli	*Co-2*	+	-	13		9	21		17	12		7	0.50	0.00
SW12	*Co-3/Co-9*	+	-	14		7	29		8	11		8	0.46	0.06
$OF10^{1000}$	*Co-10*	+	-	12		8	29		8	8		11	0.42	0.29
$OF10^{500}$	*Co-1*	+	-	16		5	28		10	15		4	0.50	0.00
$IO16^{850}$	*Co-4*	+	-	13		3	24		6	11		4	0.44	0.00
$OAK20^{890}$	*Co-6*	-	+	13		4	23		8	14		2	0.44	0.11

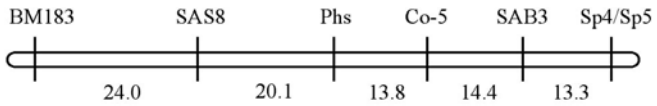

Figure 1. Relative positions of three molecular markers, two seed proteins and the *Co-5* gene, involved in the resistance against race 38.

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INTROGRESSION OF *Co-4*^{2} AND *Co-5* ANTHRACNOSE RESISTANT GENES INTO "CARIOCA" COMMON BEAN CULTIVARS WITH THE AID OF MAS

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In our backcross breeding program for creation of common bean cultivars resistant to anthracnose, incited by *Colletotrichum lindemuthianum*, one of the donor parents is cultivar G 2333. The complementary action of *Co-4*^{2} and *Co-5*, the major anthracnose resistance genes of G 2333, is effective against all known *C. lindemuthianum* races. This cultivar showed resistance to 50 *C. lindemuthianum* pathotypes collected in several common bean growing regions in Brazil (Alzate-Marin and Sartorato 2004). The recurrent parent "Rudá" is a "carioca" type cultivar with good yield potential but susceptible to most races of anthracnose. The objective of this work was to develop "carioca-type" resistant lines carrying separately the *Co-4*^{2} and *Co-5* genes aided by molecular markers, and to characterize these lines with different races of *C. lindemuthianum*.

Obtaining lines with *Co-4*^{2} gene: Selection of plants genetically closer to recurrent parent "Rudá" and containing the *Co-4*^{2} gene was conducted along three backcrossing generations, based on (1) resistance to race 73 of *C. lindemuthianum*, (2) genetic distances determined with RAPD markers and (3) presence of RAPD markers linked to the *Co-4*^{2} gene, and (4) phenotype evaluation. Amplification with RAPD markers $OPH18_{1200C}$ and $OPAS13_{950C}$ (Alzate-Marin *et al.*, 2001, Young *et al*, 1998) showed that all BC_2F_1 and BC_3F_1 plants harbored DNA fragments linked to the *Co-4*^{2} gene. BC_3F_2 resistant plants carrying *Co-4*^{2} and segregating for one anthracnose resistance gene (3:1) were identified and selfed. These lines were again analyzed with RAPD markers $OPH18_{1200C}$ and $OPAS13_{950C}$ to ensure the *Co-4*^{2} gene was present after selfing. Progeny tests were performed and 10 non-segregating lines derived from plants 341-22-3-19-1 and 341-22-3-19-11 were identified. These lines were selfed for three consecutive generations.

Obtaining lines with *Co-5* gene: For selecting BC_1F_1 plants carrying only the *Co-5* gene, the absence of RAPD marker $OPH18_{1200C}$ linked to *Co-4*^{2} was used as selection criterion. Only one plant did not harbor this marker. This plant generated a BC_1F_2 population that was inoculated with *C. lindemuthianum* race 89. The presence of RAPD marker $OPB03_{450C}$ linked to the *Co-5* gene (Young *et al.*, 1998) and the absence of $OPH18_{1200C}$ were tested in this population (Figure 1). BC_1F_2 resistant plants carrying *Co-5* and segregating for one anthracnose resistance gene (3:1) were identified and selfed. Progeny tests with *C. lindemuthianum* race 89 were performed for identification of non-segregating lines genetically closer to the recurrent progenitor Rudá. Seven lines were selected and selfed for two generations.

Resistance spectrum: Inoculation of BC_3F_6 and BC_1F_5 lines with C. lindemuthianum races 7, 55, 64, 65, 73, 81, 87, 89, 119, 453, and 2047 (only lines BC_3F_6), demonstrated that the new "carioca-type" lines are resistant to all of them. This same spectrum was observed for black seeded cultivars Selection 1308 and TU which possess the same resistance genes Co-4^2 and Co-5 (Arruda et al., 2001; Rava et al., 1994). The genes present in Selection 1308 and TU provide resistance to 11 and 26 C. lindemuthianum races characterized in Brazil (Arruda et al., 2001; Rava et al., 1994).

Final selection of lines: Pairwise genetic distances between recurrent parent "Rudá" and the "carioca-type" resistant BC_3F_6 and BC_1F_6 plants were determine to select lines genetically similar to the

recurrent parent. All BC_3F_6 plants showed genetic distances of zero in relation to Rudá (Table 1). The genetic distances between the selected BC_1F_6 lines and recurrent progenitor "Rudá" varied from 27.86 to 29.50% (Table 1). Using this methodology it was possible to select ten BC_3F_6 lines carrying the *Co-4*^{2} gene and three BC_1F_6 lines carrying *Co-5* gene genetically similar to the recurrent parent "Rudá". These lines can now be used in common bean programs that aim at pyramiding anthracnose resistance genes and/or as adapted sources of genes in Brazil.

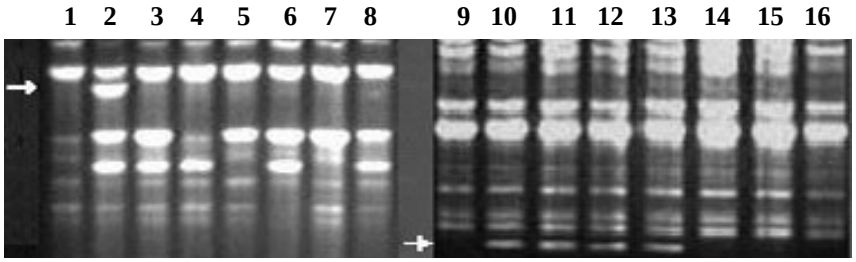

Figure 1. Electrophoretic analysis of DNA amplification products obtained with primers OPH18 (A) and OPB03 (B). Lanes are as follows: 1 and 9, Rudá; 2 and 10, G 2333; 3-5/11-13, BC_1F_2 plants resistant to *C. lindemuthianum* race 89; and 6-8/14-16, BC_1F_2 plants susceptible to *C. lindemuthianum* race 89. The arrows indicate bands of 1,200 and 450 bp amplified with primers OPH18 (A) and OPB03 (B), linked in coupling phase to *Co-4*^{2} and *Co-5* anthracnose resistance genes, respectively.

Table 1. Pairwise relative genetic distances between recurrent (Rudá) and donor (G 2333) parents, and BC_3F_6 and BC_1F_5 anthracnose resistant lines.

BC_3F_6 lines			**BC_1F_5 lines**		
Genotype	**Rudá**	**G 2333**	**Genotype**	**Rudá**	**G 2333**
G 2333	100.00	-	G 2333	100.00	-
341-22-3-19-1-1	0.00[a]	100.00	1-46-1	31.14	68.85
341-22-3-19-1-2	0.00[a]	100.00	1-46-2	40.98	59.01
341-22-3-19-1-3	0.00[a]	100.00	1-46-3	37.70	62.29
341-22-3-19-1-4	0.00[a]	100.00	1-46-5	27.86[a]	72.13
341-22-3-19-1-5	0.00[a]	100.00	1-46-6	29.50[a]	70.49
341-22-3-19-1-6	0.00[a]	100.00	1-46-7	27.86[a]	72.13
341-22-3-19-1-7	0.00[a]	100.00	1-46-8	34.42	65.57
341-22-3-19-11-1	0.00[a]	100.00			
341-22-3-19-11-2	0.00[a]	100.00			
341-22-3-19-11-3	0.00[a]	100.00			

[a]Selected lines.

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Anthracnose Resistance Spectra of Breeding Lines Derived from the Dry Bean Landrace Andecha

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Andecha is a very large white seeded landrace susceptible to races 6 and 38 and with moderate resistance to races 3, 19 and 102 of *C. lindemuthianum*. These five races are the most commonly found in Northern Spain (Ferreira et al., 1998). To protect Andecha against local races of anthracnose, four parallel backcross breeding programs were developed using germplasm lines A252, Sanilac, A321 and A493 as resistance donors and Andecha as the recurrent parent (figure 1). After 6 backcross generations, and selection for resistance to race 38, lines A1258, A1183, A1231 and A1220 were obtained. Molecular marker analysis carried out previously on the parents and the breeding lines, revealed that only a segment of the B4 linkage group (including the *Co-3/Co-9* gene) proceeding from donors A321 and A493 was present in lines A1231 and A1220, respectively, whereas only a segment of linkage group B11 (including the *Co-2* gene) proceeding from donors Sanilac and A252 was present in lines A1183 and A1258, respectively (Méndez-Vigo, 2001; Méndez-Vigo et al., 2005). Lines A2438 and A1699, were obtained from single crosses between lines A1183, A1220 and A1258 (see fig. 1), followed by marker assisted selection in order to pyramid the resistance genes present in linkage groups B4 and B11. The six lines obtained recovered all the phenotypic characteristics present in the recurrent parent Andecha. In this work, we describe the resistance spectra against 18 races of *C. lindemuthianum* displayed by the parents and the resistant lines obtained in the breeding program.

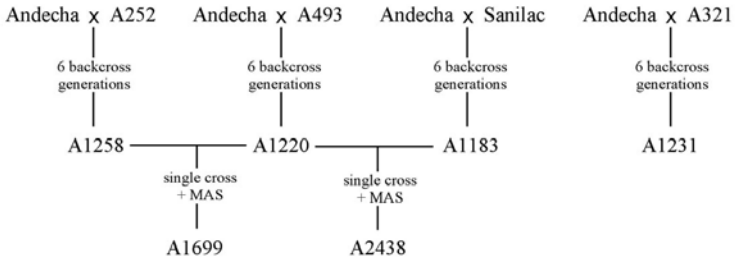

Figure 1. Origin of the breeding lines developed to protect Andecha against local races of anthracnose.

Eighteen races of *Colletotrichum lindemuthianum* were used: races 7, 31, 39, 65, 65 brazil (65b), 73, 81, 357, 449, 1545, 1933 and 2047 from the collection of the Plant and Soil Department (Michigan State University, US), and races 3, 6, 9, 19, 38 and 102 from local isolates of the SERIDA collection. A minimum of ten plants of each line (Andecha, A252, Sanilac, A321, A493, A1258, A1183, A1231, A1220, A2438 and A1699) were inoculated according to standard methods (Pastor Corrales et al., 1994). The responses of the plants were evaluated after 7-9 days using a 1-9 scale where 1 is no visible symptoms and 9 very severely diseased or dead (Van Schoonhoven and Pastor-Corrales, 1987).

Table 1 shows the results of the resistance tests. The comparison of the resistance spectra of each breeding line and its corresponding parents makes possible to predict the most likely locations of some of the genes conferring resistance to the different races.

- **A1258**. Resistance specificities to races 3, 6, 19, 31, 38, 39, 65b and 102 are located on the B11 block, introgressed in line A1258 from donor parent A252, since resistance to these races is added or improved when compared to the Andecha resistance spectrum. Resistance specificities to races 81 and 357, present in A252, were not introduced in A1258, being located somewhere else in the A252 genome.
- **A1183**. Resistance specificities to races 3, 6, 19, 38, 65b, 102, 357 and 449 are located on the B11 block, introgressed in line A1183 from donor parent Sanilac, since resistance to these races is added or

improved when compared to the Andecha resistance spectrum. Resistance specificities to races 39 and 81, present in Sanilac, were not introduced in A1183, being located somewhere else in the Sanilac genome.

- A1220 and A1231. No differences were found in the resistance spectrum showed by these two breeding lines. The resistance spectra of the corresponding donors (A493 and A321, respectively) were also identical. These two donor lines carry the resistance to races 3, 6, 19, 38, 39 and 357 in the B4 block, introgressed in lines A1220 and A1231, since resistance to these races is added or improved when compared to the Andecha resistance spectrum. Resistance to race 31 would be provided by other resistance gene, as it was not introgressed in the breeding lines. Recurrent parent Andecha carries a gene conferring partial resistance to race 449 on B4 linkage group, since resistance to this race disappears in breeding lines A1231 and A1220 when replaced by the introgressed B4 block, comming from their corresponding donor parents A321 and A493, respectively. Moreover, Andecha has a gene (or genes) conferring resistance to races 65b and 1933, not located on B4, as breeding lines A1231 and A1220 remain resistant to these races after the replacement of the B4 block from their corresponding donors (which are susceptible to these two races).

- A2438 and A1699. These two lines were the result of pyramiding resistance genes in lines A1183 and A1220 (A2438) and in lines A1258 and A1220 (A1699). The evaluation of A1699 for resistance against some of the races is still under progress. The resistance spectrum in both lines seems to be the addition of the resistance specificities of the corresponding parent lines. The resistance analysis carried out in this work revealed that all parent lines have at least two different genes or clusters conferring resistance to the 18 anthracnose races tested.

Table 1. Resistance spectra against 18 races of *C. lindemuthianum*, displayed by the materials involved in the breeding program shown in figure 1. RP: recurrent parent; DP: resistance donor parents; BL: breeding lines; PL: lines obtained by pyramidation. R: resistant; R*: partial resistance; S: susceptible

		Races										
	Lines	7, 9, 65, 73, 1545	3, 19, 102	6, 38	31	39	65b	81	357	449	1933	2047
RP	Andecha	R	R*	S	S	S	R*	R*	S	R*	R	S
DP	A252	R	R	R	R	R	R	R	R	R*	R	S
BL	A1258	R	R	R	R	R	R	R*	S	R*	R	S
DP	Sanilac	R	R	R	S	R	R	R	R	R	R	S
BL	A1183	R	R	R	S	S	R	R*	R*	R	R	S
DP	A321	R	R	R	R	R	S	R*	R	S	S	S
BL	A1231	R	R	R	S	R	R*	R*	R*	S	R	S
DP	A493	R	R	R	R	R	S	R*	R	S	S	S
BL	A1220	R	R	R	S	R	R*	R*	R*	S	R	S
PL	A2438	R	R	R	S	R	R	R*	R*	R	R	S
PL	A1699	-	R	R	R	-	-	-	R*	-	-	-

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UNEXPECTED RESISTANCE GENES FOR ANTHRACNOSE UNCOVERED

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Genetic improvement in dry bean is achieved through a combination of traditional and molecular selection techniques. The resulting cultivars are pure lines, which are improved for one or more specific traits. Despite the homozygous nature of bean cultivars, sources of genetic variation may be mutations, insect cross pollination or the cultivars' heterogeneity (Traka-Mavrona et al., 2000). In the absence of direct selection, many pure-line cultivars may be heterogeneous for major resistance genes. The following are case studies in which new resistance genes were unexpectedly uncovered in pure line cultivars.

Black Magic

The black bean cultivar, Black Magic (BM), was reported to carry resistance to races beta, gamma and delta of *C. lindemuthianum* (Kelly et al., 1987). In the currently used nomenclature for race classification, these are races 130, 102 and 23 respectively (Melotto et al., 2000). Black Magic, however, unexpectedly segregated for resistance to race 7 (Melotto, 1999). Although BM carries resistance to anthracnose races 23, 102 and 130, anthracnose resistance was not a specific goal in cultivar development (Kelly, personal communication). Our hypothesis is that the resistance to race 7 in BM is conferred by a single dominant gene. To determine the number of genes conferring resistance to race 7 in BM a F_2 population was generated from the cross BM (resistant to race 7; R7) x BM (susceptible to race 7; S7). The resulting population was inoculated with race 7 (Table 1). The F_2 population, BM (R7) x BM (S7), segregated in a 15:1 ratio of resistant to susceptible individuals (p-value = 0.936) (Table 1) indicating that the resistance to race 7, in the cultivar BM, is conditioned by two dominant resistance genes. Allelism tests must next be carried out to determine if these genes are independent of the other previously described anthracnose resistance genes. We believe that the unexpected resistance to anthracnose is a result of the cultivars natural heterogeneity which exists for specific traits which are not under direct selection during cultivar development.

Mexico 222

The anthracnose differential cultivar Mexico 222 is believed to carry a single dominant gene, *Co-3* which conditions resistance to anthracnose (Kelly and Vallejo, 2004). To determine if the anthracnose resistance in the MSU breeding line, MSU 7, is allelic to *Co-3*, a F_2 population was generated from the cross Mexico 222 x MSU 7-4. The resulting population was inoculated with race 7, which results in an RxR reaction in the parents (Table 1). The Mexico 222 x MSU 7-4 F_2 population segregated for resistance at a ratio of 63:1 (p-value = 0.979), resistant to susceptible individuals. This indicates that there are three genes segregating for resistance to race 7 in this population. Since race 7 elicits a RxR reaction in the parents (Table 1), and it was previously determined that MSU 7-4 carries only one gene conditioning resistance to race 7 (Vallejo and Kelly, unpublished data), we conclude that there must be two anthracnose resistance genes segregating from the cultivar Mexico 222. One of the anthracnose resistance genes in Mexico 222 is known to be *Co-3* and the other remains unknown. Neither of these anthracnose resistance genes are allelic to the gene carried by MSU 7-4. Heterogeneity within the cultivar can be used to explain this unexpected discovery of an additional anthracnose resistance gene in Mexico 222. Alternatively, with the abundance of different races of *C. lindemuthianum*

available, and the implicit gene-for-gene relationship between resistance in the plant and pathogenicity in the pathogen, it is not surprising if some anthracnose resistance genes go undetected in certain population/race inoculation experiments. Additional characterization of the second anthracnose resistance gene in Mexico 222 is needed to determine if it is independent of the previously reported anthracnose genes and what spectrum of resistance it offers.

Table 1 F2 populations inoculated with race 7 of *C. lindemuthianum*.

Population or Genotypes	Race Inoculated	Disease Reaction	Observed Ratio[a]	Expected Ratio[a]	p-value
BM (R7) x BM (S7)[b]	7	-	87:6	15:1	0.936
Mexico 222 x MSU 7-4	7	-	249:4	63:1	0.979
Mexico 222	7	R	-	-	-
MSU7	7	R	-	-	-

[a] Ratio of resistant to susceptible individuals
[b] R7 = resistant to race 7, S7= susceptible to race 7

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Initial dissection of the anthracnose resistance in the landrace cultivar G 2338

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INTRODUCTION

Anthracnose, is a seedborne disease of common bean (*Phaseolus vulgaris*), caused by the hemibiotrophic fungus, *Colletotrichum lindemuthianum*. The devastating effect of anthracnose on yield, particularly in tropical production areas, makes it one of the most economically important diseases of bean. Several disease management strategies are available, however, planting genetically resistant cultivars is the most effective and least expensive way for growers to prevent yield loss due to anthracnose (Pastor-Corrales et al., 1995). The best breeding strategy to generate resistant cultivars is by pyramiding anthracnose resistance genes with complementary spectra of resistance (Kelly and Vallejo, 2004). The highly variable nature of the pathogen, however, requires that new sources of resistance are constantly being characterized. The landrace cultivar G 2333, from Chipas, Mexico, is the most resistant cultivar of the anthracnose differential series and carries three independent resistance genes (*Co-4*^{2}, *Co-5* and *Co-7*). Recently, a highly virulent *C. lindemuthianum* race of Middle American origin (Costa Rica) has been identified which overcomes the resistance in G 2333. Interestingly, the landrace G 2338, also from Chiapas, Mexico is resistant to this race (J. Kelly personal communication). Since both landraces are from the same region of Mexico, we hypothesize that the differential resistance is conferred by a small difference in their allelic composition, therefore, we postulate that G 2338 carries a similar anthracnose gene pyramid. The objective of this study was to initialize the molecular and genetic characterization and dissection of the anthracnose resistance genes carried by G 2338.

MATERIALS AND METHODS

Initial characterization of the anthracnose resistance in G 2338 was carried out using molecular markers linked to known anthracnose resistance genes (Table 1). Additionally, G 2338 was previously tested for the presence of several SCAR markers linked to the *Co-4* locus and the *Co-4*^{2} allele (Table 2) (Awale and Kelly, 2001). The number of genes conditioning resistance to anthracnose in G 2338 was determined by inoculating a F_2 population of 201 individuals from the cross La Victorie (susceptible) x G 2338 (resistant) with race 7. Based on the results of the initial characterization with molecular markers linked to anthracnose resistance genes, a SCAR marker was used to begin the molecular dissection of the gene pyramid in G 2338.

RESULTS AND DISSCUSSION

The initial characterization of the anthracnose resistance genes carried by G 2338, based on analysis with molecular markers linked to various known genes, indicated that G 2338 carries the *Co-5* and *Co-4* loci (Table 1). Since allele specific primers exist for the *Co-4* locus, we wanted to determine if the G 2338 carries the same allele as G 2333 at the *Co-4* locus. The results displayed in Table 2 indicate that G 2338 does not carry the same allele at the *Co-4* locus as G 2333. To determine the number of genes segregating for resistance to anthracnose a F_2 population (La Victorie x G 2338) was inoculated with race 7 which yields a SxR reaction in the parents. Out of the 201 F_2 individuals inoculated, 9 were lost to lethal combinations of dwarfing genes due to the intergene pool nature of the cross. The remaining progeny segregated in a 63:1

ratio of resistant to susceptible indicating that three genes are segregating for resistance to race 7 (p-value= 0.244). Based on our preliminary gene characterization using molecular markers we believe that two of the genes are *Co-5* and *Co-4*. To begin the molecular dissection of this gene pyramid, we used the SCAR marker SAB3 linked to the *Co-5* locus to select against this locus among the surviving progeny of the F2 La Victorie x G 2338 population. Unfortunately, we were not able to elucidate the identity of the third gene conditioning resistance to anthracnose in G 2338. However, based on the similar origin of both of these landraces and the fact that they both have three genes conditioning resistance to anthracnose two of which are the same, we speculate that the third gene in G 2338 is very likely to be *Co-7*. Given the allele complexity at the *Co-4* locus we believe that the resistance differential between G 2333 and G 2338 is due to differences in the allelic composition at the *Co-4* locus.

Table 1. RAPD markers linked to anthracnose resistance genes

Molecular marker	Linked resistance gene	Linkage phase	+/-[a]
$F10_{530}$	*Co-1*	repulsion	-
$Q4_{1440}$	*Co-2*	coupling	-
$H20_{500}$	*Co-2*	coupling	-
$AB3_{400}$	*Co-5*	coupling	+
$AH1_{780}$	*Co-6*	coupling	-
$AK20_{890}$	*Co-6*	repulsion	-
$AS13_{950}$	*Co-4*	coupling	+
SB12	*Co-9*	coupling	-

[a] Presence (+) or absence (-) in G 2338

Table 2 SCAR markers linked to the *Co-4* locus or specific alleles.

SCAR marker	Locus	G 2333	G 2338
SH18	*Co-4*^{2}	+	-
SAS13	*Co-4*	+	+
SBB14	*Co-4*	+	+

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Characterization of the anthracnose resistance gene in common bean cultivar Michelite

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Introduction

The Mesoamerican Michelite is used to characterize *Colletotrichum lindemuthianum* races, being considered universal susceptible differential cultivar. Previous works have revealed that Michelite differential cultivar demonstrated different mechanisms of resistance to distinct physiological races of the pathogen. It was resistant to races alpha, beta, gamma and some races from Mexican groups, being characterized as a dominant monogenic action (Yerkes Jr. and Ortiz, 1956; Cárdenas et al. 1964; Tu, 1984; Kelly et al. 1994). It has been noticed in Brazil that Michelite is also resistant to races 8, 64, 72, and 102 (Rava et al., 1994). According to the same authors, the races 8, 64 and 72 belong to Mexican Group I and race 102 to Gamma group. There is no enough information about inheritance and independence of the gene present in Michelite in relation to genes previously characterized. The objective of this work was to investigate the inheritance of anthracnose resistance and characterize the independence of the gene present in Michelite using the race 64.

Material and Methods

The common bean differential cultivar Michelite was crossed with Michigan Dark Red Kidney (MDRK), Kaboon, Perry Marrow, AND 277, Widusa, Cornell 49-242, TO, TU, AB 136, BAT 93, Ouro Negro, PI 207262 (all resistant genotypes), and Mexico 222, susceptible to race 64. Fourteen days old seedlings with fully developed first trifoliate leaves were spray-inoculated with a spore suspension (1.2×10^6 spores ml^{-1}) of race 64 of *C. lindemuthianum*. After a 48 hour incubation period in a mist chamber, seedlings were evaluated for their disease reaction using a scale of 1 to 9 (Balardin et al. 1990). Plants with disease reaction that scored of 1-3 were considered resistant, whereas plants that rated from 4-9 were considered susceptible.

Results and Discussion

The inheritance studies demonstrated a 3R:1S ratio in the F_2 population from the cross between Michelite and Mexico 222 ($p = 0.70$), indicating the presence of only one resistant gene in Michelite differential cultivar.

According to Table 1, the allelism tests in 11 F_2 populations from the crosses of Michelite and other previously characterized cultivars supported an expected 15R: 1S ratio. These results indicate the action of two independent dominant genes conferring resistance to anthracnose. Allelism tests in the crosses involving Michelite with MDRK, Perry Marrow, Kaboon, AND 277, Widusa, Cornell 49-242, TO, TU, AB 136, BAT 93 and Ouro Negro cultivars, fitted a 15R:1S ratio in F_2 population, indicating that each of the cultivars carry an independent dominant resistance gene. This result supports the independence of the gene in Michelite from other Andean and Middle American resistance genes.

The segregation of 63R:1S ratio ($p = 0.73$) was demonstrated by F_2 population from the cross Michelite x PI 207262 supporting the hypothesis of three dominant genes in segregation. PI 207262 differential cultivar possesses two dominant genes: *Co-4*^{3} and *Co-9* (Alzate-Marin et al., 2002; Geffroy et al. 1999; Gonçalves-Vidigal et al., 1997), which segregated independently from that one presented in Michelite.

The allelism tests demonstrated that the gene present in Michelite is independent of the prior characterized genes that are: *Co-1, Co-1*^{2}, *Co-1*^{3}, *Co-1*^{4}, *Co-1*^{5}, *Co-2, Co-4, Co-5, Co-6, Co-9, Co-4*^{3}, and *Co-10*. Therefore, the authors propose that the anthracnose resistance gene present in Michelite should be designated with the symbol *Co-11,* and this characterization will contribute providing information to breeders that carry out researches involving common bean differential cultivars to identify *C. lindemuthianum* races.

Table 1. Cross, number of plants evaluated and expected phenotypic ratios in F_2 populations for resistance to race 64 of *Colletotrichum lindemuthianum*

Cross	Reaction*	Number of Plants		Expected Ratio	χ^2	*P value*
		R	S	R:S		
Michelite x México 222	R x S	106	38	3:1	0.1481	0.70
Michelite x MDRK	R x R	110	9	15:1	0.3501	0.55
Michelite x Kaboon	R x R	96	7	15:1	0.0524	0.81
Michelite x Perry Marrow	R x R	108	8	15:1	0.0827	0.77
Michelite x AND 277	R x R	99	4	15:1	0.9844	0.32
Michelite x Widusa	R x R	158	8	15:1	0.5799	0.45
Michelite x Cornell 49242	R x R	101	9	15:1	0.7006	0.40
Michelite x TO	R x R	49	3	15:1	0.0205	0.88
Michelite x TU	R x R	69	6	15:1	0.3920	0.53
Michelite x AB 136	R x R	75	5	15:1	0.0000	1.00
Michelite x BAT 93	R x R	135	9	15:1	0.0000	1.00
Michelite x Ouro Negro	R x R	70	7	15:1	0.0024	0.96
Michelite x P I 207262	R x R	155	3	63:1	0.1161	0.73

*S= Susceptible, R= Resistant.

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USE OF MOLECULAR MARKERS LINKED TO COMMON BEAN ANTHRACNOSE RESISTANCE IN A REAL BREEDING SITUATION

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Some diseases cause severe damages to the common bean (*Phaseolus vulgaris* L.), reducing productivity and burdening the production cost. For this reason, resistance to different pathogens is one of the main objectives of bean breeding programs. Molecular markers linked to the resistance genes can be a useful tools in these programs as they allow selection for resistance in the absence of the pathogen. Besides, markers linked to several different resistance genes could be useful in breeding programs seeking resistance to multiple diseases (gene pyramiding) as marker assisted selection can be easily incorporated in the breeding process (Kelly et al., 1995). Molecular markers $SCARF10_{1050}$ and $OPX11_{550}$ have been frequently used in the BIOAGRO/UFV bean breeding program. Marker $SCARF10_{1050}$ is used to monitor genes conferring resistance to anthracnose (*Co-10*) and to rust (*Ur-ON*) (Corrêa et al., 2000), while $OPX11_{550}$ has been used as a marker for gene *Ur-ON* (Faleiro et al., 2003). These genes are present in the cultivar Ouro Negro and were identified in a population derived from a cross between Ouro Negro and Rudá. Although $OPX11_{550}$ has not been used to monitor anthracnose resistance, it is possible that this marker, besides tagging a rust resistance gene, also tags *Co-10* (Faleiro et al., 2000).

The present work aimed at evaluating the markers currently being used by BIOAGRO/UFV and to determine their selection efficiency in a population which was not specifically prepared for marker assisted selection. The population derived from a cross between cultivars Ouro Negro and Pérola was conducted by the bulk within-family selection method. Initially, 150 $F_{3:7}$ lineages were phenotypically characterized by artificial inoculation with *C. lindemuthianum* race 89. At the same time, each lineage was genotyped with markers $SCARF10_{1050}$ (genes *Co-10* and *Ur-ON*) and $OPX11_{550}$ (gene *Ur-ON*).

Out of the 150 lineages tested by artificial inoculation, 68 were resistant to race 89 of *C. lindemuthianum*. Analysis of the same lineages with $SCARF10_{1050}$ showed a 78% selection efficiency when compared to the inoculation results. Considering that this marker is 19.6 cM apart from the resistance gene, the expected selection efficiency would be 72%. Analysis of the lineages with marker $OPX11_{550}$ showed a larger efficiency (85%). This was expected because this marker is closer (11.6 cM) to the gene block conferring resistance to anthracnose and rust present in cv. Ouro Negro. Moreover, it was observed that marker $OPX11_{550}$ can also be used for monitoring the anthracnose resistance gene. Although these two markers are relatively distant from the resistance genes, they provided a good selection efficiency. Figures 1 and 2 show the amplification of the marker bands in some of the 150 lineages. It is clear that in some cases, the use of the molecular markers as the only mean to phenotype the plants would lead to mistakes as some of the susceptible plants harbor the marker bands (Figures 1 and 2). In spite of the long distance between these markers and the resistance genes, the selection efficiency obtained demonstrate that they can be useful in the real situation of a common bean program.

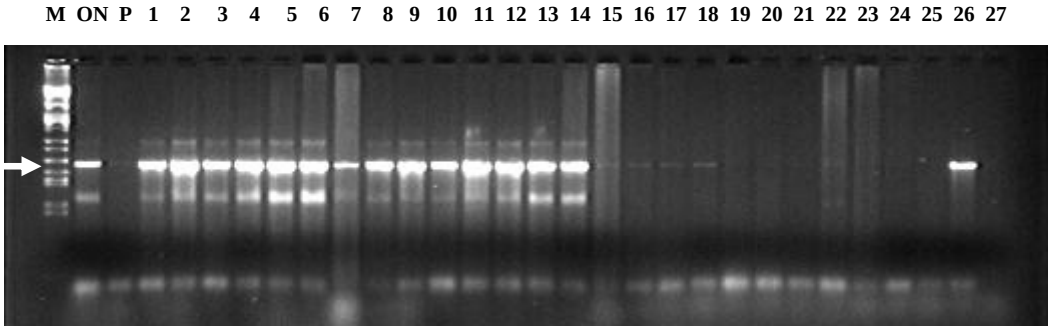

Figure 1. Amplification of marker SCARF10 in the parents (Ouro Negro x Pérola) and in derived $F_{3:7}$ lineages. The first lane (M) contains lambda phage DNA digested with *Eco*RI, *Bam*HI and *Hin*dIII (size markers). The other lanes are as follows: Ouro Negro (ON), Pérola (P), anthracnose resistant lineages (1 to 14), and susceptible lineages (15 to 27). The arrow indicates the marker $SCARF10_{1050}$.

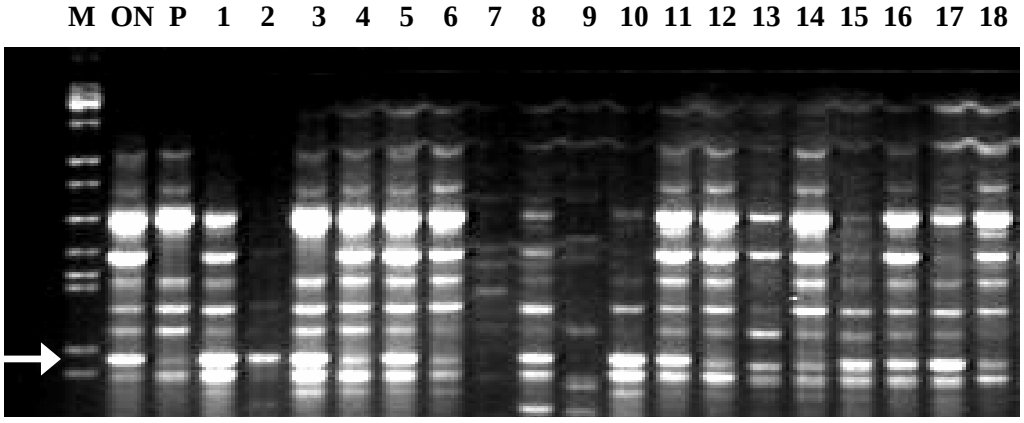

Figure 2. Amplification of marker $OPX11_{550}$ in the parents (Ouro Negro x Pérola) and in derived $F_{3:7}$ lineages. The first lane (M) contains lambda phage DNA digested with *Eco*RI, *Bam*HI and *Hin*dIII (size markers). The other lanes are as follows: Ouro Negro (ON), Pérola (P), anthracnose resistant lineages (1-5, 8, 10, 11, 13-16), and anthracnose susceptible lineages (6, 7, 9, 12, 17 and 18). The arrow indicates the marker $OPX11_{550}$.

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Marker-Assisted Selection of Common Beans for Multiple Disease Resistance

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Abstract

Common bacterial blight (CBB), bean common mosaic virus (BCMV) and anthracnose are three major diseases of common bean (*Phaseolus vulgaris L.*) in Canada and around the world wherever they are grown. Since they are seed-borne diseases and there is no effective way to control them. The most efficient and environmentally sound way to control them is the use of resistant cultivars. Simultaneous screening of bean plants for resistance to these three diseases in the greenhouse is very difficult. In addition, disease screening results are often affected by experimental and environmental factors. Marker-assisted selection (MAS) can overcome these disadvantages. MAS can be used to simultaneously screen for resistance to these three diseases without affecting the growth of plants. Since molecular markers tightly linked to each of these disease resistance genes are available, this study was conducted to transfer the three disease resistance genes into navy, black, small red, red kidney, and pinto bean cultivars by MAS. We screened various generations, such as F_1, backcrosses and advanced breeding populations for the presence of molecular markers linked to the three disease resistance genes. Individual plants that had all the marker bands and show resistances to all three diseases were identified. The problems of using molecular markers in a breeding program were also discussed.

Introduction

Two major quantitative trait loci (QTLs) for resistance to CBB from Tepary bean (*P. acutifolius*) entries PI440795 and PI 319443 were tagged by sequence characterized amplified region (SCAR) markers BC73 and UBC 420, respectively. BC73 explained 45% of the phenotypic variation of a mapping population derived from an interspecific cross involving PI 1440795 (Bai et al. 1997). It is about 9.7 cM from the major QTL. UBC420 explained 63% of the phenotypic variation of a recombinant inbred line (RIL) population derived from the cross HR67/OAC95-4 and is about 7.1 cM from the CBB QTL (Yu et al. 2004). SW13 SCAR marker is about 1 to 5 cM from the *I* gene for BCMV resistance based on three F_2 populations (Melotto et al. 1996). This marker can detect the *I* gene from different gene pools of common beans. It was used to pyramid the *I* gene with other recessive genes like *bc-3,* which conditions resistance to bean common mosaic necrosis virus (BCMNV). Among the 10 genes conferring resistance to various fungal races for anthracnose (*Co-1* to 10 except *co-8*), the $Co\text{-}4^2$ gene in SEL1308 provides the broadest resistance and has no recombination with SAS13 marker (Young et al. 1998). Breeding line H4642, developed at Harrow Research Centre from the crosses of HR67*2//Envoy/Sel1308, contains all three disease resistance genes. HR45 has both UBC420 and SW13 bands and OAC Rex has both BC73 and SW13 bands. The goal of this project is to transfer all three disease resistance genes into different market classes of beans using MAS and involved collaboration among researchers in Harrow ON, Morden MB and Lethbridge AB.

Materials and Methods

Materials: The following AAFC-developed cultivars were used as recurrent parents, Navy bean (AC Compass, AC Trident, AC Crusier, Morden003), GN (AC Polaris), black bean (AC Black Diamond, Black Violet and AC Harblack), red kidney bean (AC Calmont, and AC Elk),

small red (AC Redbond), and pinto bean AC Pintoba. Breeding lines H4642 and HR45, and cultivar OAC Rex with resistance genes were used as the male donor parents. Backcrosses, three-way and double crosses were made.

Disease screening: CBB and anthracnose reactions were evaluated for all pollen donors and advanced breeding lines at Harrow whenever possible.

Marker assisted selection: Before pollination, all pollen donors were screened with the four linked molecular markers. Only those plants with the target genes based on the presence of the markers were used for pollination.

Results and Discussion

Most recurrent parents lacked the disease resistance genes tagged by UBC420, BC73 and SAS13. For white bean cultivars, if HR45 was use as the donor in the cross, progenies with both BC73 and UBC420 could be recovered. If H4642 was in the pedigree, UC420, SW13 and SAS13 marker bands could be found in some of the progenies (Table1). In black beans, five out of 44 F_2 plants have all four marker bands. In red kidney bean and small red beans, 42 out of 67 F_4 plants had all three markers but not BC73. However, only one out of 30 plants from BC_3 had the SW13 band. For pinto bean, one out of 15 in BC_1 and one out of 41 in F_2 had the marker bands linked to all three diseases. Although it is possible to transfer CBB, BCMV and anthracnose resistance genes into one cultivar for each class, more effort is needed to increase the population size of the backcrosses. Even though SW13 and SAS13 are tightly linked to single dominant genes and are more reliable to monitor the transfer of resistance, we did find that SAS13 marker showed false positive results in some market class backgrounds. UBC420 is tightly linked to the major QTL from HR67 (HR45 or Xan159), its MAS results matched with the phenotypic screening data most of the time. However, BC73 is more problematic because it is not as specific as UBC420. MAS with reliable markers can help breeders to identify resistant plants more accurately.

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Table 1. Marker screening for progenies from different generations

Recurrent parent	Donor parent and generation	No. of plants	UBC420	BC73	SW13	SAS13
AC Polaris	OAC Rex BC_3	23 (4)§	-	6	14	-
AC Cruiser	OAC Rex/H4642† F_1	4(2)	2	3	2	4
AC Compass	H4642 F_4	81(19)	61	-	59	43
OAC Rex	H4642 F_4	58(30)	30	47	58	58
AC Black Diamond	OAC Rex BC_2	25(11)	-	14	21	-
AC Harblack	OAC Rex/H4642 F_2	44(5)	20	9	20	6
AC Redbond	OAC Rex BC_3	30(1)		29	1	
AC ELK	M4‡ F_4	67(42)	48	-	66	60
AC Pintoba	H4642 BC_1	15(1)	2	-	2	2
AC Pintoba	OAC Rex/H4642 F_2	41(1)	16	4	10	10

† H4642 was an F_5 line derived from HR67*2//Envoy/SEL1308.

‡ M4 was an F_4 line with Xan159 and Sel1308 in the pedigree.

§ Number inside parentheses is the number of plants with all tested markers present.

MOLECULAR MARKER ASSISTED BACKCROSSING FOR DEVELOPING LINES WITH CV. PEROLA GENETIC BACKGROUND, RESISTANT TO RUST, ANTHRACNOSE AND ANGULAR LEAF SPOT

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Introduction

The common bean (*Phaseolus vulgaris* L.) is one of the main protein sources in human diet in many countries, especially in Brazil. The diseases attacking this culture are among the factors that restrain its productivity and performance. Rust, anthracnose, and angular leaf spot incited by *Uromyces appendiculatus*, *Colletotrichum lindemuthianum*, and *Phaeoisariopsis griseola*, respectively, are among the most important of these diseases. The introgression of disease resistance genes in elite-cultivars has been one of the targets in bean improvement programs throughout the world. It has been shown that molecular markers are an important tool, mainly for pyramiding these genes. The objective of this study was to transfer the resistant genes to the rust, anthracnose and angular leaf spot, from cultivar Ouro Negro (*Ur-ON*, *Co-10* and *Phg-ON*, respectively), to the "carioca-type" cultivar Perola through backcrossings assisted by molecular markers.

Material and Methods

Cultivars Perola and Ouro Negro were crossed under greenhouse conditions. The hybrid nature of the F_1 seeds was phenotypically confirmed by the flower color. Three backcrossing cycles were conducted using cv. Perola as recurrent parent. In populations F_1BC_n, the resistant plants were selected on the basis of sequential inoculations with the causal agents of the diseases of interest. 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 resistance phenotypes were confirmed by using molecular markers linked to the genes *Ur-ON*, *Phg-ON* and *Co-10* (Table 1).

Molecular markers were also used in populations BC_nF_1 to determine the genetic similarity between these plants and the recurrent parent. For this purpose, the leaf DNA from the selected resistant plants was extracted according to Doyle and Doyle (1990), and amplified with 14 RAPD primers taken at random in each BC generation (Williams et al., 1990). Genetic similarities were based on the simple coincidence coefficient, which is obtained by dividing the number of DNA bands common to two given individuals by the total number of DNA bands.

Results and Discussion

Resistant BC plants which were selected based on inoculation and on the presence of molecular markers linked to the three disease resistance genes were fingerprinted with RAPD markers. The average recovery of the recurrent parent´s genome among the selected plants as determined by the molecular fingerprinting were 79%, 93% and 98% at the first, second and third backcrossing cycles, respectively. The progenies selected in the BC_3F_1 population (Figure 1) were selfed and the BC_3F_2 population was conducted by the genealogical method until generation $BC_3F_{2:5}$ in order to increase their degree of homozygozity. In each cycle, the plants showing to be resistant to the three diseases and presenting the markers linked to the three genes of interest were selected (Table 1).

From the selected $BC_3F_{2:5}$ plants resistant to rust, anthracnose and angular leaf spot non-segregating individuals will be multiplied for subsequent evaluation for agronomic performance under field conditions. Based on the results obtained, it can be concluded that the use of molecular markers in association with conventional breeding methods allowed a fast and simultaneous transfer of the resistance genes from cultivar Ouro Negro to cultivar Perola.

Table 1. Molecular markers linked in coupling phase to resistance genes for rust, anthracnose and angular leaf spot present in cultivar Ouro Negro.

Markers	Distance	Resistance Genes	Diseases	References
$OPX\text{-}11_{550}$	5.8 cM	*Ur-ON, Co-10*	rust and anthracnose	FALEIRO et al. (2000)
$OPF\text{-}10_{1,050}$	6.9 cM	*Ur-ON, Co-10*	rust and anthracnose	FALEIRO et al. (2000)
$OPAA\text{-}19_{400}$	10.0 cM	*Phg-ON*	angular leaf spot	CORRÊA et al. (2001)
$OPM\text{-}02_{425}$	5.6 cM	*Phg-ON*	angular leaf spot	CORRÊA et al. (2001)
$OPBA\text{-}16_{669}$	9.7 cM	*Phg-ON*	angular leaf spot	FALEIRO et al. (2000)
$_{SCAR}BA\text{-}08_{560}$	6.0 cM	*Ur-ON, Co-10*	rust and anthracnose	CORRÊA et al. (2000)
$_{SCAR}F\text{-}10_{1,050}$	6.9 cM	*Ur-ON, Co-10*	rust and anthracnose	CORRÊA et al. (2000)

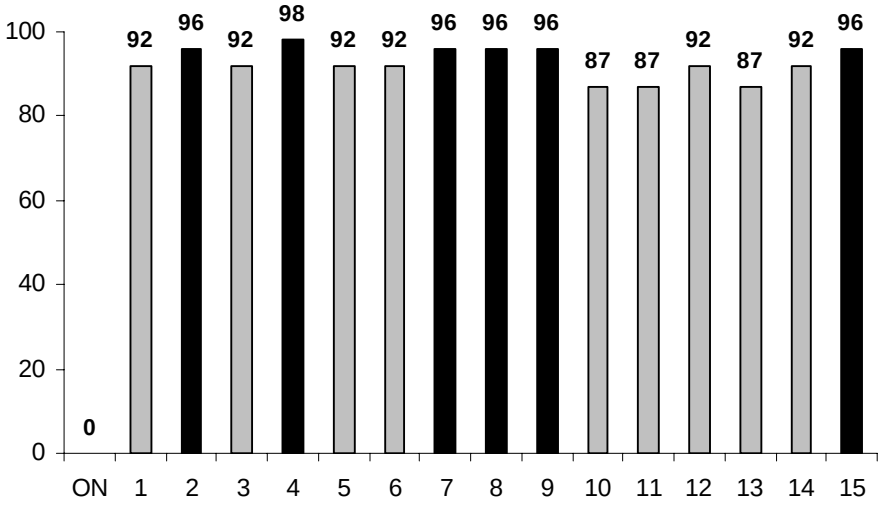

Figure 1. Relative genetic similarity (%) between BC_3F_1 plants (1 to 15) and cv. Ouro Negro (ON) in relation to the recurrent parent Perola as determined by RAPD markers. The black columns represent the plants that were closer to the recurrent parent.

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ORIGIN OF SPORES TO START AN ANGULAR LEAF SPOT EPIDEMIC

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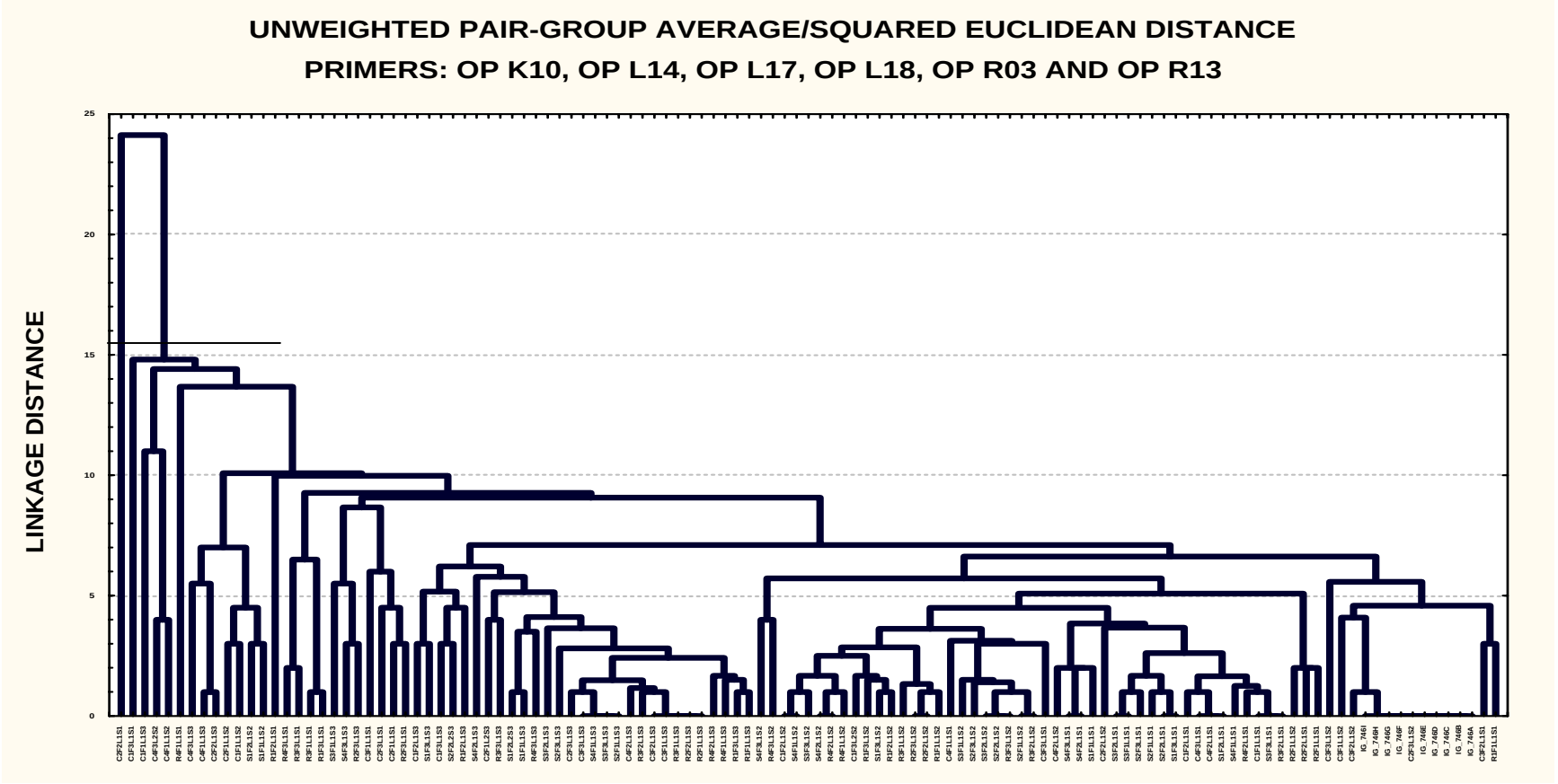
Common bean is one of the most popular leguminous crop cultivated in Brazil. It is the host of innumerous diseases including angular leaf spot, caused by the fungus *Phaeoisariopsis griseola*, which is responsible for up to 70% yield losses in the field. The objective of this work was to study the origin of *P. griseola* spores to start an angular leaf spot epidemic.

A randomized complete block design with 3 treatments (control, resistant and susceptible cultivar) and 4 replications were used. To provide a source of inoculum 3 bean plants was transferred to the center of each plot (except in the control plot) 44 days after sowing. The inoculum source plants stayed in the center of each plot for 7 consecutive days. Before been transferred to the field, these plants were inoculated in the greenhouse, in the V3 development stage, with a inoculum concentration of 2 x 10^4 spores ml^{-1} of the isolate Ig 746,. Three leaflets showing angular leaf spot symptoms were collected from each plot 57, 64 and 75 days after sowing. From each leaflet it was prepared a monosporic isolate. DNA from each monosporic isolate was extracted and amplified using the RAPD technique and the primers OPK 10, OPL 14, OPL 17, OPL 18, OPR 03 e OPR 13. Amplification reactions were performed in a thermocycler model PTC-100™ in a reaction volume of 25 μL containing 25 ng of DNA, 0.1 mM of each dNTP, 2.0 mM of $MgCl_2$, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 0.4 μM of one primer decamer and 1 unit of *Taq* DNA polymerase. Each amplification cycle consisted of 15 seconds at 94°C, 30 seconds at 35 °C and 60 seconds at 72 °C. After 40 cycles, a final extension step of 10 minutes at 72 °C was accomplished. Amplification products were separated by electrophoresis in a 1.5% agarose gel and photographed with the Eagle Eye II photosystem. The DNA bands amplified in the RAPD reactions were then scored according to their presence (1) or absence (0) for each pathogen isolate. The matrix so generated was submitted to cluster analysis, which was performed by the unweighted pair-group average and the Squared Euclidean distance methods.

The fungus *P. griseola* presented great genetic diversity (Figure 1). At a genetic distance of 15% arbitrary limit it was possible to divide the isolates in three groups. The first two groups are formed by one isolate each. The third group presented a total of 106 isolates. It is also possible to observe that the subgroup that contains the isolate used as a control (Ig 746) presented six other isolates very similar to those from the control, probably, indicating that these isolates had their origin from the isolate Ig 746, or that in the field there were some isolates very similar to the control isolate. The fact that angular leaf spot is seed transmitted is not relevant in this pathosystem since the percentage of disease transmission is no higher that 2,5%. All other isolates of the third group were divided in other subgroups, indicating that they are different from each other and from those belonging to the control isolate subgroup. As a conclusion, one could say that the isolates of *P. griseola* that reached the experimental area are genetically different from the control isolate (Ig 746) suggesting that an angular leaf spot epidemic starts from spores that comes from the outside of a bean field.

Figure 1. Dendogram of 108 isolates of *Phaeoisariopsis griseola* based on the random amplified plymorphic DNA (RAPD) method using six primers (OPK 10, OPL 14, OPL 17, OPL 18, OPR 03 and OPR 13).



RESISTANCE OF ANDEAN AND MESOAMERICAN COMMON BEAN GENOTYPES TO *Phaeoisariopsis griseola*

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In Brazil, common bean is the most important protein source for the low-income people. In this country, this leguminous crop is the host of innumerous fungus diseases including angular leaf spot caused by the fungus *Phaeoisariopsis griseola*. Losses due to this disease can be as high as 70% depending on the cultivar genetic background, environment conditions and the pathogenicity of its causal agent. Earlier studies have demonstrated that this fungus is pathogenically highly variable. In Brazil it has already been identified about 51 pathotypes. This disease can be controlled by cultural practices, fungicide spraying and host resistance. The objective of this study was to determine the resistance of 179 bean genotypes of both Andean and Mesoamerican origin to eight pathotypes of *P. griseola* under greenhouse conditions.

Bean seeds were sown in aluminum pots containing 2.0 kg of soil and five seeds per pot, 14 to 16 days before inoculation. Spores for inoculation were obtained by culturing the fungus on bean leaf-dextrose-agar medium in a Biochemical Oxygen Demand chamber at 24 ± 2^{o}C. After 14 days, inoculum was prepared by adding 5-10 ml of sterile distilled water to each plate and scraping the surface of culture. The obtained spore suspension was then filtered through a double layer of cheesecloth and the inoculum concentration adjusted to 2 x 10^4 conidia ml^{-1}. Bean plants were then inoculated at the V3 development stage. Inoculated plants were incubated in a moist chamber (>95% RH) for 36-48 h. Plants were transferred to greenhouse benches for another 14-18 days and evaluated for symptoms according to the 1-9 descriptive scale. Plants rating from 1 (no visible disease symptoms) to 3 (a few small non-sporulating lesions) were considered as resistant and from 4 (presence of several small sporulating lesions) to 9 (presence of abundant large sporulating lesions) as susceptible. When inoculated plants in the greenhouse showed symptoms but no sporulation, they were transferred to a moist chamber for 20-24 h. After this period of time, plants exhibiting non-sporulating lesions were considered resistant.

From the 179 genotypes tested, only 39 (Table 1) showed resistance to one or more pathotypes. Most genotypes were resistant to a few pathotypes. However, genotypes Ouro Negro, LM 202202530, Ecuador 299, Requinte and Cornell 49242 were resistant to 8, 7, 6, 5 and 4 pathotypes, respectively. Only the variety Ouro Negro presented resistance to all *P. griseola* pathotypes used. Most germplasm showed resistant/susceptible (R/S) reaction to the majority of the pathotypes. If any of these germplasm show other agronomic characteristic of interest to the breeders, they could be selected for their angular leaf spot resistance and could be introduced in the breeding program again to generate new common bean cultivar.

Table 1. Reaction of 39 genotypes to *Phaeoisariopsis griseola*, the causal agent of angular leaf spot in common bean. Embrapa Rice and Beans, Goiânia, GO, Brazil.

GENOTIPE	PATHOTYPES							
	63-39	63-23	63-15	31-31	63-55	63-63	63-31	63-47
AND 061	S[1]	S	S	S	S	R[2]	S	S
AND 081	S	S	R	S	S	S	R	S
AND 083	S	R	S	R	S	S	S	S
AND 135	S	R/S[3]	S	X[4]	S	R	X	X
AND 137	S	R	S	R	S	S	R	S
AND 139	S	R	S	R	S	S	S	S
AND 141	S	R	S	S	S	S	S	S
AND 163	S	S	R	R	S	S	R	S
AND 181	S	R/S	S	R	S	S	R	S
AND 240	S	S	R/S	R	S	S	R	S
AND 667	R/S	S	R/S	X	X	X	X	X
AND 673	R/S	S	S	R	S	S	R	S
AND 696	R/S	R/S	R/S	X	X	X	X	X
CNFC 9504	S	S	S	S	S	S	R/S	S
CORNELL 49242	S	R/S	R/S	R	S	S	R	S
ECUADOR 299	S	R	R/S	R/S	R/S	S	R	R
GRAFITE	S	R	S	S	S	S	R/S	R/S
LM 202202530	R/S	R/S	R/S	R/S	R/S	S	R/S	R/S
LM 202202858	R/S	S	S	S	S	S	R	S
LM 202204185	S	S	S	S	S	S	R/S	R/S
LM 202204189	R	S	S	S	S	S	R/S	S
LM 202204502	R	S	S	S	R	S	R/S	S
LM 202204503	R	S	S	S	S	S	R	R
LM 202204511	S	S	S	S	R/S	S	R	R/S
LM 202204518	R	S	S	S	R	S	R	S
LM 202204525	R/S	S	S	S	S	S	R	R/S
LM 202204595	R/S	S	S	S	R/S	S	R/S	S
LM 202204612	S	S	S	S	S	S	R	R/S
LM 202204614	R/S	S	S	S	S	S	R/S	S
LM 202204624	S	S	S	S	R/S	S	R/S	R/S
LM 202204641	R/S	S	S	S	S	S	S	R
LM 202204676	S	S	S	S	R	S	R	R/S
MAR 002	R	S	S	S	S	S	S	S
MAR 003	R	R	S	R	S	S	S	S
MÉXICO 168	S	S	R	S	S	S	S	S
OURO NEGRO	R/S	R	R/S	R	R	R/S	R	R/S
PIATÃ	S	R/S	S	S	R/S	S	S	S
PONTAL	S	R	R	S	S	S	R	S
REQUINTE	S	R	R/S	R/S	S	S	R	R/S

[1]S=Susceptible; [2]R=Resistante; [3]R/S=Resistante/Susceptible; [4]No information available.

A NEW INOCULATION PROCEDURE TO EVALUATE ANGULAR LEAF SPOT DISEASE IN BEAN PLANTS (*Phaseolus vulgaris* L.) FOR BREEDING PURPOSES

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Introduction - Angular leaf spot incited by the fungus *Phaeoisariopsis griseola* (Sacc.) Ferraris is a severe disease in common bean (*Phaseolus vulgaris* L.). This disease has been considered one of the main problems responsible for the low yield of this crop in Brazil. Pyramiding resistance genes to overcome diseases caused by pathogens has been proposed as an strategy to obtain durable and wide spectrum resistance. However, pyramiding can be a difficult task when only conventional inoculation techniques are used to evaluate the plant symptoms (Faleiro et al., 2003). The objective of this study was to develop an alternative method for inoculation of *P. griseola* using excised bean leaves.

Material and Methods - *P. griseola* race 63.23 was obtained from a monosporic culture. The fungus was grown in petri dishes containing tomato juice agar medium. The resulting spores and mycelia were scrapped smoothly with a spatula and filtered through gauze and the spore concentration was adjusted to 2.0×10^4 conidia/mL. For the excised leaf method, which was based on Tu (1986), 18 days after germination the middle follicle of the first trifoliate leaves of each plant was excised when they had reached approximately two-thirds of their full development. The detached leaves were inoculated by immersion into a spore suspension and placed in petri dishes (90 x 15 mm) on a filter paper moistened with 3.0 mL of fresh distilled water. The petri dishes were incubated for 18 days in a BOD kept at 19°C, under a 12 hour daily light regime (Phillips® TLT 20W/75RS) at about 28 μmoles $m^{-2}s^{-1}$. The two remaining follicles of the first trifoliate leaves were sprayed with a spore suspension on both leaf surfaces and incubated in a mist chamber (20-22°C; 100% relative humidity). To test the inoculation methods the 12 angular leaf spot differential cultivars (Table 1) and cultivars Vermelho (susceptible control) and AND 277 (resistant control, gene *Phg-1*) (Carvalho et al., 1998) were used. One hundred and fifty-six F_2 plants from a cross between cultivars AND 277 and Vermelho were inoculated with *P. griseola* race 63.23 by the two inoculation methods. The data obtained in the evaluations were submitted to the chi-square test to determine the efficiency of both methods to detect the segregation of one dominant resistance gene (*Phg-1*) in the F_2 population (Table 2).

Results and Discussion -The symptom evaluation showed that the proposed inoculation procedure was efficient to evaluate angular leaf spot resistance/susceptibility (Figure 1). The 12 common bean differential cultivars and the cultivars Vermelho and AND 277 presented the same scores when inoculated by both methods (Table 1). The F_2 plants used in this work also presented the same phenotypes when tested either with the proposed inoculation procedure or with the conventional inoculation method (Table 2). Out of 156 F_2 plants, 120 were resistant and 36 were susceptible to *P. griseola* race 63.23. The chi-square test support the hypothesis that a single dominant gene is responsible for resistance of AND 277 to the *P. griseola* race 63.23 (Table 2). The excised leaf inoculation procedure shows some advantages when compared with the conventional inoculation method. The alternative procedure is non-destructive. Susceptible plants can be kept alive even after their reaction to the pathogen is determined. In addition, different pathotypes can be simultaneously evaluated with leaves from the same plant. The proposed inoculation procedure is now being used in association with molecular markers to aid the selection of resistant plants in the bean breeding program of the Federal University of Viçosa. This program aims to pyramid anthracnose, angular leaf spot and rust resistance genes in common bean elite-cultivars.

Table 1. Reaction of the bean angular leaf spot differential series and of control cultivars inoculated by two

different methods with *P. griseola* race 63.23. Each value represents the evaluation of four different plants.

Cultivars	Binary value	Reaction degree and phenotype		
		Conventional method	Excised leaf method	Phenotype[c]
Don Timoteo	1	9	9	S
G 11796	2	9	9	S
Bolon Bayo	4	9	9	S
Montcalm	8	9	9	S
Amendoin	16	9	9	S
G 5686	32	9	9	S
Pan 72	1	9	9	S
G 2858	2	9	9	S
Flor de Mayo	4	9	9	S
Mexico 54	8	1	1	R
BAT 332	16	9	9	S
Cornell 49-242	32	1	1	R
[a]AND 277	-	1	1	R
[b]Vermelho	-	9	9	S

[a]Resistant control; [s]Susceptible control; [c]Resistant (R) and Susceptible (S).

Table 2. Angular leaf spot resistance/susceptibility of F_2 plants derived from a cross between Vermelho and AND 277, inoculated by *P. griseola* race 63.23, using the excised leaf and conventional inoculation methods.

Inoculation method	Expected ratio[a]	Observed ratio	χ^2	P-value[b]
Excised leaf	3:1	120:36	0.307	57.91%
Conventional	3:1	120:36	0.307	57.91%

[a]3:1 (R_:rr), expected ratio for the segregation of one dominant resistance gene in the F_2 population; [b]Estimated probability value.

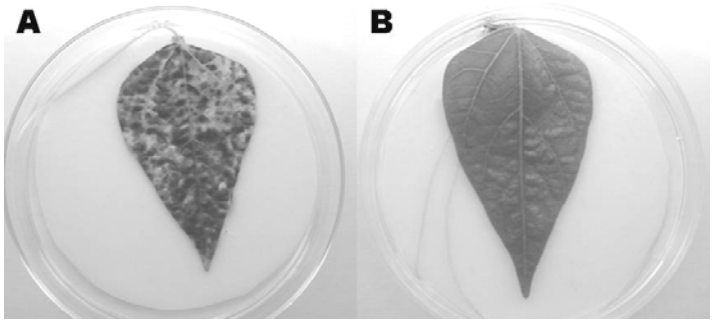

Figure 1. Angular leaf spot symptoms observed on leaves of cultivars **(A)** Vermelho (susceptible control) and **(B)** AND 277 (resistant control) inoculated by the excised leaf method.

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Pathogenic and molecular studies of *Phaeoisariopsis griseola* in Argentina

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Phaeoisariopsis griseola (Sacc.) Ferraris is the causative agent of Angular Leaf Spot (ALS) a disease of common bean (*Phaseolus vulgaris* L.) that causes important yield losses in many countries around the world (Saettler, 1991). Argentina is a major exporter of common beans with a cultivated area of approximately 280,000 ha and a production of 320,000 t.

ALS is one of the most important causes of yield losses of common bean in Argentina (Stenglein *et al.*, 2003). The development of a strategy to control the disease requires a prior knowledge of the pathogen diversity, variability and distribution. Therefore, the purpose of this work was to analyze, based on virulence and molecular markers, the variability among 45 isolates of *P. griseola* collected within the production area of common bean in Northwestern Argentina.

Pathogenicity was assessed by inoculating the isolates on a set of 12 bean differentials (van Schoonhoven and Pastor-Corrales, 1991) and it was found that many pathotypes of *P. griseola* coexist in the bean producing area of Argentina. In this work 13 pathotypes were detected among the isolates (Table 1). Races 63.15 and 63.63 were the most frequent and virulent, respectively. Both Andean and Mesoamerican genotypes might have angular leaf spots in the same leaf caused by different pathotypes, increasing the chances of recombination events. The results demonstrated the high level of variability among isolates of *P. griseola* in Argentina.

Table 1. Differential bean cultivars reaction inoculated with 45 isolates of *Phaeoisariopsis griseola* collected in Argentina

Andean group						**Mesoamerican group**						
a	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**k**	**l**	Pathotype
+	+	+	-	-	-	-	-	-	-	-	-	14.0
-	+	+	+	+	-	-	-	-	-	-	-	30.0
+	+	+	+	+	-	-	-	-	-	-	-	31.0
+	+	+	+	+	-	+	+	+	-	-	+	31.39
+	+	+	-	-	+	+	+	+	-	-	-	39.7
+	+	+	+	+	+	+	+	+	-	-	-	63.7
+	+	+	+	+	+	+	+	+	+	-	-	63.15
+	+	+	+	+	+	+	+	+	+	+	-	63.31
+	+	+	+	+	+	+	+	+	-	-	+	63.39
+	+	+	+	+	+	+	+	+	+	-	+	63.47
+	+	+	+	+	+	+	+	+	-	+	+	63.55
+	+	+	+	+	+	+	+	+	+	+	+	63.63

(a) Don Timoteo; (b) G 11796; (c) Bolon Bayo; (d) Montcalm; (e) Amendoin; (f) G 5686; (g) Pan 72; (h) G 2858; (i) Flor de Mayo; (j) Mexico 54; (k) BAT 332; (l) Cornell 49-242.

Two different types of molecular markers, RAPD and ISSR, were used to analyze diversity. The fingerprints generated either by RAPD or by ISSR clustered isolates of *P. griseola* as Mesoamerican or Andean. However, ISSR showed higher levels of variability than RAPD markers, and proved to be useful tools to assess diversity among fungal isolates (Fig. 1).

Amplification patterns were mostly related with the place of origin, suggesting either that not much selection pressure was exerted by the host genotypes on the fungal population or that the areas cultivated with beans are rather isolated from each other.

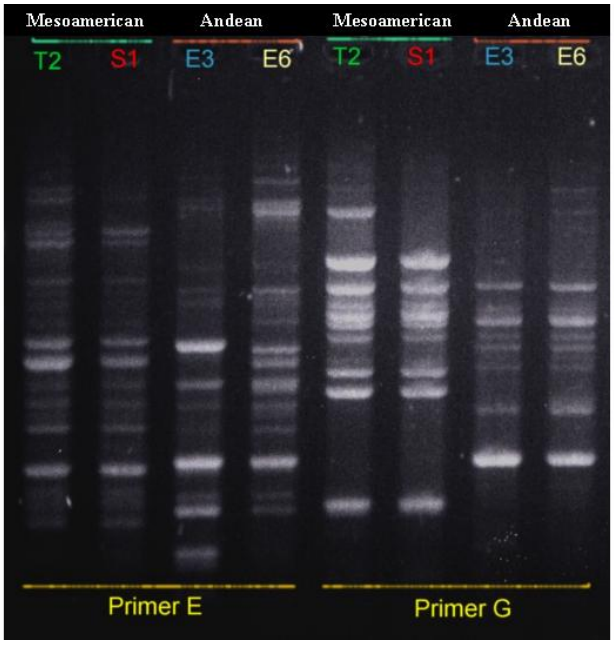

Fig. 1. Amplification patterns of Mesoamerican and Andean isolates of *Phaeoisaripsis griseola* generated with two ISSR primers.

Acknowledgments

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USE OF MOLECULAR MARKERS TO PYRAMIDING MULTIPLE GENES FOR RESISTANCE TO RUST, ANTHRACNOSE AND ANGULAR LEAF SPOT IN THE COMMON BEAN

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Rust, anthracnose and angular leaf spot incited by the fungi *Uromyces appendiculatus*, *Colletotrichum lindemuthianum* and *Phaeoisariopsis griseola*, respectively, are considered among the most important diseases affecting the common bean (*Phaseolus vulgaris* L.) in Brazil and in other parts of the world. These diseases may cause losses that can reach 100% depending on the environment and the use susceptible cultivars. The use of resistant cultivars has been considered as an efficient, safe, and inexpensive alternative accessible to bean growers. Combining or pyramiding the different resistance genes in a single cultivar may result into medium to long-term control. Recognizing the presence of different resistance genes in a specific cultivar under field conditions is extremely difficult. Molecular markers can be used to aid the identification of these genes. They can also be used be to accelerate the recovery of the recurrent parent´s genome in backcrossing programs (Faleiro et al., 2004). In this study, DNA fingerprints and molecular marker assisted selection were used to accelerate the development of common bean advanced lines simultaneously resistant to *U. appendiculatus, C. lindemuthianum* and *P. griseola*.

For the backcrosses the donor parents (cvs. Ouro Negro, TO, AB 136 and AND 277) and the recurrent parent (cv. Rudá) were used. In each backcross generation, DNA of BC_nF_1 plants were amplified by the RAPD technique to determine the relative genetic distances betweem these plants and the recurrent parent. After only three or four backcrosses, several lines resistant to rust, anthracnose or angular leaf spot genetically similar to the recurrent parent were obtained. These lines were intercrossed to pyramid the genes conferring resistance to rust, anthracnose and angular leaf spot in the "carioca-type" cultivar Rudá. The resulting F_1 (double hybrids) plants and the resistant F_2, F_3 and F_4 plants were inoculated with at least one pathotype from each pathogen to identify the susceptible plants and to reduce the number of individuals to be genotyped by molecular markers. Following this procedure it was possible to obtain in the F_4 generation plants phenotypically and genotypically very similar to the recurrent cultivar Rudá, possessing the resistance genes *Ur-ON, Co-4, Co-6, Co-10* and *Phg-1*. The $F_{4:5}$ lines were multiplied under field conditions to obtain enough seeds for subsequent evaluation of agronomic traits. The $F_{4:7}$ pyramided lines were tested against several *U. appendiculatus, C. lindemuthianum* and *P. griseola* pathotypes and demonstrated to be resistance at all of them (Table 1). These lines underwent field yield tests in two consecutive growing seasons and most of them presented a good yield performance surpassing in that sense their parents and most of the reference cultivars tested (Table 2). These lines have good potential to be released as new cultivars and besides, they constitute an excellent germplasm from which resistance genes can be easily mobilized for use in other bean breeding programs.

Table 1. Pathotypes of *U. appendiculatus, C. lindemuthianum* and *P. griseola* tested in the selected $F_{4:7}$ pyramided lines.

Pathogen	Pathotype*
U. appendiculatus	32, 45, 46, 47, 49, 50, 52, 54, 58, 59
C. lindemuthianum	7, 55, 64, 65, 67, 73, 79, 81, 83, 87, 89, 95, 102, 117, 119, 343, 453
P. griseola	31-17, 31-39, 63-19, 63-23, 63-31, 63-35

*Pathotypes of *U. appendiculatus* were characterized by Faleiro et al. (1999); pathotypes of *C. lindemuthianum* were characterized by Rava et al. (1994); pathotypes *of P. griseola* were characterized by Nietsche et al. (2001).

Table 2. Yield components evaluated for four selected common bean isolines* and three reference cultivars during two growing seasons (Fall and Spring, 2004) in Coimbra, Minas Gerais, Brazil.

Cultivar	GY[1]	PLH[2]	SEP[3]	POP[4]
Talismã	1,923.30 a	40.33 a	4.30 a	7.21 ab
Pérola	1,914.91 a	44.66 a	4.39 a	6.51 ab
Rudá	1,631.43 a	42.33 a	4.08 a	7.47 ab
R-127-10-14*	2,064.26 a	43.33 a	5.52 a	6.45 ab
R-97-13-5*	2,064.28 a	43.33 a	5.19 a	5.92 b
R-97-13-6*	2,150.30 a	44.33 a	4.07 a	8.91 a
R-127-4-13*	2,084.38 a	44.66 a	4.71 a	7.64 ab
Mean	1,976.13	43.28	4.61	7.51
CV(%)	9.53	7.59	12.52	12.40

[1] GY - grain yield in Kg/ha;
[2] PLH - plant height in cm at stage R8;
[3] SEP - number of seeds per pod;
[4] POP - number of pods per plant;
* Means followed by the same letter do not differ by the Tukey test at 5% probability.

Acknowledgement: This work was financed by grants from CNPq and FAPEMIG.

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GENE EXPRESSION PROFILING OF BEAN PLANTS IN RESPONSE TO COMMON BACTERIAL BLIGHT USING cDNA-AFLP

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Introduction

Plant diseases are kept in check by breeding strategies that have introgressed disease resistance gene in many crop species and by use of costly control measures like pesticides, antibiotics and fungicides. However, these disease causing organisms are constantly changing by adapting to new conditions, overcoming disease resistance and becoming resistant to antibiotics, pesticides etc. For these reasons, understanding the biology of plant disease is essential for the development of durable control strategies.

Common bacterial blight (CBB) caused by *Xanthomonas axonopodis* pv. *Phaseoli* (*Xap*) is one of the most important diseases affecting bean production world-wide. To understand the molecular bases of disease resistance in beans during CBB infection we used cDNA-AFLP to identify differentially expressed transcripts from *Phaseolus vulgaris* leaves subjected to *Xap* infection. Our hypothesis is that the products of induced transcripts may play a role in the plant defense response.

The cDNA-AFLP analysis of incompatible interaction between HR45 line and X*ap* resulted in more than 2448 transcript derived fragments (TDF) using thirty four different primer combinations. Ten percent of the fragments were induced or repressed after inoculation with *Xap*. Here we report the cloning and characterization of 15 differential transcript derived fragments (DTDF).

Materials and Methods

Common bean line HR45 is resistant to CBB. The source of resistance in HR45 is derived from the tepary bean (*P. acutifolius*) accession PI 319 443. The plants were inoculated at unifoliolate leaf stage using multiple needle technique. The fully expanded primary two leaflets were inoculated with *Xap* culture (10^8cfu/ml). Non symptomatic trifoliolate leaf tissue was collected from the inoculated plants at six different time points 0, 8, 12, 24, 48h, and 5 day after inoculation. Total RNA and mRNA were isolated from the collected tissues. Double stranded (ds) cDNA was prepared from mRNA. Synthesized cDNAs were used as templates for cDNA-AFLP (Bachem et al., 1998).

cDNA-AFLP analysis was done using LI-COR AFLP Expression Analysis Kit (LI-COR Biosciences). cDNA-AFLP fragments from infected and non-infected plants were separated in parallel by gel electrophoresis. The gel was scanned using Odyssey infrared scanner (LI-COR Biosciences) and the DTDF were excised from the gel for further analysis. The isolated fragments were cloned into pGEMT-Easy vector (Promega), and sequenced using IR Dye 700 labelled M13 Forward primer.

Results and Discussion

cDNA-AFLP allowed us to identify alterations in bean transcript levels in response to *Xap* infection. AFLP fragments generated by TaqI/ MseI primer pairs ranged in size from 80 bp to 400 bp. Highly reproducible banding pattern was obtained in a series of repeated experiments. The overall cDNA-AFLP profile was very similar in cDNA from healthy and infected plant tissue and only a small proportion of the fragments were

polymorphic. Thirty four different primer combinations were tested and more than 2448 TDF were obtained, of which 259 (10.6 %) showed clear variation in expression level between tissues collected before and after inoculation. Most of the polymorphic fragments were up-regulated upon pathogen infection compared to the number of down-regulated bands. In most cases activation was transient and returned back to basal level by 24h after inoculation. Twenty four reproducible and DTDF were excised from the gels. To date 15 of these isolated DTDF have been cloned into pGEMT-Easy vector, sequenced and putatively identified via database searches (Table 1). Sequences of two TDFs did not show good identity to any sequences in the database. Some of the cDNAs that may have a role in plant pathogenesis are indicated in bold in Table 1.

Table 1. Homologies of AFLP fragments after tBLASTx search in GenBank. Induced TDF (+); Repressed TDF (-).

AFLP Fragment ID #	Expre-ssion	Fragment Length (bp)	BLAST Search Homology	Accession #	Probability
M1T1-4-3	+	155	*Poncirus trifoliata citrus tristeza* virus resistance gene locus	AF506028.1	3e-04
M1T1-5-10	-	160	*P. vulgaris* putative integral membrane protein (sec61) mRNA,sec61-1 allele	AF190652	1e-18
M1T1-7-30	+	210	*A. thaliana* oxidoreductase, 2OG-Fe(II) oxygenase family protein (At3g46480) mRNA	NM_114515.2	8e-15
M1T1-8-33	-	220	*A. thaliana* oxidoreductase, 2OG-Fe(II) oxygenase family protein (At3g46480) mRNA	NM_114515.2	1e-15
M3T1-5-9	+	140	*T. monococcum* SNF2P gene	AY485644	0.65
M3T1-7-15	+	160	*P. sativum* mRNA for 33 kDa protein of the water oxidizing complex	X15350	0.002
M5T1-7-52	-	240	*A. thaliana* phosphatidyl inosital-4-phosphate 5- kinase family protein mRNA	NM 119478	1e-016
M7T1-2-4	+	145	*Piper betle* 26S rRNA gene	AY095467	2e-021
M8T1-2-28	+	110	*S. tuberosum* PPS3 mRNA for hypothetical protein involved in regulation of plant cell death	AB111942	0.052
M8T1-2-49	+	115	*A. thaliana* putative protein kinase	BT002752	5e-005
M4T2-7-10	+	220	*H. vulgare* L. (Alexis) mRNA for serine carboxypeptidase II-2	X78878	2e-20
M4T2-8-1	+	255	*A. thaliana* ubiquitin-conjugating enzyme family protein (At2g33770) mRNA	NM_179887.1	3e-20
M10T10-10-63	+	290	*A. thaliana* DNA chromosome 5, BAC clone F18D22	AL360334	0.023

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Molecular Markers Used to Validate Reaction of Elite Bean Breeding Lines to Common Bacterial Blight

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INTRODUCTION: Common bacterial blight (CBB) caused by *Xanthomonas axonopodis* pv. *phaseoli* (Smith) Dye (*Xap*) is a seed transmitted disease that causes not only reduction in seed yield but also in seed quality in the major bean production areas of Mexico. Traditional bean production in the semiarid highlands of Mexico has permitted the wide distribution of CBB despite the fact that chemicals, cultural practices, clean seed programs and genetic resistance are used to control this disease in other countries (Coyne et al., 2003). All common bean classes (black, flor de mayo, pinto, flor de junio, bayo and yellow) used across 1.2 million hectares of the semiarid highlands of Mexico, either as landraces or improved cultivars, exhibit only low to moderate levels of field resistance to CBB. This study was carried to evaluate the reaction of a group of elite breeding lines to CBB and validate the presence of SCAR markers, $SU91_{700}$ (Pedraza et al., 1997), $SAP6_{820}$ (Miklas et al., 2000) and $BC420_{800}$ (Yu et al., 2000) linked to resistance QTL in the same genotypes.

MATERIALS AND METHODS: To evaluate the field reaction to CBB, experiments were planted on July 10, 2004 at two locations, one under rainfed conditions (F.I. Madero) and the other under irrigation (Campo Experimental Valle del Guadiana -CEVAG) in Durango, Mexico. The experiments consisted of 22 common bean entries, grown in two-row, 6m long plots, with three replications. In addition, clean seed of the same bean cultivars was produced during the winter of 2003 at the Campo Experimental Valle del Fuerte, Los Mochis, Sinaloa, Mexico. Entries included elite and breeding lines, and landrace cultivars of black, flor de mayo, and pinto types, representing three major bean classes grown across the semiarid highlands of Mexico. Pinto Bayacora and N8025 were the most susceptible cultivars whereas the resistant sources included VAX 4, VAX 5 and VAX 5. Data was recorded on days to flowering and maturity, seed yield, field agronomic adaptation, and CBB (leaf and pod) infection 60, 70, and 75 days after planting using a 1-9 scale, described by Schoonhoven and Pastor-Corrales (1987). Genomic DNA was extracted from four greenhouse-grown plants of the same 22 entries grown in Michigan during winter 2004. The template DNA was used in PCR reaction to amplify SCAR markers SU91, SAP6 and BC420. Presence or absence of the resulting bp fragments of each marker was determined using agarose gel electrophoresis.

RESULTS AND DISCUSSION: The SCAR markers used in characterizing for CBB resistance were easily scored as the presence or absence of a single band on agarose gel. SU91 marker was associated with those cultivars that showed higher levels of field resistance to CBB (Table 1). On the contrary, SAP6 was found in both resistant and susceptible cultivars including the susceptible cultivars, Pinto Bayacora, FM Sol and N 8025. The use of this marker in breeding for CBB resistance will depend on the specific cross and its absence in the susceptible parent. The BC420 marker was absent in all elite bean cultivars evaluated. The three VAX lines exhibited lower CBB scores and possessed both the SU91 and SAP6 markers, but lacked the BC420 marker (Table 1). PTD 99099 was the only breeding line from Mexico that possessed the SU91 marker. The presence of the SU91 marker was a surprise as the marker is derived from a tepary resistance source but the pedigree of PTD 99099 did not indicate such a connection. Black-seeded elite cultivars, Tacana and Vizcaya, with small- and medium-size seed, respectively that lacked any of the SCAR markers could be improved using the resistant VAX lines that carry both markers. In addition, the BC420 marker could be included in these crosses as the linkage with the V locus that intensifies seed coat color would not be problematic in the black bean class. In other seed color classes such as Pinto Bayacora and P. Mestizo and FM 2000, resistance may have to be limited to QTL associated with the SU91 and SAP6 markers from the VAX lines as the V locus may produce an undesirable darker seed coat color in these classes.

Table 1. Field reaction to CBB of a group of 22 common bean cultivars and the presence (+) or absence (-) of SCAR markers linked with CBB resistance.

Cultivar	CBB Score 2004			SCAR Markers		
	CEVAG	F.I. Madero				
	Leaf	Leaf	Pod	SU91	SAP6	BC420
1 VAX4*	4	4	4	+	+	-
2 VAX5*	4	4	2	+	+	-
3 VAX6*	3	3	3	+	+	-
4 N. 8025	5	5	2	-	+	-
5 N. Tacana	5	4	4	-	-	-
6 N. Vizcaya	5	5	3	-	-	-
7 N. San Luis	6	6	5	-	+	-
8 N. Altiplano	5	5	3	-	+	-
9 N. Sahuatoba	4	5	3	-	-	-
10 P. Villa	5	5	4	-	+	-
11 P. Bayacora	6	6	5	-	-	-
12 P. Mestizo	4	4	3	-	-	-
13 P. Saltillo	5	5	3	-	+	-
14 PTD 99057	5	5	4	-	+	-
15 PTD 99092	4	4	3	-	-	-
16 PTD 99099	4	4	3	+	+	-
17 FM M38	4	5	3	-	+	-
18 FM 2000	5	5	3	-	-	-
19 FM Sol	6	6	5	-	+	-
20 FMD 99010	5	5	4	-	+	-
21 FMD 99021	5	5	4	-	+	-
22 FM Media O.	5	6	4	-	+	-
Average	4.7	4.8	3.5			
LSD (0.05)	1.1	0.98	0.96			

Resistant checks; N=Negro (black-seeded); P=Pinto; FM=Flor de Mayo seed types
CBB score: 1=resistant; 9=susceptible on 1-9 scale (Schoonhoven and Pastor-Corrales, 1987).

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Use of SCAR Markers as a Tool to Verify Presence of CBB Resistance QTL in Breeding Populations of Common Bean

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INTRODUCTION

Traditional bean production in the semiarid highlands of Mexico has resulted in the widespread distribution of common bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *phaseoli* (Smith) Dye (*Xap*) since the use of clean seed is not a common practice. Breeding for resistance offers the best alterative as landraces or improved cultivars in all common bean classes in Mexico exhibit low to moderate levels of field resistance to CBB. Resistance sources such as the VAX lines (Singh et al., 2001; Singh and Munoz, 1999) offer breeders the opportunity to enhance resistance through selection based on markers linked to major QTL controlling resistance to CBB. This study was conducted to determine the presence (+) or absence (-) of SCAR markers linked to CBB resistance in 257 of recombinant bean breeding lines developed from crosses with the VAX lines. The presence of $SU91_{700}$ (Pedraza et al., 1997) linked to a resistance QTL located on B8, $SAP6_{820}$ (Miklas et al., 2000) on B10, and $BC420_{800}$ (Yu et al., 2000) on B6 of the integrated common bean linkage map were validated in all 257 lines and checks.

MATERIALS AND METHODS

Clean seed of a series of 102 $BC_1F_{5:7}$ and 155 $F_{5:7}$ single cross bean lines was produced during the winter of 2003 under arid conditions at the Campo Experimental Valle del Fuerte, Los Mochis, Sinaloa, Mexico. These recombinant bean lines were obtained from crosses between susceptible parents N8025, DOR 500, Tacana and Pinto Villa and the CBB resistance sources, VAX 4 and VAX 6. Four seeds per entry were sown in 6" pots and grown for four weeks in the greenhouse in Michigan, before leaf tissue was obtained for DNA extraction. Genomic DNA was extracted from four greenhouse-grown plants of each of 257 bean entries and checks and was used as template DNA in PCR to detect SCAR markers SU91, SAP6, and BC420. Presence or absence of the resulting fragments for each marker was determined using agarose gel electrophoresis. The PCR protocol used was described by Melotto et. al. (1996) except that Invitrogen *Taq* polymerase was used (Invitrogen, Carlsbad, CA).

RESULTS AND DISCUSSION

Our results confirmed that SCAR marker SU91 was present in both resistant checks XAN159 and VAX5, whereas SAP6 was present only in VAX5 and BC420 was present only in XAN159. SAP6 was present in all but one susceptible parent N 8025, while none of the parents had BC420 (Table 1). As expected the CBB resistant donor parents VAX 4 and VAX 6 possessed both SU91 and SAP6, markers. Among the 257 recombinant breeding lines, SU91 was present in 50

breeding lines, SAP6 was present in 150 lines, but no lines carried BC420. Only 28 breeding lines carried both SCAR markers, SU91 and SAP6, and most of them came from the N8025/VAX6 population. The study will provide basis for advancing lines with improved levels of resistance to CBB and reducing the number of lines that need to be verified under field inoculations next season. Given the absence of QTL on B6 linked to the BC420 marker, resistance could be enhanced further in crosses with resistance sources carrying the BC420 marker.

Table 1. Recombinant $BC_1F_{5:7}$ and single cross $F_{5:7}$ bean breeding lines with presence (+) or absence (-) of SCAR markers linked with CBB resistance.

Population/Cultivar	Number of lines	SCAR Markers				
		Presence		Absence		
		SU91	SAP6	SU91	SAP6	BC420
N8025//N8025/VAX6	22	+6	+9	-16	-13	-22
DOR500//N8025/VAX4	18	+5	+4	-11	-14	-18
Tacana/VAX6//N8025/VAX6	62	+12	+43	-50	-19	-62
N8025/VAX6	146	+25	+89	-121	-57	-146
Pinto Villa/VAX4	9	+2	+5	-7	-4	-9
XAN 159*		+				+
VAX 5*		+	+			
VAX 4		+	+			-
VAX 6		+	+			-
N 8025			+	-		
Tacana				-	-	-
Pinto Villa			+	-		-
Total	257	50	150			

*Check cultivars for SCAR markers

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AFLP MARKERS ARE TIGHTLY LINKED TO THE MAJOR QTL FOR CBB RESISTANCE IN HR67

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Abstract

Common bacterial blight (CBB) of common beans (*Phaseolus vulgaris L.*), caused by *Xanthomonas axonopodis pv. Phaseoli* (*Xap*), is one of the major diseases in bean production areas in North America. The common bean line HR67, which was derived from the crosses Centralia/3/HR13-621//XAN159/OAC Rico, is highly resistant to CBB. The main objective of this study is to find additional molecular markers tightly linked to the major quantitative trait loci (QTL) for CBB resistance in HR67, to map the QTL with flanking markers and to estimate its effects. Five SSR, two SCAR and 58 AFLP markers loci were mapped to a recombinant inbred line (RIL) population of 82 lines. Seven linkage groups were constructed and one major QTL was located on linkage group1 (LG_1). This QTL was mapped to a 9 cM genomic region covered by 5 molecular markers which can explain 30% to 49% of the phenotypic variations individually. Stepwise model selection among these five markers showed that eAAGmCAG183 and eAAGmCAG333 together can explain 51.1% of the phenotypic variations. Conversions of these AFLP markers into sequence tagged site (STS) or SCAR markers are under way.

Materials and Methods

Phenotypic and genotypic data: Disease screening of 82 F_6 recombinant inbred lines (RILs) from the cross HR 67/OAC 95-4 were conducted as described by Yu et al. (2000a). One SSR and two SCAR markers associated with the CBB resistance reported previously were re-screened. Thirty-two more SSR markers (Yu et al., 2000b) were screened for parental polymorphism. The STS marker OD12S linked to V gene was screened as well. Eight *EcoR*I and eight *Mse*I primers with three nucleotide extension forming 64 combinations were screened with the parental and the bulked DNA samples (the resistant vs. the susceptible). Thirteen primer pairs with clear polymorphisms on parents and/or on the two bulks were mapped to the RILs.

Data analysis: The associations between the markers and the CBB rating were analyzed by SAS PROG GLM (version 8, 1999). Genetic maps were constructed by MapMaker 3.0 using Kosmobi function. QTL analyses were conducted by QTL Cartographer. Genetic maps and QTL intervals were drawn with MapChart.

Results and Discussion

Molecular marker and genetic maps: Five SSR, 2 SCAR and one STS marker and 13 AFLP primer pairs with 58 polymorphic loci were mapped to the 82 RILs. Seven genetic linkage groups were constructed covering 411.7 cM of the bean genome (Fig. 1).

Marker and trait association: Seventeen of the 20 markers in linkage group1 (LG_1) are significantly associated with the CBB resistance. Thirteen markers can explain 20% to 49% of the phenotypic variations individually determined by SAS PROG GLM.

QTL location and effect: One major QTL was detected by QTL Cartographer. It is located on LG_1 within a 9 cM genomic region covered by 5 markers. These five markers can explain 30 to 49% of the phenotypic variation individually determined by SAS PROC GLM. Genetic model including markers eAAGmCAG.183 and eAAGmCAG.333 can explain 51.1% of the phenotypic

variations. This major QTL is from HR67 (Fig. 1). Seed coat color gene V and its linked STS marker OD12S.490 were located on B_6 (McClean et al. 2002; Nodari et al. 1992). Jung et al. (1997) found that the V locus was linked to the RAPD marker BC420.900 which was associated a major QTL for CBB resistance from the cross PC50/Xan159. The BC420.900 was converted to SCAR marker UBC420.900 and it is linked to the major QTL for CBB resistance from the cross HR67/OAC95-4 (Yu et al. 2000a; 2004). The LG_1 included both UBC420.900 and OD12S.490. Therefore, LG_1 is probably aligned with B6. Yu et al. (2004) suggested that this QTL may be on B_7. More SSR markers will be screened to confirm the location of this QTL.

Cloning and conversion of the AFLP markers to STS or SCAR markers is under way. These converted markers should be more useful for marker-assisted selection (MAS) in bean breeding programs and they also provide a starting point for physical mapping of the QTL region and map-based cloning of the major CBB resistant QTL in the future.

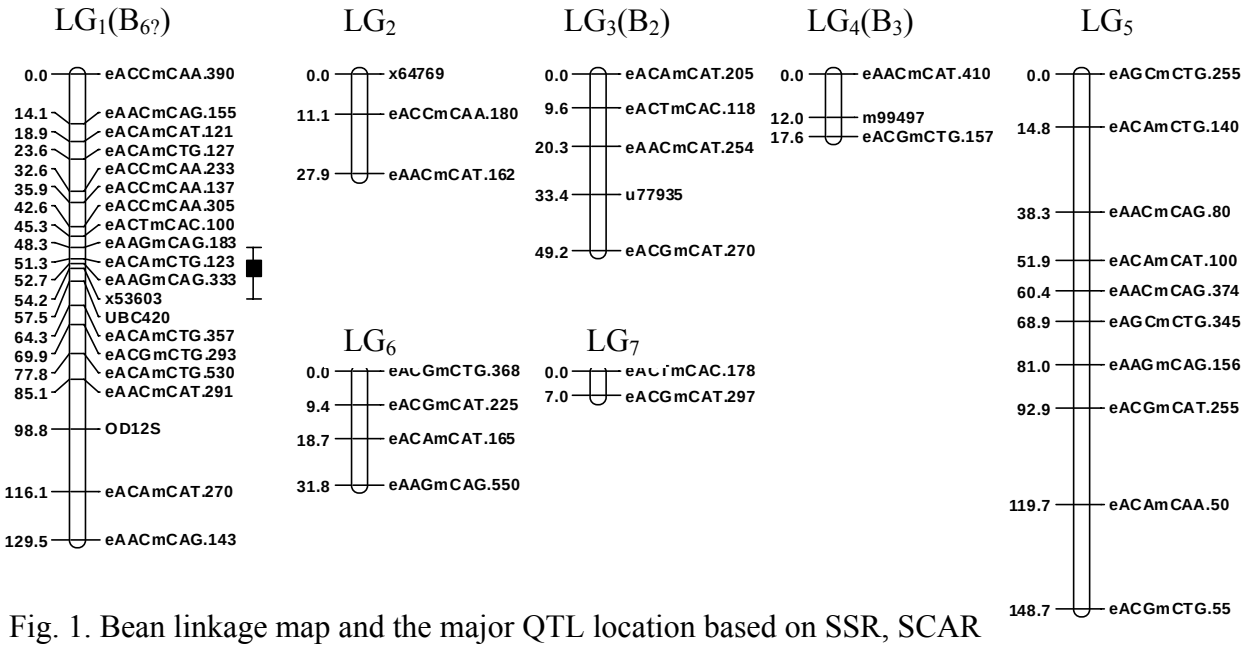

Fig. 1. Bean linkage map and the major QTL location based on SSR, SCAR and AFLP markers.

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Differential leaf reaction of common bean lines to pathogenic races of *Xanthomonas axonopodis* pv. *phaseoli* from Costa Rica, Nicaragua and Puerto Rico

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Bacterial blight of common bean, *Phaseolus vulgaris* caused by *Xanthomonas axonopodis pv. phaseoli* (*Xap*), remains an important disease worldwide. Variability in reaction of bean lines to isolates of *Xap* from Puerto Rico and Central America suggests the existence of pathogenic races. This study was conducted to demonstrate that specific interactions in response to *Xap* occurs in common bean.

Three pathogenic strains of the common type and one of the fuscans type of *Xap* were used to identify common bean lines with a differential reaction to the pathogen. The common types of *Xap* were identified as 1930 from Nicaragua, 1934 from Costa Rica and 3353 from Puerto Rico. The fuscans type was identified as 3363 from Puerto Rico. White-seeded lines from the University of Puerto Rico and red-seeded lines from Dr. James Smith, USDA-ARS Research Geneticist, were evaluated. VAX-6 (resistant) and 'Rosada Nativa' (susceptible) were included as checks. Plants were inoculated 14 days after planting using the youngest but fully expanded trifoliolate leaf. Three replications per strain were conducted using pinprick inoculations with an estimated inoculum concentration of 10^7 CFU. A 1- 10 grading scale was used to score reactions to bacterial inoculations on trifoliolate leaves. The lowest scores had the most resistant reactions. Readings were recorded at 7, 14 and 21 days after inoculation. An ANOVA was conducted and a LSD (0.05) was used to compare mean scores.

The bacterial strain 1930 from Nicaragua had the highest virulence. *Xap* from Central America as represented by strains 1930 and 1934 were higher in virulence than the strains from Puerto Rico. The lowest CV (7.1%) was observed for readings made at 21 days after inoculation. Significant interactions between strains and bean lines were observed. Four white-seeded lines from Puerto Rico had resistance only to the *Xap* strain PR 3353 (Table 1). The red-seeded line 98020-3-1-8-2/I was resistant to the *Xap* isolates from Nicaragua and Puerto Rico and susceptible to the Costa Rica and Puerto Rico fuscans type whereas 98079-1-3-1-2 was resistant to the Costa Rica and Puerto Rico *Xap* strains and susceptible to the Nicaragua and the fuscans type from PR. The red-seeded line 98020-3-1-8-2 /S and the resistant check VAX-6 were resistant to the four isolates. The results suggest that the common types of *Xap* isolates from Nicaragua, Costa Rica and Puerto Rico represent different pathogenic races of the bacterium. Although the white lines had no resistance to the fuscans type, the white lines, which derive their resistance from Nebraska # 1 sel. 27, have moderate levels of field resistance to common bacterial blight. The differential reaction of the white and red-seeded bean lines could be used to identify pathogenic races of Xap from Nicaragua, Costa Rica and Puerto Rico. The red-seeded line 98020-3-1-8-2/S and the resistant check VAX-6 could be used to improve the common bacterial blight resistance of white-seeded bean breeding lines for Puerto Rico.

Table 1. Mean scores of trifoliate leaves of common bean breeding lines inoculated in the greenhouse with different pathogenic races of *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*).

Bean line	Seed type	Days after inoculation	Mean score[1] when inoculated with different *Xap* races			
			1930-NIC	1934-CR	3353-PR	3363-PR
PR0313-58	White	7	1.8	3.3	**1.0**	2.3
		14	5.0	10.0	**1.0**	6.3
		21	9.0	10.0	**1.0**	10.0
PR0313-95	White	7	2.6	2.0	**1.3**	1.5
		14	9.0	5.0	**1.8**	5.0
		21	10.0	9.0	**2.0**	9.0
PR0309-4	White	7	1.3	2.0	**1.1**	1.3
		14	5.2	5.0	**2.0**	5.0
		21	9.0	6.3	**2.0**	9.0
PR0301-265	White	7	2.5	1.6	**1.0**	1.3
		14	5.0	5.0	**1.7**	5.0
		21	5.0	9.0	**2.0**	9.0
98020-3-1-8-2/I	Red	7	**1.0**	1.8	**1.0**	1.1
		14	**1.2**	5.0	**1.0**	5.0
		21	**1.2**	5.7	**1.0**	5.0
98079-1-3-1-2	Red	7	1.0	**1.0**	**1.0**	1.5
		14	2.3	**1.5**	**1.3**	2.8
		21	9.0	**1.3**	**1.3**	5.0
98020-3-1-8-2/S	Red	7	**1.0**	**1.0**	**1.0**	1.0
		14	**1.0**	**1.0**	**1.0**	1.5
		21	**1.0**	**1.0**	**1.0**	2.7
Rosada Nativa (Susceptible Check)	Pink	7	1.1	1.5	1.1	1.1
		14	7.0	5.0	5.0	9.0
		21	10.0	9.0	9.0	10.0
VAX- 6 (Resistant check)	Purple	7	**1.0**	**1.0**	**1.0**	**1.0**
		14	**1.0**	**1.5**	**1.0**	**1.0**
		21	**1.0**	**1.2**	**1.2**	**1.0**

[1] Rated on a scale from 1-10 where 1 = no symptoms and 10 = a completely infected leaflet and systemic infection.

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PATHOGENIC DIVERSITY OF Xanthomonas campestris pv. phaseoli STRAINS FROM MEXICO.

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Common blight of bean is an important disease in the Highlands of Mexico (López, 1991), furthermore the causal agent *Xanthomonas campestris* pv. *phaseoli* (*Xcp*) is seed transmitted. Mexico is considered as one of the primary center of origin and diversification of beans (*Phaseolus* spp.) (Miranda, 1967). It is generally known that in the same region in which a species is originated also the companion pathogens are native and highly variable (Anikster, 1979). In the case of *Xcp* there is evidence of large pathogenic variation in many regions (Ekpo and Saettler, 1976; Schuster, 1983, Opio *et al*., 1996; Navarrete y Acosta, 2000; Mkandawire *et al*., 2004). The aim of this research was to detect the pathogenic diversity of *Xanthomonas campestris* pv. *phaseoli* (Xcp) strains isolated from different bean producing areas of Mexico.

Thirty-nine strains of *Xcp* coming from different bean producing areas of Mexico were isolated from leaves and seeds on nutrient agar and identified on yeast dextrose calcium agar as Xcp. Most of the strains were isolated from cultivated and two from wild *Phaseolus vulgaris*, and one from *P. cocineus*. The inoculum was an aqueous suspension of $3x10^7$ cfu/ml (McFarland scale). The strains were inoculated by twin razor blade on trifoliate leaves of the susceptible cv. Flor de Mayo Bajío at the R5 stage. The experiment was conducted under greenhouse conditions. Disease severity was scored 13 days after inoculation using a visual scale (1 to 9) (Schoonhoven and Pastor-Corrales, 1987).

The reaction induced by the strains varied from 2.5 to 8.5 and the differences were significant ($p<0.05$) (Table 1). The strains isolated from wild *P. vulgaris* showed high severity (7.5 and 7.3), and the strain from *P. coccineus* an intermediate severity of 5.3. Both *Xcp* and *Xcp* var *fuscans* strains elicited the whole range of severity and none relationship was observed between the geographical region of origin and the severity of the strain. The large pathogenic variation of Xcp hampers the development of resistant cultivars. We also have observed that cultivars that are resistant in one area became susceptible when grown in a different region.

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Table 1. Disease severity of strains of *Xanthomonas campestris* pv. *phaseoli* from different states in Mexico inoculated on Flor de Mayo Bajio cultivar under greenhouse conditions.

Strain	State	Isolated from	***Pathovar***	Disease severity
179	Hidalgo	Leaves	*Xcp*	8.5*
130	Hidalgo	Leaves	*Xcp*	8.3
145	Morelos[1]	Leaves	*Xcp*	7.5
144	Morelos [1]	Leaves	*Xcp*	7.3
184	Durango	Leaves	*Xcp*	7.2
185	Durango	Leaves	*Xcpf*	7.0
7	México	Leaves	*Xcp*	6.7
85	------	Leaves	*Xcp*	6.7
207	Guanajuato	Leaves	*Xcp*	6.3
99	México	Seeds	*Xcp*	6.0
16	México	Leaves	*Xcpf*	5.8
104	México	Seeds	*Xcpf*	5.8
124	Puebla [2]	Leaves	*Xcp*	5.3
272	Aguascalientes	Leaves	*Xcp*	5.2
40	Chihuahua	Seeds	*Xcp*	5.2
157	México	Leaves	*Xcp*	5.2
101	Aguascalientes	Seeds	*Xcpf*	5.2
44	Chiuhuahua	Seeds	*Xcp*	5.2
164	Puebla	Leaves	*Xcp*	5.2
159	México	Leaves	*Xcp*	5.0
91	Aguascalientes	Seeds	*Xcp*	5.0
273	Guanajuato	Leaves	*Xcp*	4.7
87	------	Leaves	*Xcp*	4.7
27	Durango	Seeds	*Xcp*	4.7
142	Veracruz	Leaves	*Xcp*	4.5
75	------	Leaves	*Xcp*	4.3
134	Puebla	Leaves	*Xcpf*	4.2
132	México	Leaves	*Xcp*	3.5
90	Aguascalientes	Seeds	*Xcp*	3.5
18	México	Leaves	*Xcp*	3.3
10	México	Leaves	*Xcpf*	3.3
127	Hidalgo	Leaves	*Xcpf*	3.2
162	Puebla	Leaves	*Xcp*	3.2
24	Durango	Seeds	*Xcp*	3.2
51	Chihuahua	Seeds	*Xcp*	3.0
265	Veracruz	Leaves	*Xcpf*	3.0
6	Aguascalientes	Seeds	*Xcp*	2.8
210	Durango	Leaves	*Xcp*	2.8
218	Hidalgo	Leaves	*Xcp*	2.5

LSD (0.05)=1.467, * Average of six replicates, *Xcp*: *X. campestris* pv. *phaseoli*, *Xcpf*: var. *fuscans*, [1] isolated from wild *P. vulgaris*, [2] isolated from *P. coccineus*, ---- Unknown origin

Bacterial Wilt Resistance in *Phaseolus vulgaris* L. Germplasm

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Bacterial wilt is one of the three bacterial diseases on dry bean in Bulgaria. The disease is caused by the bacterium *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*. The bacterium causes stunting, wilting and death of the bean plants. The disease development is favored by hot weather and drought stress. Breeding varieties combining resistance to common bacterial blight, halo blight and bacterial wilt is the main strategy for control of bacterial diseases on bean in Bulgaria; this strategy is incorporated in the breeding program of DAI - General Toshevo. In our preliminary study we determined several sources of resistance to individual strains of *C.f.* pv. *flaccumfaciens* (Kiryakov and Genchev, 2000). This publication presents results on the testing for resistance of elite accessions and breeding lines of dry bean to bacterial wilt.

Material and Methods

The study included 53 accessions and 33 breeding lines of beans. The plants were sown in two rows, each 2 m long, in two replications, in randomized design of the accessions in the trial field of DAI - General Toshevo. The plants were inoculated with bacterial suspension 10^8 cfu/ml from isolate CC 96212 (yellow strain) in the cotyledonary node, after removing the cotyledons at stage V_2 (Coyne et al., 1965). The last plants in the rows were injected with sterile water and used as checkers. The bacterial wilt reaction of the accessions was rated at stages R_6 and R_8 according to the following scale: 1 - no symptoms; 2 - temporary wilting of single leaves, vigorous plant growth; 3 - wilting and shriveling of single leaves, vigorous plant growth; 4 - plant moderately stunted, without wilting of leaves; 5 - a part of the old leaves wilted and/or shriveled, vigorous plant growth; 6 - part of the old leaves wilted and shriveled, plant moderately stunted; 7 - a large part of the leaves wilted and shriveled, plant moderately stunted; 8 - severe plant stunting, wilting and shriveling; 9 - plant death.

Results and Discussion

In a preliminary study variety Ludogorie showed a susceptible response to 4 *C.f.* pv. *flaccumfaciens* isolates (Kiryakov and Genchev, 2000). In this investigation variety Ludogorie was used as a susceptible checker (Figure 1). Four breeding lines and one accession had high resistance to the pathogen. Breeding lines 95-49-106-5, 95-49-106-7, 95-49-106-6, 95-49-106-8, as well as lines 95-20-28-7, 95-20-28-2, exhibiting moderate resistance to bacterial wilt, possess resistance to common bacterial blight and halo blight (Table 1). Accessions Trudovec, Oturak, C 64 and C 31 had moderate susceptibility to *C.f*. pv. *flaccumfaciens*. Under field conditions the plant growth of the accessions was moderately stunted, without wilting. The lack of wilting typical for the disease in these accessions was probably due to a different gene control of resistance to the wilt and plant stunt.

Figure 1. Response of the susceptible variety Ludogorie (left) and the resistant line 95-49-106-5 (right) to bacterial wilt under field conditions

Table 1. Bean accessions with resistance to *C.f.* pv. *flaccumfaciens* under field conditions

Accession / Line	Disease severity index at growing stage		Growth habit	Seed colour	Weight of 1000 seeds
	Flowering (R6)	Pod filling (R8)			
Ludogorie (susceptible checker)	7.8	8.0	II a	white	180
95-49-106-5	1.0	1.0	III b	white	405
95-49-106-	1.0	1.0	III b	white	411
Raikin 1	1.0	1.0	IV b	motley	340
95-49-106-6	1.1	1.3	III a	white	359
95-49-106-8	1.6	1.6	III a	white	410
Zlaten	2.0	2.0	IV a	motley	650
Gorna Ribnica 1	2.7	2.7	I a	white	505
PMB 0127	3.3	3.3	I a	white	490
Kavrakirovo 8	3.0	3.6	I a	white	465
95-20-28-7	3.0	4.0	II a	white	210
Trudovec	4.0	4.0	I a	white	495
Oturak	4.0	4.0	I a	white	470
C 64	4.0	4.0	I a	white	350
C 31	4.0	4.0	I a	white	375
95-20-28-2	4.3	4.3	II a	white	205
Damyanitza	4.5	6.0	I a	white	404

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ENDOGLUCANASE GENES IN *Macrophomina phaseolina* ARE NOT DIRECTLY RELATED TO PATHOGENICITY IN COMMON BEANS[1]

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The fungus *Macrophomina phaseolina* (Mp) attacks common beans (*Phaseolus vulgaris*) in both arid and tropical regions of México and provokes charcoal rot disease (1). Mp shows great pathogenic (4, 5, 7) and genetic (2, 4, 7) variability which increases its adaptability to diverse environmental conditions. Two β - 1, 4 endoglucanase genes (*egl*1 y *egl*2) have been cloned and characterized from Mp. The *egl*1 gene has a cellulolytic activity and improves the penetration of charcoal rot hyphae in the host, while *egl*2 plays a major role on saprophytic activity (2). This work was conducted in order to characterize the pathogenic and genetic variability of 30 isolates of Mp from México and other countries and determine the relationship between pathogenicity and presence of the two endoglucanase genes.

Fifteen Mp isolates were obtained from different locations of Mexico and the other 15 from Italy [2 isolates], Australia [5], Argentina [2], USA [5], and Brazil [1]. Each isolate was cultured on 12 PDA Petri dishes and when Petri dishes were completely colonized 10 seeds of each common bean differential cultivar (4) were placed per Petri dish. After 5 days in incubation, pathogenicity (3) and pathotype (4) were determined. Genomic DNA was isolated (6) and 20 μg were digested overnight with enzyme *Sal*I, phenol:chloroform extracted, ethanol precipitated, dried, and separated by electrophoresis on 1.2 % agarose gels. Transfer and probing were carried out as described by Jones *et al*. (2) except that the probe was chemiluminiscent labeled by random-priming method. The genes *egl*1 and *egl*2 were supplied by Dr. Richard Jones (USDA-ARS, Beltsville, USA). Film (X-ray film, Eastman-Kodak, Rochester, USA) time exposure was one hour.

Mexican Mp isolates showed higher pathogenicity (mean = 4.4) on common bean seeds than no-Mexican isolates (mean = 2.4). Cluster analysis of pathogenicity data showed a clear differentiation of isolates on basis of their geographical origin (Fig. 1). However, *egl*1 gene was found in all no Mexican isolates and in 14 Mexican isolates; while *egl*2 was detected in 11 no Mexican isolates and 9 Mexican isolates. We found clear separation between Mexican and no Mexican isolates on basis to pathogenicity and origin of each isolate has been previously reported in *Macrophomina* (3, 4, 5, 7), but no clear differences among isolates on basis to the presence/absence of endoglucanase genes (2) Endoglucanase genes appeared to be not close associated to pathogenic ability as been previously reported (2). Although conservation of endoglucanase genes among Mp isolates was found, our results suggest that other pathogenicity factors must be involved in pathogenic/saprophytic ability of *Macrophomina*. Further works could be necessary to identify and clarify the role of those additional pathogenicity genes.

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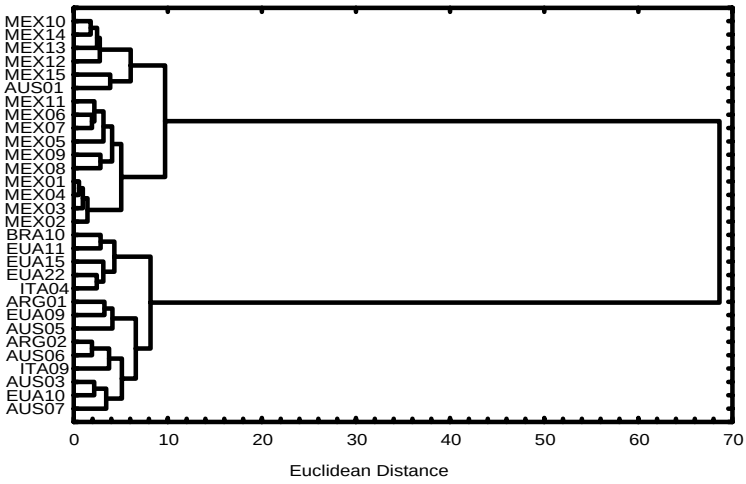

Fig. 1. Dendrogram of *Macrophomina phaseolina* isolates based on pathogenicity data.

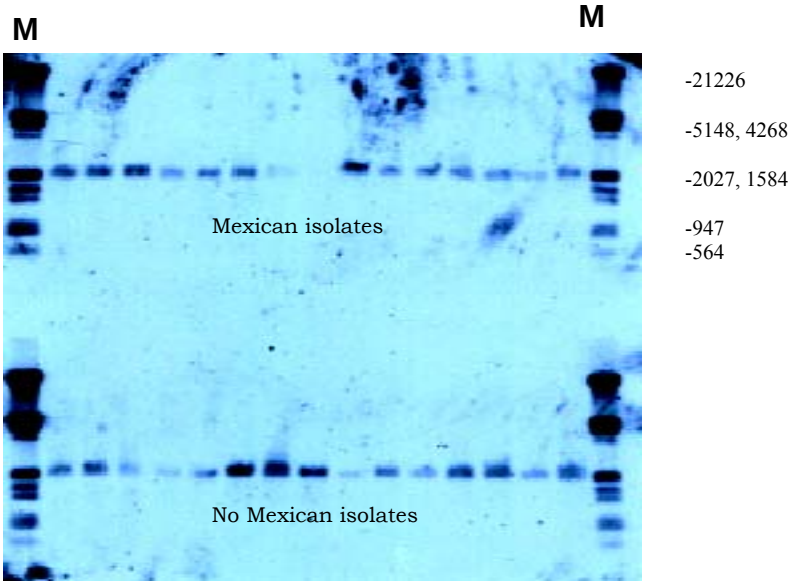

Fig. 2. Southern analysis of *egl*1 gene on 30 *M. phaseolina* isolates (M=Molecular weight ladder). Numbers indicate molecular weights of ladder in base pairs.

GENETIC VARIABILITY OF *Macrophomina phaseolina*, THE CAUSAL AGENT OF CHARCOAL ROT IN COMMON BEANS[1]

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Charcoal rot caused by *Macrophomina phaseolina* in common bean is economically important in Mexico, since the pathogen is favored by high temperature and drought stresses (1). Although a single species has been recognized in the genus *Macrophomina*, high levels of genetic variability have been found (Mayek-Perez *et al*., 2001; Su *et al.*, 2001). Genetic diversity of *M. phaseolina* (3, 6, 7) could favor survival and adaptation of the pathogen to variable environmental conditions. However, no subspecies or physiological races have been proposed, although Su *et al.* (2001) suggested a host specialization. This work was conducted in order to characterize the genetic variation of *M. phaseolina* isolates from Mexico and other countries.

The isolates were obtained from different hosts and locations in México [23 isolates], USA [23], Brazil [18], Italy [10], Japan [9], Australia [9], Colombia [2], and Argentina [2]. Each isolate was genetically purified on PDA by hyphae tip cultures from single microsclerotia. The DNA was isolated using the method of Raeder & Broda (5). The isolates were characterized using the AFLP (Amplified Fragment Length Polymorphisms) technique adapted from Vos *et al*. (8). Amplified products were visualized by electrophoresis in 6% acrylamide gels and revealed by staining with silver nitrate (Promega^{R}). AFLP data was used to calculate genetic distances using simple matching coefficient. The matrix of genetic distances was used to construct a dendrogram by UPGMA method.

The AFLP analysis was performed by using four combinations of oligonucleotides resulting in 418 amplified products of which 92.8% were polymorphic. Cluster analysis showed genetic dissimilarities ranging from 3 to 15 % and two major clusters of isolates were found, one which mainly included isolates from Mexico and Italy, and other which included the rest of isolates (data not shown). When a sub-sampling of isolates was done, a clear separation between Mexican and Asian (Japan, Australia) isolates was found (Fig. 1). A broad genetic diversity among isolates from Mexico and other countries was found, although diversity was not strongly correlated to geographical origin of each isolate. Despite high genetic diversity has been found in *M. phaseolina* isolates from specific countries (3, 6, 7), in this work we included isolates obtained from different continents in order to clarifying the perspective of variation in *Macrophomina*. Su *et al*. (6) analyzed isolates of *M. phaseolina* obtained from soybean, cotton, sorghum and maize plots and found clear separation of isolates based on host origin and RAPD analysis meanwhile Almeida *et al.* (2) indicated that monoculture reduces genetic diversity in *M. phaseolina*. The high genetic diversity levels are reflecting the polykariotic nature of *M. phaseolina* isolates or suggesting that other parasexual mechanisms for genetic exchange are interacting within and among the pathogen populations, if we assume that each microsclerotia is formed by fusion of vegetative cells or mycelia for heterokaryon formation (Mihail & Taylor,

1995; Su *et al*., 2001). In spite of the fact that AFLP analysis did not revealed a clear grouping of isolates on the basis of geographical or host origin, a clear differentiation on the basis of geographical origin was found when subgroups of isolates were re-analyzed. The lack of a strong correlation between AFLP genotype and geographical origin could suggest a high diversity level within and among populations of *M. phaseolina*, increasing the probability that two AFLP bands with identical molecular weight derived from different loci could be identified. The similarity among isolates from remote origin confirmed genetic exchange and free genetic information flux in the genus that reduce the probability for formation of delimited groups (4, 6). Our results emphasize that pathogenic specialization is unlikely due to the fact that *M. phaseolina* is a saprophytic fungus and a facultative parasite which do not involves the close host-pathogen co-evolution needed for establishing subspecies or physiological races. Genotyping based on molecular markers could be useful for monitoring the variability of fungal populations through time, geographical locations or plant hosts. However, new insights into the association between pathotype and genotype, depending on the geographical origin of isolates were revealed.

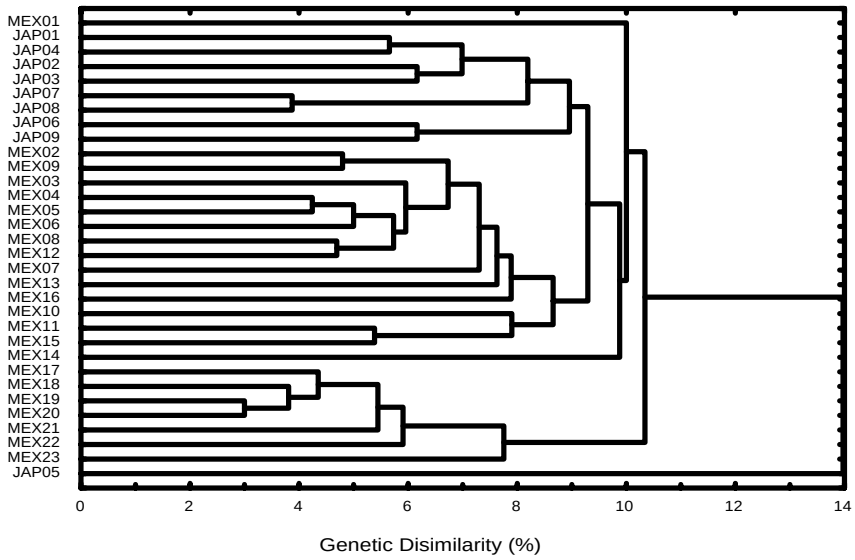

Fig. 1. Dendrogram of *M. phaseolina* isolates from México and Asia on basis to AFLP analysis.

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PATHOGENICITY OF Macrophomina phaseolina ISOLATES FROM MÉXICO AND OTHER COUNTRIES ON COMMON BEANS[1]

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Charcoal rot on common beans is caused by the fungus *Macrophomina phaseolina* and the disease shows a significant progress under drought and high temperature stresses in both tropical and arid regions of México. A high variability on genotype and pathogenicity in Mexican *M. phaseolina* isolates has been found, and in general populations from tropical lands are more aggressive than those from arid lands. A 12 common bean differential cultivar set have been proposed to the characterization of pathogenic variability and clarify the biology of populations and host specialization of *M. phaseolina* (2). In this work we characterized the response of 12 common bean differential cultivars to 96 *M. phaseolina* isolates from México and other countries under controlled conditions.

The isolates were obtained from different hosts and locations in México [23 isolates], USA [23], Brazil [18], Italy [10], Japan [9], Australia [9], Colombia [2], and Argentina [2]. Each isolate was genetically purified on PDA by hyphae tip cultures from a single microsclerotium. Each isolate was replicated on 12 PDA plates and 10 seed from each common bean differential cultivar were placed per colonized Petri dish. The differential cultivars are BAT 477, SEQ 12, G 19428, TLP 19, Negro 8025, G 4523 (resistant to charcoal rot), Pinto Villa, Pinto UI-114, Azufrado Tapatío, Bayo Durango, Rio Tibagi, Bayo Mecentral (susceptible) (2). Petri dishes were incubated for 5 days at 28 °C and the pathogenicity index (1) and pathotype (2) of each isolate were calculated.

The most aggressive *M. phaseolina* isolates were obtained from México (mean of pathogenicity = 3.9), while isolates from Japan were the least aggressive (mean of pathogenicity = 1.3). A broad range of pathotypes was found for each country, values ranged from 0 to 4095 (Table 1). The most common pathotypes were 4095 (15 isolates), 0 (9), and 2 (5), while 59 isolates showed a single pathotype. The pathogenicity data was transformed into a binary matrix where values from 0 to 2.0 = 0 while values from 2.1 to 5.0 = 1. Cluster analyses were performed and dendrograms constructed by Ward's method in order to evaluate pathogenic diversity into two ways: common bean cultivars and *M. phaseolina* isolates. The common bean cultivars were grouped in two major clusters, one included 5 cultivars pre-classified as resistant to charcoal rot (BAT 477, SEQ 12, N. 8025, TLP 19, and G 4523) while the other group included 5 cultivars pre-classified as susceptible (A. Tapatío, B. Durango, P. Villa, B. Mecentral, and P. UI-114). Two major groups of isolates were found, one group included 36 isolates (mainly from México, Colombia and Brazil) and the other included 60 isolates (data not shown). When a sub-sampling of isolates was done, a clear separation between Mexican and Asian (Japan, Australia) isolates was found (Fig. 1). Great pathogenic diversity had been found previously in *M. phaseolina* (2, 3, 5) but no clear association had been reported between pathogenicity patterns and geographical

origin. In this work, as Su *et al*. (4) reported, we found a clear differentiation on pathogenicity patterns when some isolates were sub-sampled from contrasting countries (México *vs*. Australia and Japan). Data suggest that geographical reproductive isolation could favor the formation of pathogenic groups, lineages or pathotypes clearly defined (3). Mexican isolates were more aggressive than all other isolates of *M. phaseolina*. Since the most of aggressive Mexican isolates were obtained from common beans, we suggest that incipient host specialization could be present in *M. phaseolina* (4), despite the saprophytic condition of the fungus.

Table 1. Pathogenicity and pathotypes of the 96 *M. phaseolina* isolates used in this study.

Origin of isolates	No. isolates	Mean of pathogenicity	No. Pathotypes	Range of pathotypes
Brazil	18	2.4	16	0-4094
Japan	9	1.3	4	0-1026
Australia	9	2.4	9	0-3832
Italy	10	2.3	10	0-4039
Colombia	2	3.7	2	2527-4095
Argentina	2	2.5	2	2299-2555
USA	23	2.2	22	0-4095
México	23	3.9	11	331-4095
Total/Mean	96	2.5		

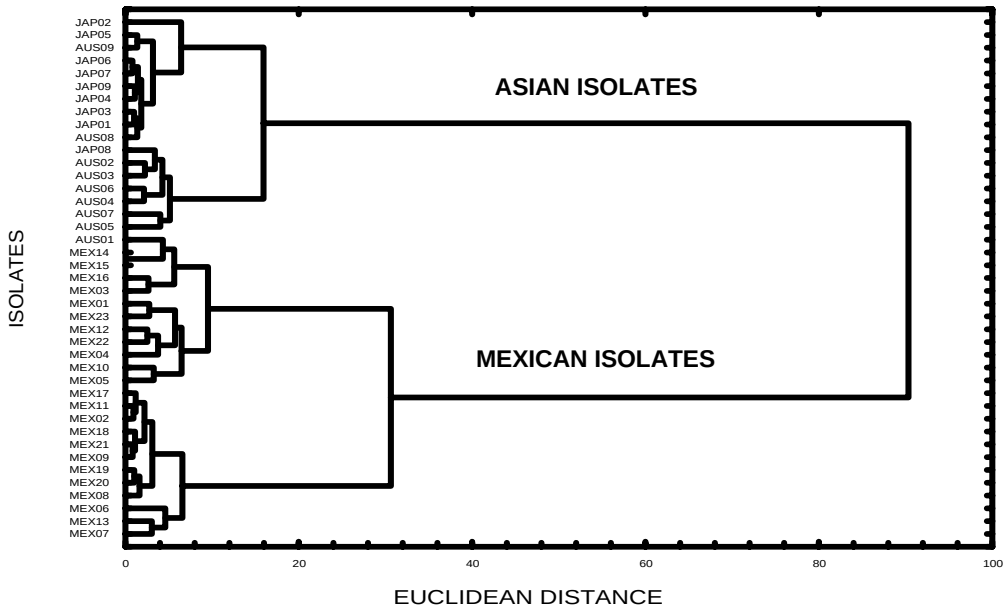

Figure 1. Dendrogram of *M. phaseolina* isolates from México and Asia on basis to the pathogenicity in 12 common bean differential cultivars.

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AFLP FINGERPRINTING FOR IDENTIFICATION OF ANASTOMOSIS GROUPS OF *Rhizoctonia solani* ISOLATES FROM COMMON BEAN IN MEXICO[1]

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Rhizoctonia solani (Rs) is a basidiomycete anamorph that does not produce any asexual spores (conidia) and occasionally produces sexual spores (basidiospores). Parmeter (4) indicated that hyphal anastomosis is a useful characteristic to identify Rs, and implies that genetically related isolates can recognize and fuse ("anastomose") with each other. Determination of anastomosis is done by microscopic monitoring of hyphal fusion which is a laborious and subjective method. Our study was carried out in order to obtain the AFLP genotype of nine *R. solani* isolates from common beans and 17 AG testers and determine the genetic relationship between anastomosis group (AG) and isolates of the pathogen; and determine the pathogenicity of RS isolates and then establish the association between anastomosis group, pathogenicity and AFLP genotype.

Nine Rs isolates were obtained from bean plants collected in Estado de Mexico and Veracruz, Mexico. DNA from each RS isolate and 17 AG testers (AG 1A, AG B1, AG 1C, AG 2.1, AG 2.2, AG 2.3, AG 3, AG 4, AG 5, AG 6, AG 7, AG 8, AG 9, AG 10, AG 11, AG 12, AG 13) was extracted (5). AFLP analysis (6) was performed and amplified products were separated in acrylamide gels and visualized using silver staining. The AG of each Rs isolate was performed on *in vitro* conditions (1). Each isolate was cultured on PDA and 10 seeds of each of the bean cultivars Pinto Villa, Río Tibagí, Pinto UI-114, Bayo Durango, Azufrado Tapatío, Bayo Mecentral, Negro 8025, SEQ 12, TLP 19, and BAT 477 were placed over the mycelia. Pathogenicity was scored three days after placing the seeds using a six level scale (from 0 to 5, where 0 = No infection and 5 = 81-100 % of infected seed). Values from 0 to 2.0 were classified as a resistant reaction and values from 2.1 to 5 as a susceptible reaction.

Five isolates of Rs were from Veracruz (RS010, RS011, RS012, RS013, RS014) and four from México (RS001, RS002, RS003, RS009). AFLP analysis produced 415 bands including 3 monomorphic bands (0.72 %) among the nine *Rhizoctonia* isolates, while among the AG testers no monomorphic bands were found (data not shown). One isolate from Veracruz (RS010) and four isolates from México (RS001, RS002, RS003, RS009) were similar to AG-2 type 3; three isolates from Veracruz were similar to AG-BI, and one isolate from Veracruz (RS013) grouped with AG-5 (Fig. 1). Test for identification of anastomosis groups *in vitro* showed the complete coincidence between the AGs determined by AFLPs and by *in vitro* tests. BAT 477 showed the highest mean of severity by Rs while the other nine bean cultivars exhibited severity ratings lower than 2.0. Common bean germplasm from races Durango and Jalisco showed a higher frequency of resistance (7 to 9 isolates) than whose from race Mesoamerica (5 to 8 isolates). Isolates 011 (AG-B1), 002 and 003 (AG 2.3) were the more aggressive in common bean seeds (Table 1). No association was found between the anastomosis group of each Rs isolate and pathogenicity on common bean seeds. An inverse resistance/susceptibility relationship as the one showed here have been found between *P. vulgaris* gene pools and *Macrophomina phaseolina* (3) and *Fusarium* (2). Resistance to Rs in Durango germplasm may have been produced through

selective pressure since the fungus is the major causal agent of root rots in cultivated *P. vulgaris* in the states of Durango and Zacatecas, México from where the germplasm was originated. The grouping of each isolate to its corresponding AG indicated that AFLP could be a reliable method for molecular identification of AGs of *Rhizoctonia* when the technique is available. The AFLP technique offers several advantages as a molecular test for identification of anastomosis groups of Rs isolates such as the short time needed to obtain results; the possibility to rapidly classify large numbers of isolates; the establishment of a data base that includes banding patterns of AGs for further identification of isolates (using automatic sequencing systems); and the elimination of the laborious identification of AGs under the microscope.

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Table 1. Resistance/susceptibility of 10 common bean cultivars to nine isolates of *R. solani*.

	Race[z]	*R. solani* isolates									Mean ± SE
		001	002	003	009	010	011	012	013	014	
Cultivar											
B. Durango	D	0.6	0.3	1.7	0	0	2.0	0.6	0.3	0	0.6±0.7
P. Villa	D	1.0	3.7	1.6	1.7	0.9	3.5	1.6	1.1	0.2	1.7±1.2
P. UI-114	D	1.0	0.9	1.8	0.6	0.5	2.1	0.6	0.2	0.9	1.0±0.6
A. Tapatío	J	0.4	0.3	0.9	0.2	0.1	1.2	0.4	0.1	0	0.4±0.4
SEQ 12	M	0	1.5	2.6	0	0	1.3	0.8	0	0.5	0.8±0.9
BAT 477	M	3.4	3.4	1.9	1.0	1.5	3.6	1.9	3.2	1.1	2.3±1.1
TLP 19	M	1.1	2.3	1.4	1.0	0.9	1.5	2.3	0.2	0.5	1.2±0.7
N. 8025	M	0.5	2.3	1.8	0.6	0.6	1.3	0.5	0.5	0.2	0.9±0.7
Río Tibagí	M	1.6	2.4	3.4	0.8	0.7	2.6	2.0	1.5	0.8	1.8±0.9
B. Mecentral	J	0.7	3.1	1.7	0.1	0.7	1.5	0.8	0.3	1.0	1.1±0.9
Mean ± SE		1.0±0.9	2.0±1.2	1.9±0.7	0.6±0.5	0.6±0.5	2.1±0.9	1.2±0.7	0.8±1.0	0.5±0.4	1.2±0.7

[z] D = Durango, J = Jalisco, M = Mesoamerica.

* Where reaction of resistance were from 0 to 2.0 and susceptibility were from 2.1 to 5.

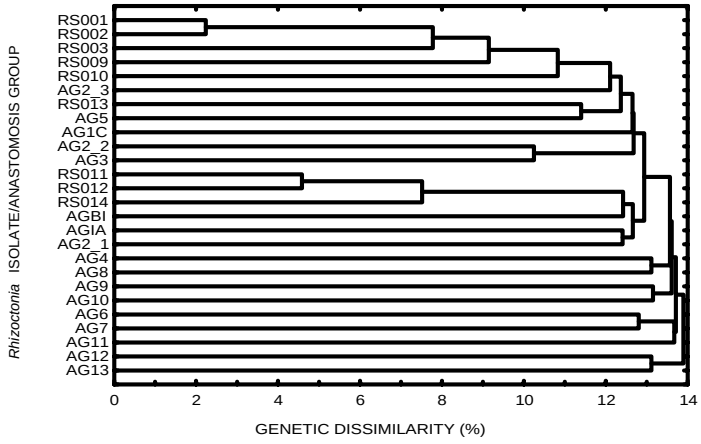

Fig. 1. Dendrogram produced from AFLP data obtained from nine Mexican *R. solani* isolates and 17 AG testers.

Effect of Three Crop Rotations With and Without Deep Plowing on Root Rot Severity and Yield of Beans

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Introduction

Root rot diseases continue to be a major factor in bean production in New York. The major root pathogens on beans include the fungi *Fusarium*, *Pythium*, *Rhizoctonia* and *Thielaviopsis* as well as the lesion nematode (*Pratylenchus* spp.). These pathogens may occur singly or in any possible combination. Over the years, we have demonstrated that most of the production practices employed including crop rotation, tillage and cover crops significantly impact root rot severity and yield of beans (1, 2). As a part of our on-going research on bean root-rot and its management, we have maintained three replicated, 4-year crop rotations (2000 – 2003) in one of the root rot fields at the Vegetable Research Farm, NYSAES - Cornell University in Geneva. The three crop rotations were #1) Bean-Bean-Bean-Bean; #2: Bean-Corn-Bean-Corn; and #3: Bean-Corn-Corn-Corn. Thus, this experimental site provided a great opportunity for additional evaluation of the influence of crop rotations on root rot development and yield of snap beans under conventional and deep plowing tillage practices. Thus, the entire site was planted to snap bean cv. "Hystyle" during the 2004 season for this evaluation.

Materials and Methods

The 3 rotations were previously arranged in a randomized block design with 4 replications, over an area of about 2 acres. Each plot (replicate)/ crop rotation was 19.2 X 32.9 meter. Each rotation plot was split into two tillage treatments (conventional vs. deep plowing/chiseling). The trial area was prepared, treated with herbicides and fertilizers, and planted to snap bean according to Cornell's guidelines for commercial bean production in New York (3). The herbicides Treflan and Eptam were applied pre-plant and Dual was applied as a spray after planting. The fertilizer (15-15-15; NPK) was applied as a broadcast at 200 lbs./A. Commercially treated snap bean seeds were planted with a 2-row Monosem vacuum-planter at a rate of 26-seeds/m row. Emergence and stand counts were made at 16 days and 9 weeks after planting, respectively. A minimum of 40 plants were carefully dug up from each plot/rotation/tillage at 6 weeks after planting and rated for root rot severity on a scale of 1 (no visible symptoms) to 9 (>75% of roots and stem tissues are affected and with considerable decay and reduced size). At harvest time, four 3 m sections/plot were harvested for determining total weight and marketable (pod) weight of beans.

Results and Discussion

Heavy and frequent rains prevailed throughout the season and night temperatures were rather low. The results presented in Table 1 clearly documented the benefit of crop rotation to bean yield at this root-rot site. Total weight (weight of above-ground plant parts) and pod weight of "Hystyle" were highest and root rot severity rating was lowest in the plots that were previously planted to 3 consecutive years to corn (Rotation #3). Stand establishment and yield of "Hystyle" grown in plots of rotation #2 (one year rotation with corn) were also good. In contrast, pod weight was the lowest and root rot severity were the highest on beans grown in the continuous bean plots with no rotation (Rotation #1). Pod weight of beans cv. "Hystyle" grown in rotation

#1 (continuous beans for 4 years), rotation #2 (Corn-Bean-Corn-Bean), and rotation #3 (Corn-Corn-Corn-Bean) averaged 1.14, 2.63, and 3.21 Tons/A, respectively.

Root rot severity ratings and yield of beans grown in the deep plowed plots were slightly higher as compared to those grown in the conventionally plowed plots, but the differences were not statistically significant. The limited benefit to deep plowing observed in 2004 might have been due to the wet weather conditions that prevailed throughout the season. We have previously observed that the benefit of deep plowing is the production of larger and deeper bean roots, thus the access to more nutrients and water in the soil profile under dry weather conditions.

The results of this experiment further document the importance of practicing 2-3 years rotation, and preferably with grain crops (out of vegetables), for improving snap bean yield and reducing root rot severity and damage. Deep plowing or ripping will contribute to reducing soil compaction and improving drainage. The latter generally results in deeper and larger roots that reduce stresses on beans and improve yield.

Table 1. Effect of selected crop rotations and deep tillage on root rot severity and yield of beans in the root rot field at the NYSAES Research Farm, Geneva 2004.

	No.plants/12m		Yield(kg)/12m		Pod Yield	Root Rot Rating
Rotation 2000-2004	Emergence	Stand	Total	Pod	(T/A)	(1-9)
CORN-CORN-CORN-BEAN	106	90	12.43	6.70	3.21	4.12
CORN-BEAN-CORN-BEAN	125	93	10.34	5.50	2.63	4.46
BEAN-BEAN-BEAN-BEAN	77	76	4.63	2.39	1.14	5.03
lsd (p=0.05)	39.3	16.5	2.34	1.285	0.62	ns
Tillage						
DEEP	100	84	9.38	4.98	2.39	4.30
CONVENTIONAL	105	87	7.98	4.25	2.03	4.73
lsd (p=0.05)	ns	ns	ns	ns	ns	ns

Acknowledgment
To the PA and NY Bean Research Associations for support and interest in the research.

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CONFIRMATION OF QUANTITATIVE TRAIT LOCUS FOR ROOT ROT RESISTANCE IN TWO INBRED BACKCROSS SNAP BEAN POPULATIONS

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Introduction: Bean root rot caused by several pathogens including *Pythium ultimum* and *Aphanomyces euteiches* f. sp. *phaseoli* (Pfender and Hagedorn, 1982a; 1982b) is an important disease of snap beans. Crop rotation has traditionally been the only control for this disease; however, the best long term solution is the development of resistant varieties. Navarro et al. (2004) identified a quantitative trait locus (QTL) in linkage group 6 that explained 46% of the variation to root rot in a recombinant inbred line (RIL) population derived from crossing 'Eagle', a susceptible Andean snap bean variety with 'Puebla 152' a resistant Mesoamerican dry bean cultivar. Other candidate QTL were mapped on linkage groups 3 and 7 of the core map (Freyre et. al., 1998). Our objective was to confirm the importance of QTL previously detected using two inbred backcross snap bean population that contain introgressed segments of the genomic regions around the QTL of interest.

Materials and Methods: 67 and 83 lines of the BC_1F_7 EPE and EPH inbred backcross (IBC) populations, generated by crossing the Eagle x Puebla 152 F_1 to the root rot Eagle and 'Hystyle' susceptible cultivars respectively. The EPE and EPH populations were evaluated at the Hancock, WI Agricultural Research Station, at a field that had been continuously planted with beans for 12 years. After including susceptible and resistant control varieties, the experiments included 72 and 96 entries randomized in a blocks within replication design. Each plot consisted of single rows of 1.14m separated 0.91m containing 15 plants. At flowering, plant vigor was evaluated on a 1-9 scale, '1' representing vigor equivalent to the resistant Puebla 152 parent and '9' equivalent to the susceptible Eagle or Hystyle parent. Total above ground plant biomass for the IBC populations were measured 75 DAP as the fresh weight in grams of a random sample of 5 plants per plot. DNA was extracted from a sample of six plants of each line of the EPE and EPH populations using a procedure developed by Jhingan (1992), and modified by Johns et al. (1997). The EPE and EPH populations were screened with 43 and 5 random amplified polymorphic DNA primers (Operon Biotechnologies, Inc., Huntsville, AL) corresponding to markers located in linkage groups associated to QTL for root rot resistance identified by Navarro et al. (2004). Confirmation of marker-QTL relationship was done by simple regression of the least square means for vigor and plant weights from the EPE and EPH populations to the presence or absence of RAPD markers previously associated to QTL identified in the RIL population (Navarro et al. 2004).

Results and Discussion: Confirmation of the association of S18.1500 with root rot susceptibility previously observed in Eagle x Puebla 152 RIL population (Navarro et al., 2004) was observed from analysis of regression of S18.1500 and AD9.950 on root rot traits on the EPE and EPH inbred backcross populations. Analyses of regression indicated that the presence of AD9.950 and the absence of S18.1500 was associated to plant weight increases of 0.27 ± 0.05*** and 0.41 ± 0.05*** kg/pt and reduction of 2.43 ± 0.43*** and -2.78 ± 0.36*** in the plant vigor scale for both the EPE and EPH inbred backcross populations (Fig.). Similarly, 33 and 44% of the variation for plant weight and 32 and 39% of plant vigor were explained by the presence of

AD9.950 and absence of S18.1500 RAPD markers in EPE and EPH. None of the other putative marker-QTL association identified in the RIL population (Navarro et al., 2004) resulted significant from the regression analyses on the EPE and EPH populations. Exploitation of the linkage disequilibria of the root rot resistant and susceptible locus with AD9.950 and S18.1500 will allow marker assisted selection for root rot resistance in early generation. The combined use of the AD9.950 and S18.1500 RAPD in marker assisted selection programs presents the additional advantage of separating root rot resistant from heterozygous genotypes in early generations. This will allow breeders to select a higher number of root rot resistant lines in the greenhouse and focus on the recovery of quality traits in the field evaluations.

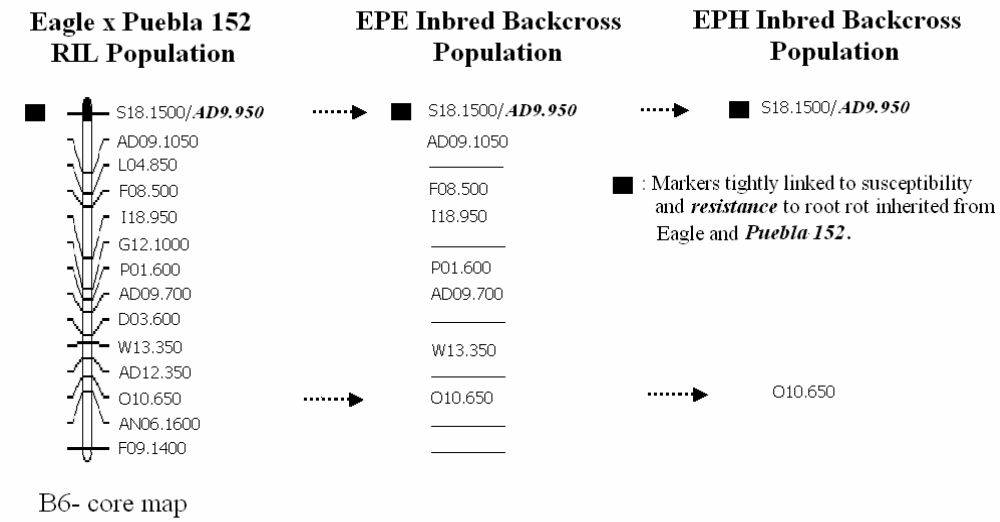

Fig. 1. Quantitative trait locus identified in the Eagle x Puebla 152 RIL population (Navarro et al., 2004) in linkage group 6 (shaded area), and confirmed evaluating the EPE and EPH inbred backcross populations at Hancock, WI under a field with high inoculum level of *A. euteiches* and *P. ultimum*.

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CHARACTERIZATION OF *SCLEROTINIA SCLEROTIORUM* ISOLATES USED IN COMMON BEAN SCREENING FROM BEAN PRODUCTION AREAS IN THE UNITED STATES

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There is a dearth of information on variability of isolates of *Sclerotinia sclerotiorum* from common bean. In addition, variability between data from collaborators screening for putative resistance has been reported (Steadman et al, this issue). Pathogen isolates were collected from collaborators from locations within the U.S. bean production areas to determine mycelial compatibility groupings (MCGs), aggressiveness, and molecular genotype.

Sclerotia of nine *S. sclerotiorum* isolates used for screening in greenhouses/labs were collected from the following U.S. sites: ID, ND, WI, WA, NE, CO, MI, OR, and NY. These nine isolates were tested for clonality using MCGs (Kull et al, 2004). One 8mm plug of one isolate was placed on one side of a plate of red PDA (Potato Dextrose Agar) and another plug of the same or a different isolate was placed 2.5 cm across from it. After 7-14 days, if the isolates formed a barrage line where the hyphae from the isolates met, then the isolates were incompatible and were considered unique isolates. If there was no evidence of mycelial interaction, the isolates were compatible, and were considered clones. The nine isolates were tested in a matrix for MCGs (Table 1). No two greenhouse/lab isolates were compatible; each isolate was unique.

The nine isolates were also tested for aggressiveness using the detached leaf test (DLT) (Steadman et al, 1997). In the DLT, a detached trifoliolate leaf was inoculated with an 8 mm agar plug containing *S. sclerotiorum* mycelium. After 48 hours of incubation in high humidity, the area of the lesion caused by the pathogen was measured by digital analysis. The most aggressive isolates used in greenhouse/lab screening were from ID, ND, and WI; these isolates were significantly more aggressive than greenhouse/lab screening isolates from NE, WA, and CO (Table 2).

We have shown that the internal transcribed spacer region (nuclear subunit rDNA) can be used for interspecific separation of *S. sclerotiorum*, *S. trifoliorum*, and *S. minor*. A preliminary study of the nuclear large subunit rDNA failed to find intraspecific variation for the nine screening isolates. Further investigation of this region as well as molecular characterization by the use of L. Kohn's microsatellites is planned to determine if polymorphisms found by molecular genotyping are related to aggressiveness of the isolates.

The variation in aggressiveness and MCGs may help explain why greenhouse/lab screening results often do not agree across different test sites. When collaborators described greenhouse/lab screening protocols, such as the straw test, variations in each protocol such as stage of plant inoculation, apex vs. petiole could also have contributed to variation in results across test sites. The selection of a universal isolate(s) for use in all screening tests may result in more consistent similarity in ranking of white mold resistance sources.

Table. 1. Results of the MCG test of the nine greenhouse *Sclerotinia sclerotiorum* bean isolates.

	NY	CO	WA	WI	ND	MI	OR	NE	ID
NY	O	X	X	X	X	X	X	X	X
CO		O	X	X	X	X	X	X	X
WA			O	X	X	X	X	X	X
WI				O	X	X	X	X	X
ND					O	X	X	X	X
MI						O	X	X	X
OR							O	X	X
NE								O	X
ID									O

(O) = Compatible MCG reaction; (X) = Incompatible MCG reaction

Table 2. Mean lesion size of nine *Sclerotinia sclerotiorum* isolates on bean line G122 using the detached leaf test.

ISOLATE-SOURCE	MEAN LESION SIZE	t GROUPING
JRS 483-IDAHO	17.5	A
JRS 274-NORTH DAKOTA	17.3	A
JRS 478-WISCONSIN	17.0	A
JRS 443-NEW YORK	15.8	A B
JRS 482-MICHIGAN	15.7	A B C
JRS 455-OREGON	14.2	A B C
JRS 152-NEBRASKA	12.8	B C
JRS 467-COLORADO	12.0	C
JRS 456-WASHINGTON	8.3	D

LSD=3.14; ALPHA=0.05

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IDENTIFICATION OF PARTIAL RESISTANCE TO *SCLEROTINIA SCLEROTIORUM* IN COMMON BEAN AT MULTIPLE LOCATIONS IN 2004

J.R. Steadman, Coordinator and Collaborator, L. K. Otto-Hanson and K. Powers (University of Nebraska-Lincoln). Data from C. Kurowski (California), R. Mainz (Minnesota), J. Kelly (Michigan), P. Griffiths (New York), K. Grafton (North Dakota), J. Myers (Oregon), P. Miklas (Washington), H. Schwartz (Colorado), S. Singh (Idaho), and A. Opplear (France).

There is no complete resistance to *Sclerotinia sclerotiorum*, cause of white mold, in common bean. The development of bean cultivars with partial physiological resistance and architectural avoidance to white mold would reduce disease losses at no cost to producers. The objective of the study was to identify bean germplasm with broad partial resistance to white mold. To accomplish this, putative sources of resistance developed by bean breeders were evaluated by greenhouse and field screening methods of multiple sites.

Field tests consisted of two rows of each entry and a common susceptible genotype, resulting in a three-row plot 4.6 m (15 ft.) long replicated three times in a randomized complete block design. Sixteen separate screening tests, seven field and nine greenhouse (straw, oxalate, and detached leaf), were rated on a scale ranging from most resistant (1) to most susceptible (12) (Table 1). Spearman and Pearson correlations were used to compare entry rankings in each test. Each test included eleven common bean lines; the *P.coccineus* cultivar Dwarf Bees was added for greenhouse screening, and CO75944 was added for field screening, were used in each test in the US and France..

The highest positive field correlations were NE and OR (r=0.735, p=0.0065) and the outlier field France and OR (r=0.604, p=0.0374). The highest positive greenhouse screening correlations were ID and ND (r=0.730, p=0.0107). The MN field and the OR, CO, NY, and WA greenhouse tests were all significantly correlated with each other: MN field and OR (straw test) (r=0.841, p=0.0012), MN field and CO (straw test) (r=0.829, p=0.0016), MN field and NY (straw test) (r=0.727, p=0.0113), MN field and WA (straw test) (r=0.839, p=0.0006), OR (straw test) and CO (straw test) (r=0.818, p=0.0011), OR (straw test) and NY (straw test) (r=0.862, p=0.0003), OR (straw test) and WA (straw test) (r=0.766, p=0.0037), CO (straw test) and NY (straw test) (r=0.653, p=0.0213), CO (straw test) and WA (straw test) (r=0.660, p=0.0195), and NY (straw test) and WA (straw test) (r=0.711, p=0.0095).

When an ANOVA was used on ranking with each test as a block and bean line (entry) as a treatment, there were significant differences (p=0.0005) among lines (Table 2). As in 2003, Dwarf Bees, Cornell 601, G122, and Cornell 501 had the lowest mean rank (=most resistant). When greenhouse and field tests were analyzed separately, G122, Cornell 601, N02 302, Cornell 501, Ex Rico (Bunsi), and USWA-6 were ranked lowest in field tests, but Dwarf Bees, IO1892-115M, AN-37, and CO75944 replaced N02 302, Ex Rico (Bunsi), and USWA-6 in the lowest rankings from greenhouse tests, where N02 302, Ex Rico (Bunsi), and USWA-6 ranked the highest (=most susceptible). N02 302, Ex Rico (Bunsi), and USWA-6 could have field avoidance to white mold.

Table 1. Comparison of rankings* of 13 bean lines for white mold reaction in 16 tests.

	Greenhouse/Laboratory									Field						
Cultivar	OR	CO	NE	NY	WA	ID	ND	MI[1]	MI[2]	MN	FR	CA	ND	NE	OR	MI
Dwarf Bees	2	1	10	1	1	-	-	-	4	-	-	-	-	-	-	-
Cornell 601	3	2	8	2	3	3	1	3	12	1	2.5	3	5	10	6	3
G122	1	5	12	4	2	7	6	9	9	2	2.5	4	1	1	2.5	7
Cornell 501	5	7	11	3	5	3	3	10	6.5	3	8	7	6	3	2.5	5
AN 37	6	8	2	5	7.5	10	10	5	1	8.5	6	10	3	7	9	9.5
Ex Rico	9	9	3	9.5	6	9	9	6.5	6.5	6	5	2	9.5	5	4	7
N02 302	11	10	9	9.5	13	12	11	11	2	10	10	1	2	2	5	1
IO1892-115M	7	3	5	9.5	4	8	3	1	9	4	7	5.5	4	8	7.5	7
USWA-6	8	6	4	9.5	12	11	-	12	11	8.5	2.5	9	9.5	6	1	3
AN-69	4	4	6	6	10	6	8	4	13	6	11	11	9.5	9	11	3
AN-1	12	11	7	9.5	11	1	6	2	4	11	2.5	5.5	9.5	4	7.5	9.5
CO75944	-	-	-	-	7.5	5	6	8	4	6	9	8	7	12	11	11
Beryl	10	12	1	9.5	9	3	3	6.5	9	12	12	12	12	11	11	12

(-) = No Data ,*Most resistant (1) to most susceptible (12),[1]Michigan straw test and [2]Michigan oxalate test

Straw test, (BIC 39:142), oxalate test, (2000 Crop Sci.40:281), and detached leaf test (BIC 40:140).

Table 2. Mean ranking of bean lines (1=most resistant) for white mold reaction in 16 separate greenhouse and field tests.

Entry	Mean Ranking	t Grouping	Seed Class	Source
Beryl	9.063	A	GN	*Check-Susceptible*
CO75944	7.875	B A	PTO	M. Brick
AN 69	7.594	B A	PTO	P. Miklas
USWA 6	7.533	B A	SR	P. Miklas
N02 302	7.469	B A	NAVY	J. Kelly
AN 1	7.063	B A C	GN	P. Miklas
AN 37	6.719	B D A C	PTO	P. Miklas
Ex Rico	6.625	B D A C	NAVY	*Check-Intermediate*
IO1892-115M	5.781	B D C	BLK	J. Kelly
Cornell 501	5.500	B D E C	SNAP	P. Griffiths
G122	4.688	D E C	CRAN	*Check-Resistant*
Cornell 601	4.219	D E	RK	P. Griffiths
Dwarf Bees	3.167	E	*P. coccineus*	Territorial Seeds

LSD=2.52, Alpha=0.05

Progress in Introgressing White Mold Resistance from the Secondary Gene Pool of Dry Bean

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Only low levels of white mold resistance occur in dry and snap bean (Phaseolus vulgaris L.). In contrast, Phaseolus species in the secondary gene pool possess high levels of resistance that must be introgressed and pyramided into dry and snap bean cultivars for effective control of white mold. Our long-term objectives are to introgress and pyramid high levels of white mold resistance from across Phaseolus species into dry bean cultivars. In this article, we shall briefly describe the progress achieved thus far in introgressing white mold resistance from the three Phaseolus species in the secondary gene pool.

A total of 482 interspecific breeding lines from eight of eleven populations between dry bean 'ICA Pijao' and *P. coccineus* (G 35171 and G 35172), *P. costaricensis* (S 33720), and *P. polyanthus*= *P. dumosus* (G 35877) produced seed in Idaho in 2002. These interspecific breeding lines were introduced from CIAT, Cali, Colombia. These were systematically screened in the field in Idaho and in the greenhouse (using the straw test) in Colorado in 2002 and 2003. While white mold pressure in the greenhouse was adequate, disease pressure in the field was erratic and uneven. Moreover, continued segregation for white mold resistance, plant type, and seed characteristics interfered and slowed down screening of interspecific breeding lines. Nonetheless, based on both field and greenhouse tests, 129 of 482 most promising breeding lines were again screened in the greenhouse in Idaho (using the petiole test) and Colorado (using the straw test) in 2004 (Table 1). Also, a much smaller set of breeding lines was screened in the field at Paterson, Washington in 2004. The field trial in Idaho did not have any white mold pressure. But, Washington nursery had heavy disease pressure that permitted identification of a few breeding lines with moderate to high levels of white mold resistance in the field as well as in the greenhouse (Table 2). In the codes for breeding lines VP10-115 (tested as IS 115), VR11-198 (tested as IS 198), VR12-283 (tested as WM 32), and VC6-507 (tested as WM 60), V=*P. vulgaris*, P=*P. polyanthus*, and R=*P. costaricensis*. Thus, all three *Phaseolus* species in the secondary gene pool seemed to impart white mold resistance, but an objective comparison cannot be made because of an unequal number of breeding lines available from different interspecific populations. Nonetheless, relatively higher proportions of interspecific breeding lines have survived from the backcross with and congruity backcross of ICA Pijao with *P. coccineus* accession G 35172. Similarly, 49% of breeding lines from the first backcross of ICA Pijao with *P. polyanthus* accession G 35877 have survived the three years of greenhouse and field screening for white mold resistance.

All 129 interspecific breeding lines are being evaluated in the greenhouse using more severe white mold pressure (i.e., three sequential inoculations: first and sixth trifoliolate leaves, and first branch) in Idaho. Inoculated plants are scored at 3, 7, and 27 days after inoculation, and near maturity. Highly resistant breeding lines will be screened in greenhouse in Colorado and Idaho, and in the field in Idaho and Washington (and hopefully in North Dakota) to identify white mold resistant interspecific breeding lines for further testing in the National White Mold Nursery and subsequent germplasm release. This project is also involved in determining the inheritance of white mold resistance in P. coccineus accessions PI 433246 and PI 439534; (2)

tagging and mapping resistance genes and QTL, (3) introgressing resistance from PI 433246 and PI 439534, (4) pyramiding resistance, and (5) transferring high levels of resistance into pinto bean.

Table 1. Sequential white mold screening of interspecific breeding lines derived from crosses of dry bean cultivar ICA Pijao and the three *Phaseolus* species in the secondary gene pool in Colorado and Idaho from 2002 to 2004.

Identification	No. in 2002	Sel. No. in 2004	White mold score in greenhouse in 2004			
			Idaho		Colorado	
			Range	Mean	Range	Mean
ICA Pijao/G 35171// ICA Pijao	24	3	4.6 - 5.0	4.8	4.4 - 7.4	5.6
ICA Pijao/G 35171// ICA Pijao///G 35171	54	4	4.8 - 5.1	5.0	3.0 - 5.9	4.4
ICA Pijao/G 35172//G 35172	11	2	4.0 - 4.8	4.4	6.1 - 7.2	6.7
ICA Pijao/G 35172// ICA Pijao	48	19	7.7 - 10	8.6	5.0 - 9.0	6.6
ICA Pijao/G 35172// ICA Pijao///G 35172	31	26	4.5 - 9.2	7.9	4.9 – 8.5	6.4
ICA Pijao// ICA Pijao/G 35877	127	62	1.5 – 9.5	6.6	4.4 - 9.0	6.8
ICA Pijao/S 33720	43	1		5.2		7.1
ICA Pijao// ICA Pijao/S 33720	144	12	2.9 -6.5	5.0	3.0 - 8.6	6.2
Total	**482**	**129**				

Table 2. Mean white mold scores for four interspecific breeding lines derived from crosses of ICA Pijao with *Phaseolus* species in the secondary gene pool screened in greenhouse in Colorado (straw test) and Idaho (petiole test) and in the field at Paterson, Washington in 2004.

Identification	Idaho	Colorado	Washington
VP10-115	3.5	-	5.8
VR11-198	1.7	-	4.3
VR12-283	0.6	3.0	5.7
VC6-507	2.8	6.2	2.7
Checks			
I 9365-25	1.0	5.2	9.0
Othello	3.9	8.7	8.7

FUNGICIDE, ROW WIDTHS AND PLANT DENSITIES AFFECTING WHITE MOLD INTENSITY

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Introduction

White mould caused by *Sclerotinia sclerotiorum* has increased on irrigated bean in the past 10 years in Brazil, especially during the fall-winter growing season. Low temperature, high humidity and plant canopy and/or soil surface wetting favour disease progress. Therefore, wider row and/or plant spacing may provide less favourable environmental conditions due to better light penetration into plant canopy and soil, and more ventilation. The objective of this research was to study the implementation of combined strategies against white mould, which included plant density adjustments and fungicide application.

Material and Methods

Experiments were carried out in Viçosa (State of Minas Gerais, Brazil) in a bean field naturally infested with sclerotia of *S. sclerotiorum*. Seeds of the cultivar 'Pérola' were sown on May (end of fall) of 2002 and 2003. The trials were conducted as a 2x2x2 factorial: two row widths (0.50 or 0.75 m), two plant densities in the rows (6 or 12 plants/m) and two fungicide treatments (with or without application). A randomised complete-block design with six replications was used. Plot size was 15 m² and each row 5 m long. The trials were sprinkler irrigated. The fungicide fluazinam (0.5 l/ha) was applied at 45 (early bloom) and 55 days after emergence (DAE). An area of 1.2 m² of each plot was separately harvested for disease evaluation at 90 DAE. Incidence of white mould was evaluated considering % of plants with symptoms on stem or branches. Plants were rated for severity with a scale from 0 to 4 (Hall & Phillips, 1996). Yield and 100-seeds weight were also evaluated.

Results and Discussion

Disease intensity was higher in 2002 than in 2003. Fungicide reduced disease intensity in both years (Tables 1 and 2), but plant arrangements were only significant in 2002 (Table 1). In 2002, fluazinam also reduced rust severity. Depending on year, larger width row, lower plant density, and fungicide application increased 100-seed weight (Tables 1 and 2). An interaction between row widths and fungicide treatments on yield occurred in 2002 (Table 3). When fungicide was applied, bean yield was higher at 0.50 m (3018 kg/ha) than at 0.75 m (2650 kg/ha). When fungicide was not applied, there was no significant difference between row widths. In 2003, fungicide did not increase yield significantly. Results show that lower plant density (6 plants per meter) does not decrease bean yield, regardless of white mold intensity, and that reduction of disease intensity by fluazinam does not mean higher yield in years not favourable for white mold. Larger row width could be used in areas where disease is not serious or no fungicide is used.

Table 1. White mold intensity, rust severity, 100-seed weight and grain yield at two row widths, two plant densities, and with or without fluazinam (Viçosa, Brazil, 2002)

Row width	Density	Fungicide	White mold incidence[1]	White mold severity	Rust severity[2]	100-seed weight (g)	Yield (kg/ha)
50			46.1** (51.9)	1.54**	2.00*	25.5*	2599
75			34.0 (34.6)	0.94	2.25	26.3	2428
	6		34.9* (35.6)	0.95**	2.19^{ns}	26.1^{ns}	2572^{ns}
	12		45.2 (50.9)	1.54	2.06	25.7	2455
		With	29.3** (26.8)	0.66**	1.33**	26.9**	2834
		Without	50.8 (59.7)	1.83	2.92	25.0	2193

[1] Between parenthesis are untransformed mean percentage of incidence.
[2] 1 = no symptoms, 3 = low severity.
** = significant at 1% level, * = significant at 5% level, ns = not significant.

Table 2. White mold intensity, 100-seed weight and grain yield at two row widts, two plant densities, and with or without fluazinam (Viçosa, Brazil, 2003)

Row width	Density	Fungicide	White mold incidence[1]	White mold severity	100-seed weight (g)	Yield (kg/ha)
50			41.3^{ns} (43.6)	0.79^{ns}	26.1**	2251^{ns}
75			46.5 (52.4)	1.02	27.8	2067
	6		44.1^{ns} (48.3)	0.80^{ns}	28.1**	2131^{ns}
	12		43.7 (47.7)	1.01	25.8	2188
		With	39.6** (40.9)	0.61**	27.4^{ns}	2192^{ns}
		Without	48.3 (55.1)	1.20	26.5	2126

[1] Between parenthesis are untransformed mean percentage of incidence.
** = significant at 1% level, * = significant at 5% level, ns = not significant.

Table 3. Interaction between row width and fungicide treatments on bean yield (kg/ha) (Viçosa, Brazil, 2002)

Row width	Fungicide		Difference
	with	without	
0.50 m	3018	2180	838**
0.75 m	2650	2206	444**
Difference	368**	26^{ns}	
C.V. (%)	12.2		

** = significant at 1% level, ns = not significant.

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IDENTIFICATION OF NEW SOURCES OF RESISTANCE TO WEB BLIGHT OF COMMON BEAN

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Web blight, caused by the fungus *Rhizoctonia solani* Kühn is an important disease of common bean (*Phaseolus vulgaris* L.) in the tropical lowlands of Latin America and Africa where high temperatures and abundant rainfall prevail (Gálvez, et al., 1989). Only moderate levels of resistance to web blight are currently found in common bean. A portion of what appears to be web blight resistance in common bean may be disease escape due to erect architecture and an open leaf canopy. In Honduras, web blight is also found at higher altitudes (> 1000 m) where wild and cultivated scarlet runner bean (*P. coccineus* L.) populations exist. Due to this disease pressure, it was postulated that some *P. coccineus* and *P. polyanthus* accessions may serve as sources of resistance to web blight.

Ninety-nine accessions of *P. coccineus* and *P. polyanthus* from the CIAT bean germplasm collection[1] were screened for web blight reaction using a detached-leaf inoculation technique. This screening technique was used to identify accessions with physiological resistance to web blight rather than traits associated with disease escape. The AG 1-1B isolate, *R. solani* 12, from the fungus collection maintained at the University of Puerto Rico Mayagüez Campus was used to inoculate the detached leaves. The first and third fully-expanded trifoliates of the *P. coccineus* and *P. polyanthus* plants and the susceptible check 'Morales' were cut and the petioles were placed into orchid tubes filled with distilled water. Under aseptic conditions, agar discs (4 mm diameter) were cut from the edges of 4-day-old cultures of *R. solani*. The discs were placed at random on two of the three leaflets of the *P. coccineus* and *P. polyanthus* accessions and on the susceptible check. Immediately after inoculation, the trays were placed inside plastic bags to maintain high humidity and maintained under constant light at temperatures ranging from 26 to 30ºC. The degree of leaf damage was evaluated at 24, 48 and 72 h after inoculation according to the CIAT scale where: 1 = no visible symptoms, 2 = < 5% leaf area affected, 3 = 6-10% leaf area affected, 4 = 11-20% leaf area affected, 5 = 21-30% leaf area affected, 6 = 31-40% leaf area affected, 7 = 41-60% leaf area affected, 8 = 61-85% leaf area affected and 9 = > 85% leaf area affected (Schoonhoven and Pastor-Corrales, 1991).

All of the *P. polyanthus* accessions and most of the *P. coccineus* lines were susceptible to web blight with mean leaf damage scores ≥ 7 at 48 hours after inoculation. G 35163 was the most resistant *P. coccineus* accession, with less leaf damage and smaller lesion size than the susceptible *P. vulgaris* check 'Morales'. Mean leaf damage scores of the first trifoliate of G 35163 were 1 at 24 and 48 hours after inoculation and 3 after 72 hours. The mean leaf damage scores of the third trifoliate of G 35163 was 3 at 48 hours after inoculation and 6 at 72 hours.

When G35163 was evaluated in a second experiment using the same inoculation technique, mean leaf damage scores were 1.8 and 2.3 at 60 and 72 hours after inoculation, respectively. The *P. coccineus* accessions G 35007 and G 35066 expressed moderate levels of resistance to web blight. G35007 had mean leaf damage scores of 5.0 and 6.8 at 72 hours after inoculation for the first and third trifoliates, respectively. G35066 had mean leaf damage scores of 5.0 for the first trifoliate and 6.3 for the third trifoliate at 72 hours after inoculation. Takegami and Beaver (2000) evaluated the web blight reaction of another group of *P. coccineus* accessions from the CIAT bean germplasm collection in the field. Although the accessions had a prostrate growth habit, the web blight scores at 15 days after inoculation were 2.5 for G 35006 and G 35066. When evaluated using the detached-leaf inoculation technique, both lines had mean scores of 2.5 at 60 h after inoculation. In 2000, F.H. Ferwerda at the University of Florida made an interspecific cross between 5-593 and G 35006 and another interspecific cross between 'ICA Pijao' and G 35006. During the summer of 2004, 43 $F_{3:4}$ lines from these crosses were evaluated in the field at Isabela, Puerto Rico for reaction to web blight. The plots were inoculated twice with the AG 1-1B isolate of *R. solani* using techniques described by Takegami and Beaver (2000). Twenty-five of the lines had web blight scores ≤ 2 at 14 days after the second inoculation whereas the susceptible check Morales had a mean score of 5. No further readings were made because tropical storm Jeanne destroyed the nursery. In Honduras in 2001, F_2 plants from the interspecific crosses were crossed with the common bean cultivars 'Tío Canela 75' and 'Amadeus 77'. In 2003, F_3 lines derived from these crosses were screened in the field in Jamastrán, Honduras for web blight reaction. Several lines in the trial had low levels of web blight infection with mean scores < 3.0. These lines have been distributed to cooperators in the Central American region to test their resistance to different isolates of the web blight pathogen.

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Characterization of virulence diversity of the bean rust pathogen *Uromyces appendiculatus* in wild bean populations as a tool for effective resistance gene deployment

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Bean rust is a limiting factor for bean production in tropical and subtropical regions. The bean rust pathogen, *Uromyces appendiculatus,* is characterized by high diversity of virulence phenotypes in both natural and agricultural systems. High virulence diversity of the bean rust pathogen in Honduras supports the hypothesis that this area is a center of diversity for this fungal pathogen. Also, the occurrence of commercial varieties, landraces and wild beans growing in close proximity make this an appropriate region to study the host–pathogen interaction. Understanding virulence variability in natural populations can give insights about the origin and evolution of pathogen virulence. Virulence diversity in plant pathogen populations is a major constraint in the management of plant diseases, especially using host resistance, which is the most economic and environmentally sustainable disease management strategy.

During 2002 and 2003 rust infected leaves were collected from wild beans including *Phaseolus vulgaris, P. coccineus and P. augusti* in Honduras. Samples areas were selected based on the CIAT GIS map for wild bean probability (Beebe., *et al.,* 1997). Field samples were increased on bean rust susceptible lines then inoculated into the 12 bean rust differentials and Pinto UI 114 as the susceptible control. The 12 bean differentials consist of 6 Andean and 6 Middle American bean rust resistance sources (Steadman *et al*., 2002). After incubation in a mist chamber for 24 hours, the differentials were placed in a greenhouse fabric enclosed chamber until pustules were visible but before the epidermis ruptured to release spores. Single pustules were collected and re-inoculate on primary leaves. Single pustule isolates representing all the diversity of pustule sizes in each differential were increased onto the primary leaves of selected bean rust differential lines. Disease reaction of each pathotype was determined 14 days after inoculation of the 12 differentials using uredinium size (J.R. Stavely *et al.,* 1983).

A total of 289 isolates were characterized and 77 pathotypes were determined on the 12 differentials. Of the 77 pathotypes, 44 were unique to one isolate. The most common pathotype was virulent in 9 of the 12 differentials with Aurora (*Ur-3*), Mex 235 (*Ur-3*$^+$) and PI 181996 (*Ur-11*) showing resistance. Considering all pathotypes, 12% (6.5 % of all isolates) were virulent on *Ur-11* and 15% (11% of all isolates) were virulent on *Ur-3*$^+$ (Table 1a). However, only two pathotypes (less than 1% of all isolates) were virulent on both *Ur-3*$^+$ and *Ur-11*. Of the 77 pathotypes identified, nine were virulent on PI 181996 (*Ur-11*) (Table 1b).

Development of resistant bean varieties can provide a low cost disease management option for both large and small landholders. Based on the results from Honduras, bean breeding programs for Honduras and Middle America should incorporate the resistance source present in Mex 235 (*Ur-3*$^+$) to the elite lines. The *Ur-3*$^+$ gene pyramided with other sources of resistance specifically *Ur-11* can provide a broader and more durable resistance for Honduras and other areas in Middle America and the Caribbean. Preliminary data from samples from *U. appendiculatus* isolates collected from Guatemala and the Dominican Republic during 2004 and 2005, showed the presence of rust isolates that are virulent on Ur-*11*, an important source of resistance for Latin America and the Caribbean.

Table 1a. Reaction of *Uromyces appendiculatus* pathotypes from Honduras on the 12 bean rust differential lines.

Rust Differential Line	Host Gene Pool	Rust Resistance Genes	% of pathotypes with a virulent reaction
Early Gallatin (EG)	A*	*Ur-4*	75
Redlands Pioneer (RP)	A	Unknown	63
Montcalm (Mo)	A	Unknown	94
PC 50	A	*Ur-9, Ur-12*	71
Golden Gate Wax (GGW)	A	*Ur-6*	92
PI 260418	A	Unknown	82
GN 1140	M	*Ur-7*	83
Aurora (AU)	M	*Ur-3*	36
Mex 309	M	*Ur-5*	70
Mex 235	M	*Ur-3*$^{+}$	15
CNC	M	Unknown	58
PI 181996	M	*Ur-11*	11

*A= Andean; M= Middle American

Table 1b. Virulence pattern of *U. appendiculatus* pathotypes that are virulent on the resistance gene *Ur-11*.

Pathotype		**Rust Differential Lines**											
	Number of isolates	EG	RP	Mo	PC 50	GGW	PI 260418	GN 1140	AU	Mex 309	Mex 235	CNC	PI 181996
1	4	S*	S	S	S	S	S	S	S	S	R	S	**S**
2	2	R	S	S	S	S	S	S	R	S	R	S	**S**
3	7	S	S	S	S	S	S	S	R	S	R	S	**S**
4	1	S	S	S	S	S	S	S	R	R	R	S	**S**
5	1	R	R	S	R	S	S	S	R	S	R	S	**S**
6	1	S	S	S	S	S	S	S	R	S	S	R	**S**
7	1	R	S	S	R	S	S	S	S	S	S	S	**S**
8	1	R	S	S	R	S	R	S	R	S	R	S	**S**
9	1	S	S	S	R	S	S	S	R	S	R	R	**S**

* R= uredinia absent or <300µm; S=uredinia>300µm

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INHERITANCE OF RESISTANCE IN PI 260418 AN ANDEAN BEAN RESISTANT TO MOST RACES OF THE BEAN RUST PATHOGEN

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Introduction

Rust, caused by *Uromyces appendiculatus*, is a major disease of the common bean (*Phaseolus vulgaris*) in humid and moderately humid areas of the world (4). Host resistance is a very effective strategy for the control of bean rust but the achievement of durable resistance over time and space is complicated by the high virulence diversity of the rust pathogen (2, 4). Many races (different biotypes) of the rust pathogen are usually found in a single bean field. The diversity of these races often differs from one location or year to another. Recent research shows that the races of this pathogen segregate in two different groups, one Andean and another Middle American, that mirror the diversity of their common bean host. Andean races of the pathogen are compatible (cause rust) only with Andean beans that originated in the highlands of South America. On the other hand, Middle American races are compatible with Andean and also Middle American beans from Central America and Mexico.

Although several rust resistance genes have been identified in common bean, most of these genes are present in beans of the Middle American gene pool (1). These include *Ur-3*, *Ur-5*, *Ur-7*, and *Ur-11* and others genes that have not yet being characterized or named. Rust resistance genes from Andean beans include *Ur-4* and *Ur-6*. Inoculations under greenhouse conditions of various sources of rust resistance with individual races of the rust pathogen show that rust resistance genes from Middle American beans are usually effective against a greater number of races than those from Andean beans. Middle American genes *Ur-5* and *Ur-11* are resistant to 70 and 89 races respectively, of the 90 races maintained at Beltsville, while the Andean genes *Ur-4* and *Ur-6* are resistant only to 44 and 22 races respectively, of these same races (2). Recently, an Andean plant introduction (PI) 260418 was shown to be resistant to all but one of the races of the rust pathogen maintained at Beltsville. PI 260418 is susceptible only to race 84 obtained from an Andean kidney bean in Colorado. PI 260418 is also resistant to race 108, the only known race in the Beltsville collection that overcomes the resistance of *Ur-11*. The Objective of this research was to elucidate the genetics of resistance of PI 260418 to the bean rust pathogen.

Materials and Methods

Population development. PI 260418 was crossed to Pinto 114, a Middle American bean that is susceptible to all but two races of the rust pathogen maintained at Beltsville. Pinto 114, generally used as the female parent, was crossed to PI 260418 to generate reciprocal F_1, F_2 populations and the backcross populations - F_1 crossed to the resistant and susceptible parents respectively.

Inoculations and Evaluations. Parents and check bean cultivars such as Aurora (*Ur-3*), Early Gallatin, (*Ur-4*), Mexico 309 (*Ur-5*), Golden Gate Wax (*Ur-6*), PI 1818996 (*Ur-11*) and others, were used in all inoculations. The parents, F_1, F_2, backcross populations and check cultivars were inoculated with various races of the rust pathogen using procedures already published (3). A total of 105 F_2 plants (from population 2-3761) were inoculated with eight Middle American races: 41, 44, 47, 49, 53, 67, 73, and 108 and 120 F_2 plants (from population 2-3773) with Middle American races 63 and 85. Also120 F_2 plants (from 2-3801, 2-3804, and 2- 3805 populations) were inoculated with the four Andean races: 38, 72, 89, and 105. Inoculated plants were placed

in inoculation chambers at 20^0 C at 100 % relative humidity for about 18 hours. Disease evaluations were conducted 14 days after inoculation using established grading scales.

Results and Discussion

The susceptible reaction of Pinto 114 was characterized by large (0.3-0.8 mm in diameter) and very large (larger than 0.8 mm in diameter) rust pustules. The resistant PI 260418 parent had tiny pustules measuring less than 0.3 mm in diameter. The F_1, F_2 and backcross generations exhibited a susceptible (S) reaction like Pinto 114 or a resistant (R) reaction like PI 260418. A summary of the results for of the parental genotypes, F_1, F_2, and backcross generations with 14 races of the rust pathogen is reported in Table 1. The results from the various generations evaluated indicate that the resistance in PI 260418 to the 14 races of the bean rust pathogen used in this study is controlled by a dominant allele at a single locus. However, preliminary results, not shown here, indicate that the resistance in PI 260418 to other races of the bean pathogen may be controlled by two additional unlinked loci.

Table 1. Reaction of susceptible Pinto 114 and resistant PI 260418 *Phaseolus vulgaris* genotypes and derived populations to inoculations with races Middle American races 41, 44, 47, 49, 53, 67, 73, and 108 and Andean race 38, 72, 89, 98, 99, and 105 of *Uromyces appendiculatus*, the bean rust pathogen.

Genotype	Number of plants		Expected	
	R	S	Ratio (R:S)	P value
Pinto 114	0	50	0:1	-
PI 260418	50	0	1:0	-
(P.114 x PI 260) F_1	15	0	1:0	-
(P.114 x PI 260) F_2	82	23	3:1	0.38
(P.114 x PI 260) x P.114	41	42	1:1	0.95
(P.114 x PI 260) x PI 260	70	1	1:0	-

Symptom expression was measured 14 days after inoculation. The susceptible Pinto 114 parent had large (0.5-0.8 mm) and very large (larger than 0.8mm in diameter) pustules while the resistant PI 260418 parent had tiny pustules measuring less than 0.3 mm in diameter. Symptom expression in the F_1, F_2 and backcross generations were either a susceptible (S) reaction like Pinto 114 or a resistant (R) reaction like PI 260418.

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ALTERNATIVE INOCULATION METHOD FOR EVALUATING COMMON BEAN REACTION TO *Uromyces appendiculatus*

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Introduction – The pyramiding of resistance genes (R) to overcome diseases incited by different pathogens has been proposed as a strategy to obtain wide spectrum durable resistance. However, this procedure is difficult to accomplish when conventional inoculation techniques are used to evaluate the plant-pathogen interaction (Faleiro et al., 2003). Conventional inoculation methods, based on multiple or sequential inoculations, do not allow the distinction of the symptoms caused by each pathogen, preventing the identification of different R genes (TU, 1986; Faleiro et al., 2003). DNA markers have been used to aid the pyramiding of different resistance genes. However, in many cases markers for these genes are not readily available. In the present work we tested an alternative inoculation method with *Uromyces appendiculatus*, causal agent of rust in common bean (*Phaseolus vulgaris* L.), using excised leaves. This method would give more flexibility to breeding programs using the gene pyramiding strategy and in addition would represent a fast and straightforward way to monitor bean growing regions for the presence of *U. appendiculatus* pathotypes.

Material and Methods – The rust resistance sources cv. Golden Gate Wax (gene *Ur-6*) and Ouro Negro (gene *Ur-ON*) were used as genitors to obtain F_2 populations to be evaluated for rust resistance/susceptibility to *U. appendiculatus* pathotype 10 (Faleiro et al.,1999). This pathotype 10 is incompatible with Ouro Negro and compatible with Golden Gate Wax. Two hundred and seventeen F_2 plants and four plants each of cultivars Golden Gate Wax, Ouro Negro and US Pinto 111 (susceptible control) were evaluated. For the alternative methodology which was based on Tu (1986), the cotyledonary leaves were excised when they reached approximately two-thirds of their full development, ca. ten days after sowing. The detached leaves were inoculated by immersion into a suspension containing spores of *U. appendiculatus* pathotype 10 (2.0×10^4 spores/mL). Then each leaf was placed in a petri dish (90 x 15 mm) over a filter paper previously moistened with 3.0 mL of fresh distilled water. The dishes were incubated in a BOD incubator at 20°C, under a 12 hour daily light regime (Phillips® TLT 20W/75RS) at about 28 μmoles $m^{-2}s^{-1}$. Three days after inoculation each filter paper was moistened again with 1.5 mL of fresh distilled water. For the conventional pathogenicity assays, the remaining cotyledonary leaves attached to the plants were sprayed on both surfaces with a suspension of pathotype 10 spores (2.0×10^4 spores/mL) with the aid of a De Vilbiss n° 15 atomizer powered by an electric compressor (Carrijo et al., 1980). The plants were transferred to a mist chamber (20 ± 1°C and >95% relative humidity) where they were kept for 2 days, under a 12 hour daily light regime. In both inoculation procedures, symptom evaluation was based on a 1-to-6 symptom scale (Stavely et al., 1983). Plants with reaction degrees 3 or lower were considered resistant and those with degrees 4 or higher, susceptible. The data obtained in the evaluations with the two methods were submitted to the chi-square test to determine the efficiency of both methods to detect the segregation of resistance gene *Ur-ON* in the F_2 population (Table 1).

Results and Discussion – Both methods of inoculation tested were efficient to evaluate the reaction of the common bean to *U. appendiculatus* pathotype 10. Cultivars Ouro Negro and Golden Gate Wax (Figure 1), and control cultivar US Pinto 111 (data not shown) showed the same scores in both methods. Segregation analyses of the F_2 population by the two methods confirmed the dominant nature of the rust

resistance gene *Ur-ON* present in cultivar Ouro Negro (Table 1). The only difference observed on the results obtained by the two methods was that the number of pustules on the leaves inoculated by the conventional method was higher (Figure 1). This result suggests that the conventional inoculation method is more appropriate for spore multiplication. The excised leaf method can be used in common bean breeding programs where one plant needs to be assayed several times. In this case, the tested plants are still able to produce healthy seeds. Another advantage of this procedure refers to costs and safety, the whole method can be conducted in the laboratory without the need of exposing other plants to the pathogen. Our results show that both inoculation methods tested can be used to evaluate common bean rust symptoms with similar precision.

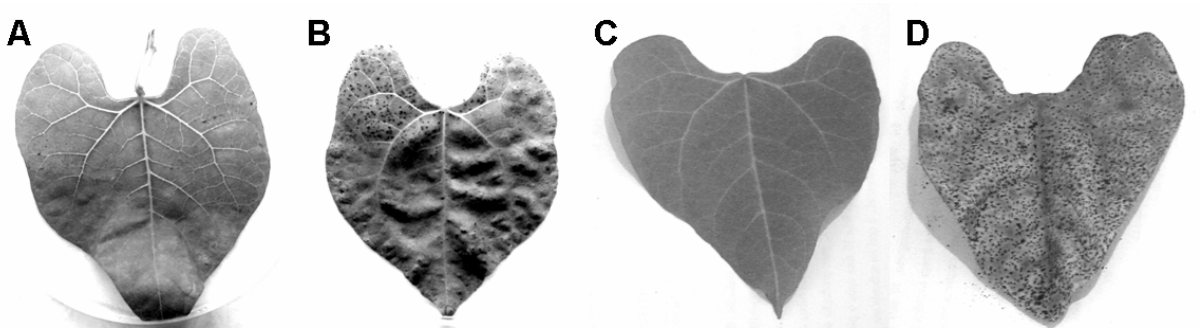

Figure 1. Rust symptoms observed on leaves of cultivars Ouro Negro (**A** and **C**) and Golden Gate Wax (**B** and **D**) inoculated by the excised leaf (**A** and **B**) and conventional methods (**C** and **D**).

Table 1. Rust resistance/susceptibility of F_2 plants derived from a cross between Ouro Negro and Golden Gate Wax, inoculated by *U. appendiculatus* pathotype 10 using the excised leaf and conventional inoculation methods.

Method	Expected ratio[a]	Observed ratio	χ^2	P-value[b]
Excised leaf	3:1	161:56	0.0752	78.38%
Conventional	3:1	165:52	0.1244	72.43%

[a]3:1 (R_:rr), expected ratio for segregation of a single dominant resistance gene; [b]Probability value.

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DEVELOPMENT OF "CARIOCA-TYPE" COMMON BEAN LINES RESISTANT TO RUST WITH THE AID OF MOLECULAR MARKERS

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For many years the BIOAGRO/UFV common bean breeding program has used the cultivar Ouro Negro as the only rust resistance source. However, new sources have been tested and characterized. Mexico 309 and Belmidak RR-3 have been shown to present a wide resistance spectrum to pathotypes of *Uromyces appendiculatus*, rust common bean causal agent, in Brazil. Allelism studies involving these three cultivars showed that Ouro Negro harbors a distinct resistance gene from those present in Mexico 309 (gene *Ur-5*) and Belmidak RR-3 (gene *Ur-11*) (Alzate-Marin et al., 2004). The main goal of this work was to develop advanced common bean lines with "carioca-type" grains genetically close to the recurrent parent "Rudá" and containing the rust resistant genes *Ur-5* and *Ur-11*.

Two populations were obtained from the crosses Rudá vs. Mexico 309 and Rudá vs. Belmidak RR-3. The hybrid nature of the F_1 seeds was confirmed by the flower color, when possible, or by molecular markers according to Alzate-Marin et al. (1996). The F_1 plants were backcrossed to Rudá and in each backcross generation BC_nF_1 plants were inoculated with a spore suspension of a mixture of races of *U. appendiculatus* (2.0×10^4 spores/mL). The plants were then incubated for two days in a mist chamber kept at 20-22^{o}C and 100% relative humidity (Carrijo et al., 1980). Fourteen days after inoculation the plants were scored visually for the disease symptoms using a 1-to-6 scale (Stavely et al., 1983). Leaf DNA was extracted from the progenitors and from the resistant BC_nF_1 plants by a mini-prep procedure based on Williams et al. (1990). Amplification reactions were according to Vasconcelos et al. (1996) using different RAPD primer sets in each backcross cycle. Relative genetic similarities and cluster analyses were determined with the aid of the Genes program (Cruz, 1997).

Plants, which were genetically closer to the recurrent parent, were used in the next backcrossing cycle. The relative genetic similarities in relation to the recurrent parent varied between 80.8% and 98.2% for BC_3F_1 resistant plants (Figures 1 and 2). After only three backcrosses, two plants with genetic similarities of 97.6 and 98.1 (harboring gene *Ur-5*) and four plants with genetic similarities of 97.4 and 98.2 (harboring gene *Ur-11*) in relation to the recurrent parent were obtained (Figures 1 and 2). The seeds of these plants were sown and the resulting plants were selfed for obtaining homozygous lines for both genes *Ur-5* and *Ur-11*. This breeding strategy using molecular fingerprints considerably decreased the time normally required to recover the genome of the recurrent progenitor and will allow the fast pyramiding of these genes into lines developed by the BIOAGRO/UFV breeding program, which already contain the Ouro Negro rust resistance gene, and genes conferring resistance to anthracnose and angular leaf spot.

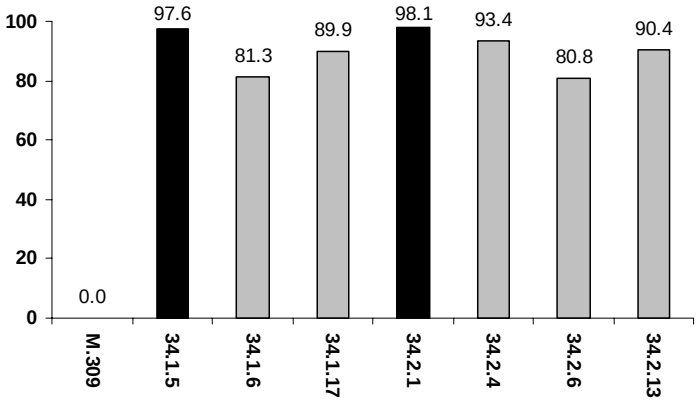

Figure 1. Relative genetic similarities (%) between BC_3F_1 plants (34.1.5 to 34.2.13) and Mexico 309 (M.309) in relation to the recurrent parent Rudá. The black columns represent plants that were closer to Rudá.

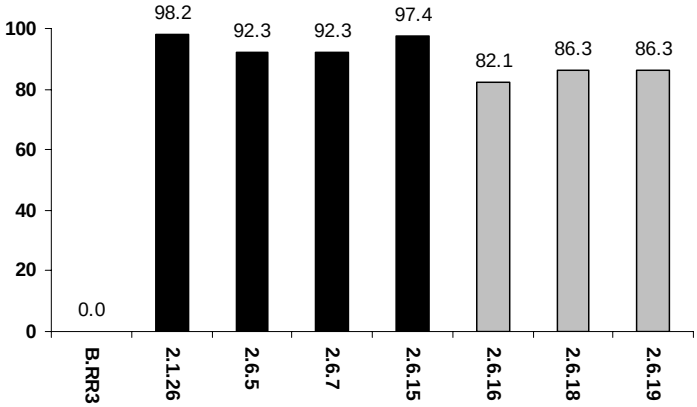

Figure 2. Relative genetic similarities (%) between BC_3F_1 plants (2.1.26 to 2.6.19) and Belmidak RR-3 (B.RR3) in relation to the recurrent parent Rudá. The black columns represent plants that were closer to Rudá.

Acknowledgement: This work was supported by grants from CNPq and FAPEMIG. Thiago Lívio P. O. de Souza was supported by an M.S. scholarship from CNPq.

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Water relations responses to salinity during early vegetative stage in common bean (*Phaseolus vulgaris* L.)

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Physiological processes such as water status are highly sensitive to salinity and are, therefore, dominant in determining the plant's response to stress. Water stress induced by salinity may influence plant growth by adverse effects on dry matter partitioning, cell extension, cell division, leaf photosynthesis and/or transpiration (Munns, 2003). The objectives of this study was to understand the salt-stress-induced mechanisms, at the whole plant level, that cause growth reduction by analyzing how salinity affects plant water relations in *P. vulgaris*.

MATERIALS AND METHODS

Two wild (PI325687 and G11024) and two cultivated genotypes (G4017 and G21981) of common bean were used. Plants were grown in nutrient solution under greenhouse conditions at Universidad Michoacana de San Nicolás de Hidalgo, Mexico between May and August 2004. Seedlings were allowed to grow with no salinity stress until the emergence of the first trifoliate leaf, when several NaCl treatments were added to the solutions (0, 30, 60 and 90 mM). A randomized complete block design with a split-plot arrangement of salt treatments and four replications was used. Predawn water potential (Ψ_w) at 9, 14 and 19 days after transplanting was measured with a pressure chamber. Leaf solute potential (Ψ_π) was measured with a Wescor-5500 vapor pressure osmometer. Readings were converted to pressure units by using the van't Hoff equation. Turgor potential (Ψ_p) was determined using the relationship: $\Psi_p = \Psi_w - \Psi_\pi$. Plants were harvested at 10, 15 and 20 days after transplanting and separated into roots, stem and leaves. Data were analyzed using the GLM procedure (SAS Institute, Cary, NC, 1985). Four replicates per salinity treatment per species per harvesting date were used for growth analyses and water relations. Two-way analysis of variance was used to determine significant differences among accessions for various traits. Treatment means were compared using protected LSD test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Salinity significantly affected leaf water, osmotic and water potentials (Fig. 1). Differences among cultivated and wild accessions were significant at any salt concentration, except for osmotic potential. Overall, wild accessions (PI325687, G11024) had less negative values of water potential at 0, 30 and 60 mM NaCl than cultivated accessions. At 90 mM NaCl cultivated G4017 and G21981 had significantly lower water potential than wild accessions. Osmotic potential was unaffected by increased salinity. Osmotic potentials of all accessions ranged between –0.82 to –1.24 MPa at the same salt concentration. Leaf turgor potential was unaffected by 30 and 60 mM NaCl, but was increased between 0.26 and 0.48 MPa at 90 mM NaCl. Leaf water potential (Ψ_w) gradually declined during the first 14 days after salinization (-0.47 to –0.78 MPa), thereafter, a steady state was attained, and except at 90 mM NaCl, which decreased Ψ_w further. Salinity also decreased leaf osmotic potential. This difference was reflected in average turgor potentials which increased at 90 mM NaCl. Water status is highly

sensitive to salinity and is, therefore, dominant in determining the plant responses to stress. Our data indicate that the decrease in leaf osmotic potential always exceeded that of leaf water potential. As turgor potential was maintained or enhanced by salinity, osmotic adjustment was maintained. Although there is not a rigorous mechanistic analysis of salt tolerance in these species, it appears that salt tolerance in *P. vulgaris* is associated a better stomatal control through osmotic adjustment. This generalization appears to hold for *Phaseolus* species because of accumulation of high levels of inorganic ions, predominantly Cl^-, Na^+ and K^+ in their leaves (Bayuelo-Jiménez et al., 2003). In these leaves, the osmotic potential decreased as the concentration of total inorganic ions rose. However, higher inorganic ion concentrations could also lead to problems of ion compartmentation and a decline in leaf function.

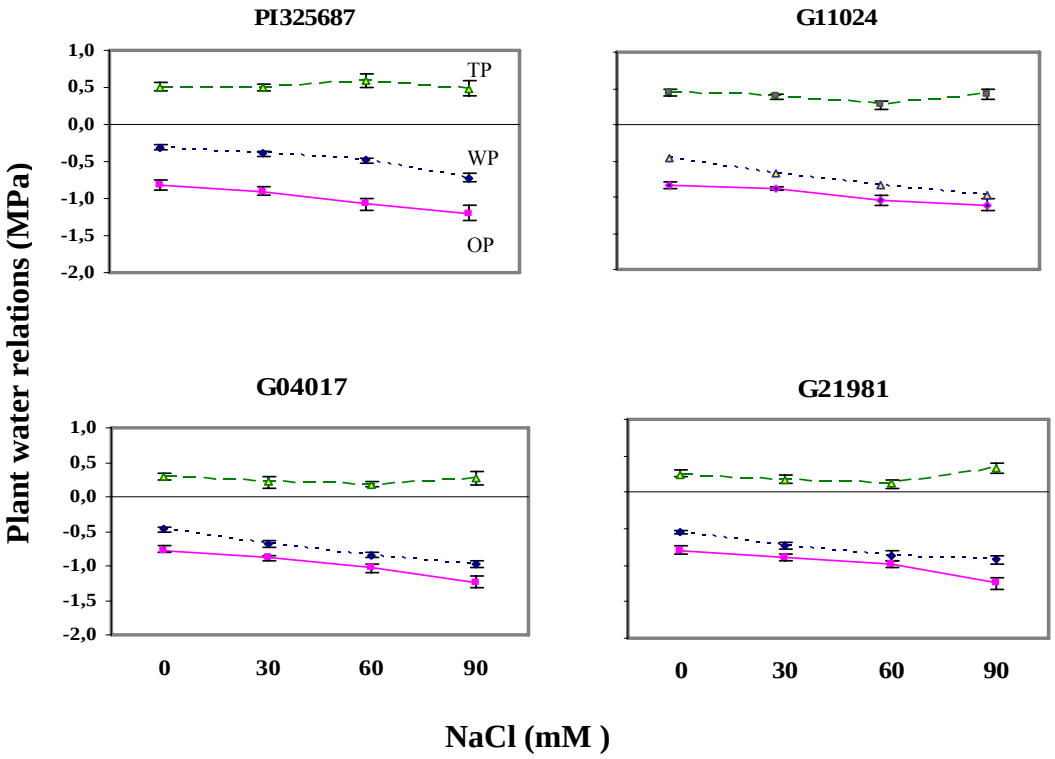

Fig. 1. Effects of increasing NaCl concentrations in the growth medium on leaf water potential: (…..) WP; osmotic potential, OP (—); turgor potential, TP (---) (in MPa) of *Phaseolus* species. Data correspond to the average of four measurements on different individual plants. Standard errors, when larger than symbols, are shown as vertical bars.

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Growth and partitioning responses to salinity during early vegetative stage in common bean (*Phaseolus vulgaris* L.)

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Common bean (*Phaseolus vulgaris* L.) is extremely sensitive to salinity and suffers yield losses at soil salinity less than 2 dS m^{-1} (Läuchli, 1984). Although there are several studies demonstrating the effects of salinity on bean growth, there is limited genetic variation in cultivated bean germplasm for salinity tolerance (Moreno et al., 2000). Wild accessions of *P. vulgaris*, however, showed a wide range of variation in their salinity tolerance during vegetative growth (Bayuelo-Jiménez et al., 2003). The objectives of this study was to understand the salt-stress-induced mechanisms, at the whole plant level, that cause growth reduction by analyzing how salinity affects relative growth rate and its components in *P. vulgaris*.

MATERIALS AND METHODS

Two wild (PI325687 and G11024) and two cultivated genotypes (G4017 and G21981), representing *P. vulgaris* L. were used. Plants were grown in nutrient solution in a greenhouse at Universidad Michoacana de San Nicolás de Hidalgo, Mexico between May and August 2004. The plants were grown in a control solution until the emergence of the first trifoliate leaf, at which time several concentrations of NaCl were added to the solutions (0, 30, 60 and 90 mM). A randomized complete block design with a split-plot arrangement of salt treatments was used. Plants were harvested at 10, 15 and 20 days after transplanting and separated into roots, stem and leaves. Absolute growth (actual dry matter produced) and relative growth were determined. Growth parameters such as mean relative growth rate RGR (g g^{-1} d^{-1}), unit leaf rate on a leaf area basis, ULR (g m^{-2} d^{-1}), leaf area ratio, LAR (m^{-2} g^{-1}), leaf weight ratio, LWR (g g^{-1}), and leaf weight ratio, LWR (g g^{-1}), were calculated. Data were analyzed using the GLM procedure (SAS Institute, Cary, NC, 1985). Four replicates per salinity treatment per species per harvesting date were used for growth analyses. Two-way analysis of variance was used to determine significant differences among accessions for various traits. Protected LSD test at $P \leq 0.05$ was calculated for comparison of individual means

RESULTS AND DISCUSSION

Salinity significantly reduced RGR, ULR, LAR (Fig. 1a, 1b, 1c), and SLA whereas LWR showed no definite trend (data not shown). In all accessions, except in the cultivated G21981 accession, ULR, but not LAR, was significantly correlated with RGR, indicating that ULR was an important factor underlying the salinity-induced differences in RGR among accessions. In the cultivated G21981 accession, high salinity reduced SLA, and consequently LAR. The significant correlation of SLA and LAR with RGR suggested that the growth components reflecting leaf area extension were the primary factors explaining the inhibition of growth in this accession. Salt-induced growth reductions in most *Phaseolus* accessions during vegetative growth were due to a decrease in the specific activity of the leaves (ULR). On the other hand, a reduced leaf area expansion per unit of plant biomass (LAR), primarily caused by a decrease in SLA, played an

important role in determining RGR of salt-stressed G21981. The lower ULR of salt-stressed plants may be the result of decreased photosynthesis due to stomatal closure.

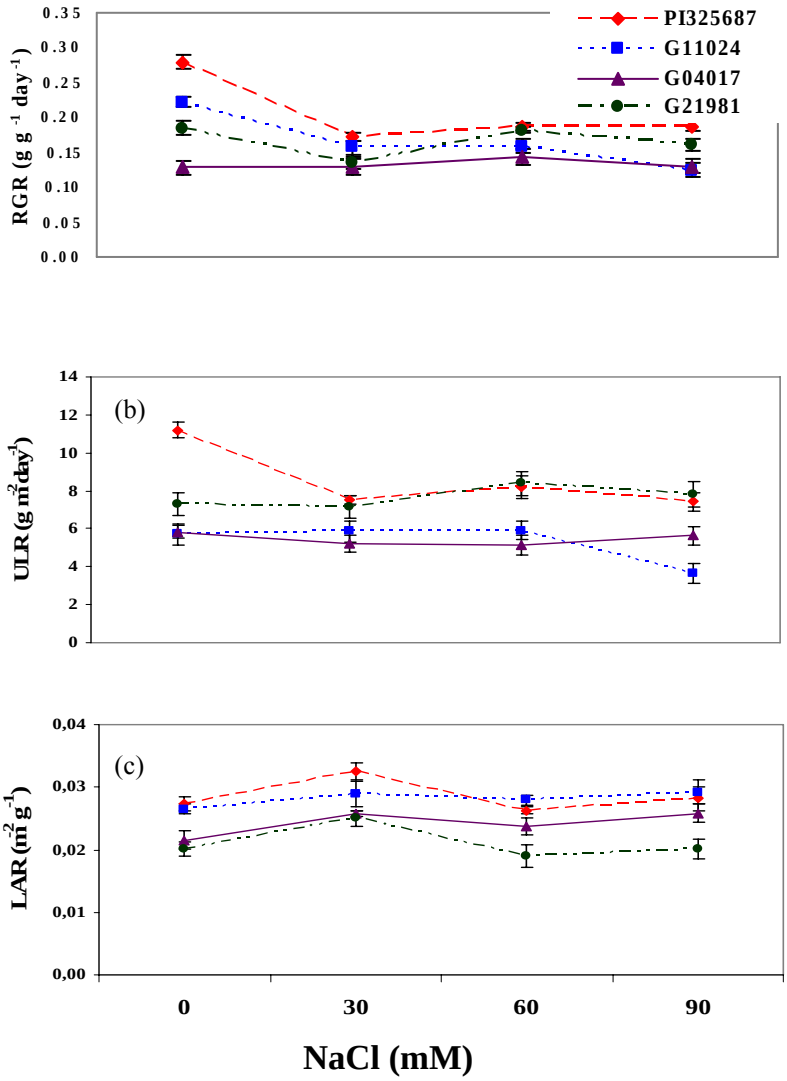

Fig. 1. Effects of external NaCl on relative growth rate (RGR) (a); unit leaf rate (ULR) (b); and leaf area ratio (LAR) (c) of *Phaseolus* species following a 20-day exposure to NaCl. Each point represents the mean of four measurements on different plants. Standard errors, when larger than symbols, are shown as vertical bars.

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Drought Resistance, Water Use Efficiency and Nutrient Uptake by Old and New Dry Bean Cultivars

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Introduction

Moderate to severe drought with increased precipitation deficit has been observed throughout the western United States in the recent years (Cook et al., 2004). In dry beans, drought stress reduces biomass, seed yield and harvest index (Ramirez-Vallejo and Kelly, 1998; Terán and Singh, 2002). Water use efficiency (WUE) was positively correlated with grain yield and harvest index and negatively correlated with drought susceptibility index (DSI) in wheat (Solomon and Labuschagne, 2003). The effect of drought-stress on nutrient uptake by dry bean cultivars is not known with few exceptions. Our objectives were to determine response of old and new dry bean cultivars to drought, WUE, and nutrient uptake.

Materials and Methods

Sixteen medium-seeded dry bean landraces and cultivars of great northern, pinto and red market classes were evaluated under non-stressed (NS) and drought-stressed (DS) conditions at Kimberly, Idaho in 2003. The NS treatment received seven and DS only four gravity irrigations, each of 12-hr run. Each plot consisted of 8 rows, 25 ft long with 4 replicates arranged in a randomized complete block design. The distance between rows was 22 inches. Pre-plant soil samples were taken for nutrient and moisture analyses. Also, plant (10-plants plot^{-1}) and seed (100-seeds plot^{-1}) samples were analyzed for 16 nutrients. WUE was measured for six cultivars under NS and DS conditions. Hansen data-loggers and watermark sensors were installed at three depths (0.23, 0.46, and 0.92 m) to record water potential. In addition, gravimetric soil samples were taken at 11 depths up to 2 m before and after each irrigation. Data were recorded for growth habit, days to flower, days to maturity, 100-seed weight, and seed yield.

Results and Discussion

Large differences among dry bean cultivars were observed for seed yield, WUE, percent yield loss due to drought stress (PR), and DSI. The data for three contrasting cultivars, namely Common Red Mexican, UI 259 and UI 320 are given in Table 1. The Common Red Mexican, a landrace grown by the Native Americans in the western United States for thousands of years, had high seed yield and WUE in both NS and DS conditions. Common Red Mexican had drought susceptibility index of 0.88 suggesting that, on average, it yielded higher under DS conditions than other cultivars. Its seed yield in NS was also comparatively high. As summarized for six of 16 elements in Table 1, Common Red Mexican had higher seed uptake of nitrogen, phosphorus, iron, zinc, copper, and manganese in DS and NS conditions than drought-susceptible UI 259 and UI 320. These results may suggest that important genes and/or quantitative trait loci (QTL) that impart drought resistance in Common Red Mexican have been inadvertently lost in some modern cultivars. It is therefore essential to identify and tag the major drought resistance genes and/or QTL present in Common Red Mexican. Common Red Mexican should also be utilized to improve drought resistance, nutrient uptake, and WUE of cultivars such as UI 259 and UI 320.

Table 1. Seed yield (kg ha^{-1}), water use efficiency (kg ha^{-1} mm^{-1} water), seed nutrient uptake (kg ha^{-1}), reduction due to drought stress (%), nutrient uptake and drought susceptibility index (DSI) of old and new dry bean cultivars under non-stressed and drought-stressed conditions at Kimberly, Idaho in 2003.

	Non-stressed								Drought-stressed								Reduction due to drought (%)							
Cultivar	Y[1]	W	N	P	Fe	Cu	Zn	Mn	Y	W	N	P	Fe	Cu	Zn	Mn	Y	N	P	Fe	Cu	Zn	Mn	DSI
CRM[2]	2162	5.1	80	10	53	9	26	12	1164	3.5	50	6	39	5	18	7	46	38	40	26	46	31	44	0.8
UI 320	1850	3.2	48	6	36	5	17	8	471	1.5	33	4	24	4	14	5	75	30	34	33	19	18	40	1.3
UI 259	1407	2.2	79	9	60	8	25	12	502	1.4	19	2	18	2	7	3	64	76	74	70	76	72	75	1.1
Mean[3]	1792	3.6	69	9	49	8	24	11	753	2.1	36	4	27	4	14	5	59	48	50	45	54	42	56	1.0
LSD (0.05)	204	1.1	7	1	8	1	3	1	630	1.3	6	3	21	3	10	4	10	14	13	14	13	15	13	0.2

[1]Y=seed yield, W=water use efficiency, DSI=drought susceptibility index. [2]CRM=Common Red Mexican landrace.
[3]Average for 16 cultivars with exception of water use efficiency that was recorded only for 6 cultivars.

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PREDAWN LEAF WATER POTENTIAL EQUILIBRIUM IN COMMON BEAN UNDER SOIL WATER DEFICIT

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Introduction. Predawn leaf water potential (Ψ_{lp}) has been used to estimate soil water potential (Ψ_s) based on the assumption that Ψ_{lp} equilibrates with the Ψ_s around the roots (Slatyer, 1967; Donovan *et al*. 2003). However, the relationship of Ψ_{lp} and Ψ_s is at present controversial. Consequently, the objective of the present study is to determine whether predawn water potential equilibrium is achieved in all parts of the common bean plant, as a consequence of equilibrium with the soil during drying. When this occurs the Ψ_{lp} of leaves at different levels on the main stem should be equal or approximately equal.

Material and Methods. Greenhouse-grown potted plants of *Phaseolus vulgaris* L. type III, cv. Bayo Madero (Acosta, 1982) were subjected to two moisture treatments: irrigated (**I**) and non-irrigated (**NI**). In the second treatment, irrigation was suspended when the 3rd compound leaf had just unfolded. At each sampling date, Ψ_{lp} of the main stem leaves and their transpiration rate, evapotranspiration (ET), wilting progression were determined, as well as soil water content and atmospheric vapor pressure deficit (VPD).

Results and discussion In all sampling dates Ψ_{lp} equilibrium was reached in **NI** and **I** (Fig. 1a and 1b). Therefore, it can be assumed that equilibrium was reached in all parts of the plant. In **NI**, Ψ_{lp} reached equilibrium in spite of increasing soil moisture deficit, a certain amount of nocturnal transpiration, stomatal conductance and VPD. From the 6th day (d) after irrigation suspension, the rate of ET during the day and night diminished and reached a minimum at the 9th d (Fig. 1c and 1d). Afterwards water absorption probably stopped or became minimal and the ET value was due mainly to evaporation. Soil field capacity was reached at the 6th d and permanent wilting percentage at the 14th d after irrigation was suspended (Fig. 1e). On the other hand, in the **I** treatment the soil moisture was maintained at close to field capacity during the entire experiment. Equilibrium between the Ψ_{lp} and the Ψ_s was not reached in any treatment (Fig. 1b). In the 3rd compound leaf, visual symptoms of the onset of permanent wilting condition were closely related to a Ψ_{lp} of -1.5 MPa. Therefore, the value of Ψ_{lp} is a good quantitative estimator of the onset of the plant permanent wilting condition.

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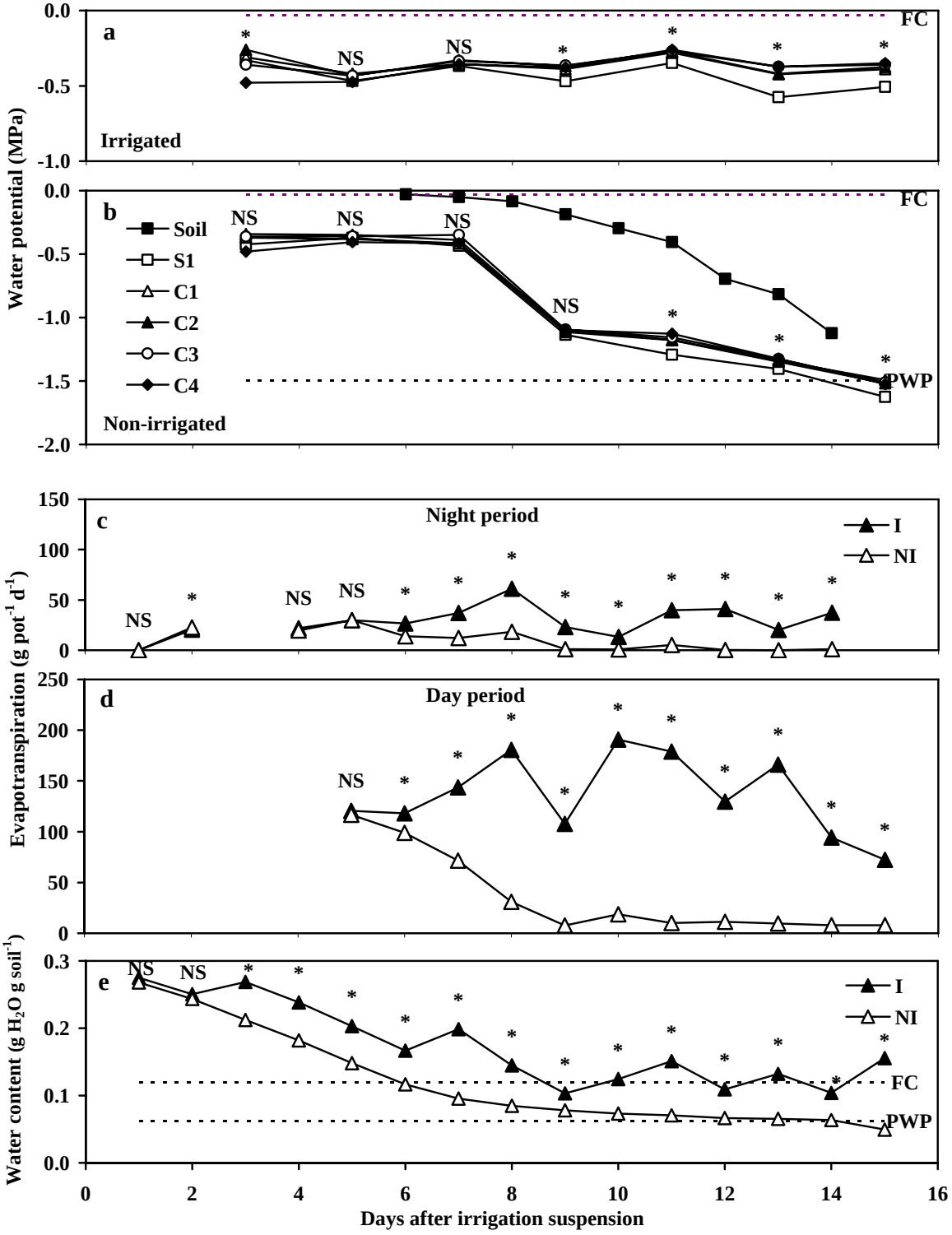

Figure 1. Predawn water potential of leaves (a), leaves and soil (b), evapotranspiration rates (c and d), and soil water content (e), after irrigation was suspended. FC=soil field capacity, PWP=soil permanent wilting percentage, SL=simple leaf, C1…C4=compound leaf number, **I**=irrigated, **NI**=non-irrigated, NS=non-significant and *=significant at P≤0.05.

Bean Production under High Temperature and Sub-irrigation: why it is feasible?

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Bean production at high temperature is possible when water is not limiting. Leaf transpiration reduces temperature of the leaves. This makes possible to grow bean seed production in semi desert with irrigation in many part of the world, e.g., along the Snake River/Id or Imperial Valley/CA, where peak temperature reaches beyond 40°C. Common diseases can not proliferate in the lowland tropics when irrigation water is supplied by raising the water table or sub-irrigation and low humidity and no rainfall during the crop season also prevent the contamination of diseases in the field. Furthermore, the soil is inundated for more than 4 months during the rice production in the rainy seasons, eliminating most of disease propagules in the soil. Few or no plant protection chemicals are needed; hence the production cost can be reduced significantly appropriated for seed production or bean production. High temperature modifies physiological and morphological characteristics of beans and only few commercial cultivars can adapt this environment. The bean growth cycle is reduced by 10 days. This shorter growth cycle reduces productivity, when nutrients are not adequately available in early growth stages. The N deficiency can be seen at any growth stage in this lowland tropic, because high quantity of crop residues from the previous rice production and the rapid decomposition by soil microorganisms, that need large quantity of N for its activities. The traditional recommendation for N fertilization, where one third of the total rate is applied at planting and the rest at 15 till 21 days after emergence can not satisfy the high demand of N by the bean plant.

The soil temperature can reach up to 40^0C for 6 hours a day in the upland areas in Africa (Lal 1978) and traditional growing areas in Brazil without irrigation soil temperature at the depth of 10 cm is around 26^0C (Met. Sta Embrapa Goiania). Information on soil temperature in lowland tropic is not available; therefore in 2004 the soil temperature was monitored at 5 and 20 cm depth. The result was encouraging for bean production in this region. The mean daily soil temperatures in July and August varied between 22.6 and 26.8^0C at 10 cm depth and at 20 cm the mean soil temperature is almost constant at 25^0C. This optimal soil temperature favors root activities for nutrient uptake and promotes the life of soil microorganisms, e.g. Rhizobium for N fixation. Farmers with rudimentary cultural practices can obtain 2 Mg ha^{-1} using the commercial cultivars from the traditional bean growing areas, but this productivity level is not attractive for large scale bean production.

Earlier experiment conducted by Santos & Silva, 2002 that N rates up to 200 kg ha^{-1} still increased yield. To obtain the optimum rate of N for higher bean yield an experiment in split plot design with four replications was conducted in 2004. The main plots were the N rates (0, 45, 90 and 135 N kg ha^{-1}) applied at planting at 8 cm depth and the subplots were the 4 cultivars (Carioca, ETA, Carioca Pitouco and BRS Valente). Among the four cultivars tested Carioca produced the highest yield with 3871 Mg ha^{-1} at 135 N kg ha^{-1} and planted at 22.5 cm row spacing. Increasing N rate further from 45 to 135 N kg ha-1 increased yield, but the differences were not statistically significantly. The optimum rate was 45 N kg ha^{-1} this region.

It can be concluded that bean yield beyond 3.5 Mg ha^{-1} can be obtained in the tropical lowland with sub-irrigation with adapted cultivars such as Carioca, provided sufficient N is applied at the time of planting. Soil temperature lower than 32^0C during the crop season corroborates for bean root activity and yield. Further confirmation of these results will follow in 2005. Cultivars for this specific region must be developed to attend the demand.

Figure 1. The mean daily soil temperature at 5 and 20 cm depth.

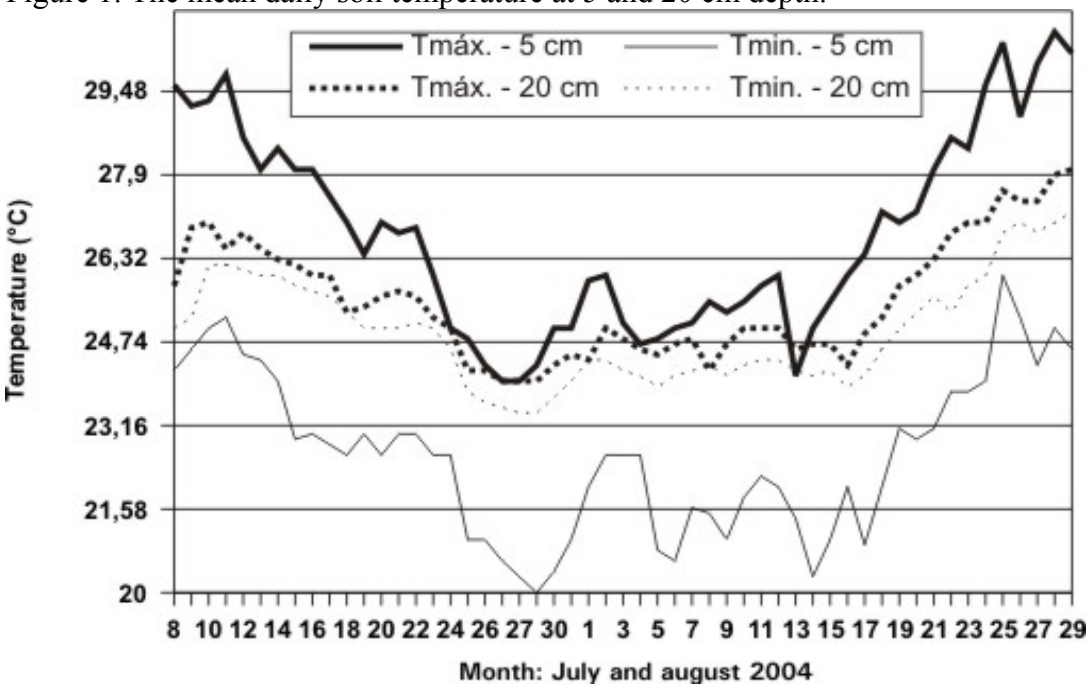

Table 1. Yield of 4 bean cultivars as affected by different N rates applied at planting grown with subirrigation system in Lagoa da Confusão-TO. 2004.

Cultivar	N kg ha^{-1}				Mean
	0	45	90	135	
Carioca	2273	2982	3358	3871	2971a
ETA	1692	2685	2682	2492	2387c
Carioca Pitoco	1514	2683	2671	2763	2408c
Valente	1725	3261	3204	3045	2809b
Mean	1801a	2903b	2979b	3043b	2681

CV=13,1%. LSD (5%)=136

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Association between stomatal conductance and yield in *Phaseolus vulgaris* and *Phaseolus coccineus*

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Introduction

Diffusion of CO_2 into the mesophyll of leaves and water vapour from the leave to the atmosphere is mainly driven by the stomatal aperture. Higher photosynthetic rates could in turn favor a high biomass and crop yield, and higher stomatal conductance appears to favor higher yields (Taiz & Zeiger, 2002). The selection of genotypes with high gas exchange may provide the development of bean (*Phaseolus vulgaris*) lines with high yield (Bressan-Smith & Pereira, 2003). The objective of the present work was to determine stomatal conductance and transpiration rate in different genotypes of *Phaseolus vulgaris* L. and *Phaseolus coccineus* L. grown under field conditions.

Material and methods

The study was carried out in Montecillo, Mexico (19º19' N, 98º54' W, 2250 m of altitude) during rainy season (June-September, 2004) and with a temperate climate. Seeds of thirteen genotypes of *Phaseolus vulgaris* L. (Bayo-18, Bayomex, Canario-107, Flor de Durazno, Flor de Mayo, Negro 98, Morito, Ojo de Cabra, Peruano, Pinto, Pinto Cabaña, Promesa, and Zacatecas) and three genotypes of *Phaseolus coccineus* L. (Ayocote blanco, Ayocote Morado, and Ayocote Negro) were sown in a soil with a pH of 6.8-7.5 and an electro-conductivity of 2-5 dS m^{-1}. The design was a random block with four replications, and a plant density of 6.25 plants m^{-2}. All the plots were fertilized with 100-100-00 NPK. Measurements of stomatal conductance, transpiration rate and leaf temperature were taken using a portable steady-state porometer Model LI-1600 (Licor Instruments, Nebraska) at pod filling stage (85 days after the sowing). Seed yield, biomass and other yield components were measured at physiological maturity

Results and discussion

The *P. vulgaris* genotypes showed a lower seed yield and biomass than the *P. coccineus* genotypes (Table 1). The *P. coccineus* genotypes had a bigger size than did *P. vulgaris* genotypes. As a result of the size *P. coccineus* had a higher seed weight, height, raceme number, and pod number. In contrast, *P. vulgaris* genotypes showed a higher seed number per pod with a low weight.

Table 1. Average of seed yield, biomass and other yield components measured at physiological maturity in genotypes of *Phaseolus vulgaris* and *Phaseolus coccineus* grown under field conditions. Montecillo, México.

	Seed yield (g m^{-2})	Biomass (g m^{-2})	100 seeds (g)	Height (cm)	Racimes m^{-2}	Pods m^{-2}	Seeds pod^{-1}
Phaseolus vulgaris	206.2 b†	393.5 b	38.6 b	52.8 b	185.0 b	203.8 b	3.6 a
Phaseolus coccineus	579.9 a¶	1568.8 a	182.3 a	137.3 a	252.5 a	341.3 a	2.5 b

†Different letters indicate statistical significant differences (Tukey, $p \leq 0.05$).

Instead of the low stomatal conductance and transpiration rate of *P. coccineus* genotypes, they had a high yield than *P. vulgaris* genotypes (Table 1, 2). Leaf temperature was decreased with a high stomatal conductance value as happened with *P. vulgaris* genotypes. Evapotranspiration at the leaf surface lowers leaf temperature, and higher stomatal conductance enhances this leaf cooling (Taiz & Zeiger, 2002).

Table 2. Average of stomatal conductance, transpiration rate and leaf temperature measured during the pod filling stage (85 days after the sowing) in genotypes of *Phaseolus vulgaris* and *Phaseolus coccineus* grown under field conditions. Montecillo, México.

	Stomatal conductance (mmoles m^{-2} s^{-1})	Transpiration rate (mmoles H_2Om^{-2} s^{-1})	Leaf temperature (ºC)
Phaseolus vulgaris	239.1 a	6.9 a	25.5 b
Phaseolus coccineus	149.1 b	4.6 b	26.4 a

Table 3. Correlation coefficients and significance between gas exchange and yield measured during pod filling stage in *Phaseolus vulgaris* and *Phaseolus coccines* genotypes grown under field conditions. Montecillo, México

	Stomatal conductance	Transpiration rate
Phaseolus vulgaris		
Seed yield	0.59*	0.63*
Biomass	0.40	0.45
Phaseolus coccineus		
Seed yield	0.88	0.82
Biomass	0.99**	0.98*

*Correlation coefficient significant at the 0.05 level of probability.
**Significance level 0.01.

When stomatal conductance and transpiration rate were associated with seed yield and biomass in each species, the results indicated a high relationship (Table 3). The *P. coccineus* genotypes with a higher stomatal conductance than *P. vulgaris* genotypes showed a stronger correlation. Recent studies of this type focusing on historical series of bread wheat (*Triticum aestivum*) have shown a remarkable positive correlation between yield increases and increases in stomatal conductance (Fisher *et al.*, 1998).

Conclusions

In conclusion, stomatal conductance was associated with biomass and seed yield in *P. vulgaris* and *P. coccineus* genotypes.

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PARTITIONING AND PARTITIONING RATE TO SEED YIELD IN DROUGHT-STRESSED AND NON STRESSED DRY BEAN GENOTYPES[1]

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Introduction. Different physiological and morphological traits have been used to evaluate drought resistance in crops. Some measurements require costly equipment and specialized hand labor. However, the evaluation of larger number of genotypes needs measurements that are easy to determine and that can explain the biological bases of adaptation and yield of cultivars in drought prone environments. Plant traits that can help to evaluate the proportion of photosynthates that are partitioned to seed yield might contribute to the selection of more efficient genotypes. Therefore, the objective of this study was to analyze the contribution of supplemental irrigation to seed yield and to determine the changes observed in above ground biomass, harvest index, growth duration and daily seed yield rate accumulation of dry bean genotypes under rainfed and rainfed plus supplemental irrigated conditions.

Materials and Methods. The study was conducted at the Research Station of Sandovales, Aguascalientes, Mexico (22° 09' N, 102° 18' W, 2000 masl) during three years. Field trials were established on June 27 in 2001; July 2 in 2002 and July 14 in 2003. Forty nine dry bean genotypes were tested, mostly belonging to the Durango and Mesoamerica races (4). Bean genotypes were from different breeding programs and 'G' accessions from the CIAT bush core collection (2). An experimental triple lattice design was utilized with two 6 m row plots and 0.76 m apart. The trials were grown under two soil moisture treatments: rainfed and rainfed plus supplemental irrigation. Supplemented plots received a light irrigation of 30 mm twice during the reproductive period. Daily precipitation was recorded from a near climatological station during the growing season. Traits recorded in each plot included days to maturity, seed yield and total aerial biomass (leaves and roots excluded). Harvest index (seed yield/total aerial biomass) and the daily rate of seed yield accumulation (seed yield/days to maturity) were estimated (5).

Results and Discussion. Accumulated rainfall during the crop cycle varied in the experimental site from 270 in 2001 to 324 mm in 2003. As expected, the rainfed crop never had enough moisture to met evaporative demand and crop growth was restricted during the whole cycle. In addition, temporary drought periods of different duration occurred each year, mainly during the reproductive period (data no shown). The uneven distribution of rainfall increases the risk for production in the area, since around 60 to 65% from the total rainfall during the cycle occurs in the vegetative period and only 35 to 40% occurs during the reproductive period (3). Average from all genotypes, lowest yield and shortest crop cycle were observed in 2002, results due to the occurrence of terminal drought. Under rainfed plus supplemental irrigation average from years and cultivars, seed yield, above ground biomass, harvest index and the rate of daily seed yield accumulation were 1014 and 1742 $kg.ha^{-1}$, 58.7 % and 11.3 $kg.ha^{-1}day^{-1}$, respectively. Whereas in the rainfed plots the observed values for those traits were, 624 and 1171 kg ha^{-1}, 52.1 % and

7.0 kg.ha^{-1}.day^{-1}, respectively (Table 1). Of those traits, biomass and the rate of partitioning were enhanced by supplemental irrigation and as a consequence seed yield raised an average of 390 kg ha^{-1}.

Outstanding genotypes under drought-stressed conditions such as G13637 and G842 achieved high yield via biomass accumulation and efficient partitioning, the former genotype being earlier by five days. Average seed yield of these two genotypes under rainfed conditions was 1223 kg.ha^{-1}, as compared to 782 kg.ha^{-1} of Pinto Villa a cultivar well adapted in the region.

On the other hand, genotypes with low above ground biomass and low rates of daily seed yield accumulation resulted in poor yielding genotypes. The opposite was true in the supplemented treatment where high yielding genotypes, such as G13637 and G22923, displayed large above ground biomass and a high rate of seed yield accumulation. These two genotypes showed similar growth cycle and an average seed yield of 1700 kg ha^{-1}. A high base yield potential under favorable conditions has been suggested to contribute to higher yields under stress (1). It is important to notice that the genotype G13637 (apetito) showed the best performance under both water treatments, suggesting that biomass accumulation and partitioning towards the developing seeds are key physiological factors in the adaptation to the semiarid conditions observed in the test site.

Table 1. Average seed yield, above ground biomass accumulation, harvest Index (HI), rate of yield accumulation (RYA) and days to maturity of 49 bean genotypes grown under two moisture conditions during three years at a semiarid location in Mexico.

Year/treatment	Seed yield kg ha^{-1}	Biomass kg ha^{-1}	HI %	RYA kg ha^{-1} day^{-1}	Days to Maturity
		Rainfed			
2001	622	1316	47.0	6.9	90.1
2002	364	766	49.8	4.4	82.0
2003	886	1432	59.5	9.7	91.6
Mean	624	1171	52.1	7.0	87.9
		Supplemented			
2001	1141	2206	51.3	12.6	90.8
2002	678	1309	53.3	8.0	84.8
2003	1223	1432	71.5	13.4	91.1
Mean	1014	1742	58.7	11.3	88.9

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DRY MATTER ACCUMULATION BY COMMON BEAN cv. BRS MG TALISMÃ

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Introduction: The dry matter accumulation analisys of the bean plant makes to know the different growth rates along the crop cycle, showing the variations in the nutrients demand and pointing strategies of rationalizing the fertilizers applications. In order to characterize the new cultivar BRS MG Talismã, recently recommended in Brazil, relative to dry matter accumulation at the different development stadiums, an experiment was carried out (winter-spring 2002) in a typical dark red latossol at experimental area of the Departamento de Agricultura, Universidade Federal de Lavras (UFLA), Lavras – Minas Gerais state, Brazil.

Material and Methods: The experimental design was randomized blocks with three replications and twelve treatments (twelve sampling times, seven days spaced, the starting from the plant emergency). Were used the rows spacing of 0,5 m, the sowing depth of 5 cm and the sowing density of sixteen seeds per meter. The plot was constituted by four 5 m rows. The experiment was conducted under conventional irrigation by aspersion. The bean cultivar was the BRS MG Talismã, a carioca commercial type originating fom the UFLA Bean Genetic Improvement Program that showed growth habit III (semi prostrated), normal cycle and resistance to the 89 race of *Colletotrichum lindemuthianum* and the gold mosaic virus (EMBRAPA, 2002). Each time, twenty plants were sampling for to determine the plant height (measure in cm), the development stadium and of the dry weigth (DW), separating the aerial part in stems, leaves, flowers+pods and grains. The collected material was dry with air circulation to 65-70^{o}C even constant weight.

Results and discussion:

Phases and stadiums of the crop cycle: In the winter-spring sowing season, the emergency was delayed, due to the low temperatures (12 days). The crop cycle, from the emergency to the maturation, had total duration of 84 days. The vegetative phase, computed from the emergency to the first floral buttons emergence (R_5 stadium), showed the duration of 35 days and the reproductive phase (between the R_5 and R_9-maturation) lasted 49 days. In the vegetative phase the stage V_4 was the longest, with duration of 14 days and in the reproductive phase the stage R_7 was the most extensive, with 21 days.

Growth curves: From emergency to the third complete leaf emission (stage V_4) the growth was slow, increasing soon after, until reaching a maximum point of total DW accumulation to 77 DAE (days after emergency), mainly due to the accentuated increase of the grain DW starting from 70 DAE (Figure 1). The maximum stems+leaves DW happened to 60 DAE, in the middle pod stuffing. Starting fom this point, ocorring significant leaves senescence and abscision, extending to the maturation, happened when about 63% of the total DW were represented by the grains DW (Figure 1). The maximum stem DW accumulation happened about 70 DAE, due to the largest length of the main stem. The H+F+Fl+V growth curve (Figure 1) reached your maximum about 63 DAE, due to the appearance of the pods.

Plant height: The plant height behavior followed the pattern presented by the stems+leaves DW, reaching a maximum point to 70 DAE and presenting, in the final stadiums, quick decrease (Figure 2).

Table 1 – Development stadies of common bean cv. BRS MG Talismã (winter-spring 2002).

Stadium	**Beginning (DAE)**	**Duration (Days)**
V0 - Germinaton	-	12
V1 - Emergency	0	7
V2 – Primary leaves	7	7
V3 – First composed leaf	14	7
V4 – Third composed leaf	21	14
R5 – Pre-flowering	35	7
R6 – Flowering	42	7
R7 – Pod growth	49	21
R8 – Grain stuffing	70	14
R9 - Maturation	84	-

$Y(H)=21,6–3,38x+0,14x^2-0,0011x^3$ (R^2=96,95%)
$Y(H+F)=10,53–2,59x+0,25x^2-0,0025x^3$ (R^2=94,09%)
$Y(H+F+Fl+V)=49,62–7,75x+0,4x^2-0,0036x^3$ (R^2=95,68%)
$Y(Total)=26,87–3,95x+0,25x^2-0,0018x^3$ (R^2=99,09%)

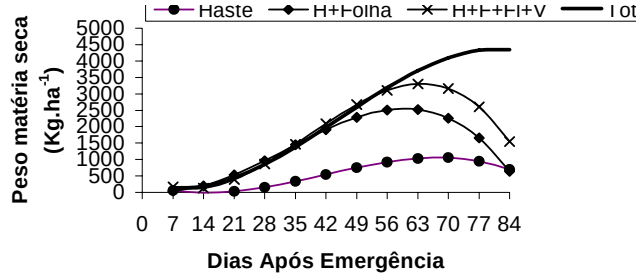

Figure 1 – Dry matter (DW) accumulation by the bean plant cv. BRS MG Talismã in the winter-spring 2002. H=stems DW, H+F=stems + leaves DW.

$y=12,803502–1,115137x+0,087867x^2-0,000746x^3$ (R^2=97,76%)

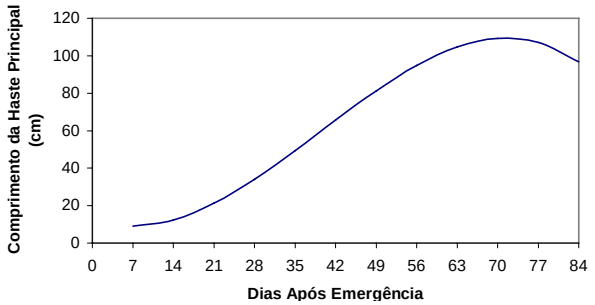

Figure 2 – Common bean growth curve according the ster lenght.

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Breaking the paradigm in lowland tropics under sub irrigation system: Nitrogen application and bean yield.

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The lowland tropics supposed to be not appropriated for bean production, because of high temperature and normally the domain of the rice production during the rainy seasons. The expansion of bean production to lowland tropics with subirrigation (raising the water table) is attractive. Common bean diseases do not develop under these conditions because of the relative low humidity and dry period during the whole crop cycle and the producing healthy bean seeds due to less chemical application for plant protection. Average bean yield is around 2 Mg ha^{-1} with the technologies adopted from the highland area using the same cultivars and fertilizer rates. Split N applied at planting and the side dressing at 21 days after emergence (DAE) as practiced in highland regions not proved adequate to meet the N requirement during the peak demand. High soil organic matter (SOM) and stubble left by the previous crop (rice) induces high microorganism activities, hence fixed temporary the applied N. Reducing the rice stubble by burning is forbidden recently due to the new pollution or environment law, aggravating N deficiency.

The objectives of these experiments were 1. to obtain the optimum rate of N at planting in 4 cultivars, 2. the optimum N rate applied at planting in combination of different dates of side dressing with 45 kg N ha^{-1} as urea; and 3. the interaction between N and P on bean yield.

Experiments were conducted in 2004 in split plot design with four repetitions with net plot of 10 m^2. The main plots are the basal treatments at planting and the subplot either the cultivars or the side dressing time. Potassium was applied at the rate of 60 K_2O kg ha^{-1} and 80 P_2O_5 kg ha^{-1} to all plots in the first and second experiments. In the third experiment 60 K_2O kg ha^{-1} as potassium chloride was given as basal application and varying the N and P doses. The soil characteristics are shown in Table 1. The soil was high in organic matter and phosphorus, but deficient in Mn. The best cultivar obtained from the first experiment was cv. Carioca at the dose of 90 N kg ha^{-1} and urea as the N source. The second experiment showed that 90 N kg ha^{-1} as urea applied before planting without any side dressing gave the highest yield. This means that total N applied at planting was sufficient to obtain the highest yield in this environment without causing burning to the plants during the germination or excessive vegetative growth before flowering period. Very late side dressing as 25 DAE proved to be ineffective.

The third experiment showed the highest bean yield was obtained from the combination of 30 P_2O_5 kg ha^{-1} and 90 N kg ha^{-1}. Higher doses than this did not increased yield significantly. These results concludes that under lowland tropics with subirrigation system a total N dose up to 90 N kg ha^{-1} can be applied before planting without causing burning to the seedlings and phosphorus up to 30 P_2O_5 kg ha^{-1} proved to be adequate rate for this environment.

These experiments showed that there are still room for yield improvement through better cultural practices e.g., N application at planting time and better adapted cultivars for this region. It will be verified whether the same result can be obtained in the second year on the same site, so that this practices can be recommended to all farmers of the region.

Table 1. Soil chemical characteristics of the lowland tropic, 2004.

Depth	pH	Ca	Mg	Al	H + Al	P	K	Cu	Zn	Fe	Mn	O.M.
(cm)	Water	$mmol_c/dm^3$				mg/dm³						g/dm³
0-10	5,8	43,2	11,2	1	90	32,5	145	1,7	4,2	82	16	54
10-20	5,9	42,0	10,7	1	91	30,6	78	1,6	3,5	85	17	50

Table 2. The effect N levels applied at planting on yield of 4 cultivars in the Lowland Tropic, 2004.

N* kg ha^{-1} at planting	Cultivar				
	Carioca	ETA*	Carioca Precoce	BRS Valente	Mean
			Yield (kg ha^{-1})		
0	1.707	1.244	985	1.074	1.252 c
45	2.660	2.324	2.337	2.480	2.450 b
90	3.116	2.547	2.574	2.709	2.736 a
135	2.742	2.480	2.402	2.386	2.502 b
Mean	2.556 a	2.149 b	2.074 b	2.162 b	-

LSD (5%): 108; CV (%): 10.9; * Urea fertilizer

Table 3. The effect of N levels at planting and time of side dressing on yield of cv Carioca grown in the Lowland Tropic, 2004.

N* kg ha^{-1} at planting	Side dressing in days after emergence				
	Not applied	0*	10	25	Mean
			Yield (kg ha^{-1})		
0	1.707	2.585	2.074	1.508	1.969 c
45	2.660	2.621	3.115	2.713	2.778 b
90	3.116	2.967	3.148	3.026	3.064 a
135	2.742	2.987	3.003	2.677	2.852 b
Mean	2.556 b	2.790 a	2.835 a	2.481 b	-

LSD (5%): 157; CV (%): 8.2. Side dressing with 45 N kg ha^{-1}, urea fertilizer.

Table 4. Combinations of N and P doses on yield of Carioca in Lowland Tropic, 2004.

N kg ha^{-1} at planting	P_2O_5 (kg ha^{-1})			
	0	30	80	Mean
			Yield (kg ha^{-1})	
0	2.105	1.870	1.707	1.894 c
45	2.630	3.048	2.660	2.779 a
90	2.563	2.804	3.116	2.828 a
135	2.280	2.562	2.742	2.528 b
Mean	2.394 b	2.571 a	2.556 a	-

LSD (5%): 124; CV (%): 15.5

GROWTH AND DRY MATTER ACCUMULATION IN BEAN CULTIVARS.

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INTRODUCTION: The analysis of growth is one instrument to better knowledge of plant how biology entity and permit to handle, rationally, the cultivate species to express your yield potential. Beside, permit to evaluate the growth of plant how completeness and the contribution of organ different to the totality growth. With dates of growth, evaluate physiologic activity, estimate the cause of change growth between plants genetically different or between plants same growing on different environment. Thus, the analysis of growth show the conditions morphophysiologics of plant and evaluate your liquid yield, derived to photosynthetic process, consisted the result of performance of assimilate system during well-known period of duration. Over there mass of dry matter (of plant and, or your organs, fruit, shoot, leaves and other), the leaf area is also determined (Benincasa, 2003). The objects this work was characterize the growth and grain yield of common bean (*Phaseolus vulgaris*, L) cultivars Ouro Negro and Talismã, cultivated in a field experiment.

MATERIALS AND METHODS: The vegetative growth and the grain yield Ouro Negro and Talismã cultivars were evaluated. The work was realize in period of November/03 until February/2004 in area of Coimbra Station Experimental, pertaining Viçosa Federal University (UFV), estate of Minas Gerais, Brazil. The soil of area was classified Yellow Red Argissolo Distrófico, clayey texture (EMBRAPA, 1999). The chemical analyze of soil sample (0.0-0.2 m class) show the value: pH (H_2O)= 5,06; P= 9,3 mg dm^{-3}; K= 57 mg dm^{-3}; Ca^{+2}= 1.75 $cmol_c$ dm^{-3}; Mg^{+2}= 0.53 $cmol_c$ dm^{-3}; Al^{+3}= 0.20 $cmol_c$ dm^{-3}; $H^+ + Al^{+3}$= 5.3 $cmol_c$ dm^{-3}; SB= 2.43 $cmol_c$ dm^{-3}; t= 2.63 $cmol_c$ dm^{-3}; T= 7.73 $cmol_c$ dm^{-3}; V= 31.40%; m= 7.6%; M.O.= 3.66 dag kg^{-1}; P-rem= 28.5 mg L^{-1}; Zn^{+2}= 0.73 mg dm^{-3}; Fe^{+2}= 22.1 mg dm^{-3}; Mn^{+2}= 22.9 mg dm^{-3} and Cu^{+2}= 2.36 mg dm^{-3}. It was installed two experiments, with one different cultivars: Ouro Negro (black class, Type II/III) and another cultivar Talismã (Carioca class, Type III). The fertilization were defined with base on analyze of soil and yield waited from 1,200 to 1,800 kg ha^{-1} (COMISSÃO..., 1999). Was utilized 20-80-20 kg ha^{-1} of N, P_2O_5 and K_2O + 30 kg ha^{-1} of N in cover 20 days after the emergency (DAE). The soil received 1,700 kg ha^{-1} of dolomitic calcareous and were fertilizer in moment of seeding with 3 kg ha^{-1} of Zn and 60 g ha^{-1}de Mo (on leaf, 20 DAE). Were utilized ammonium sulfate, superphosphate simple, chlorine of potassium, zinc sulfate and molibdato of ammonium. The experimental design was the randomized blocks with five replications and six treatments corresponded the sample age realize in 12, 22, 36, 51, 65 and 76 days after emergency (DAE - after stage V1). It was harvest, randomized, three plant/sample. Both experiments, the plants were retreated on central three line (one of each line) of parcel. The parcel were constituted seven lines of 5.0 m of length and between lines 0.50 m. On each experiment, three sample plants on each epoch and each replication were cut on level of soil and separated in shoot, leaves, pods and grains. The growth was characterized by dry matter accumulation of shoot, leaves, pods and grains, absolute growth rate and relative growth rate.

RESULTS AND DISCUSSION: The statistics analyses to each experiment (cultivar) were realized to follow recommendation of CAMPOS (1984), utilized the programs SAEG, version 8.0 (RIBEIRO JÚNIOR, 2001), at UFV and TABLECURVE. In choice of better model of regression to accepted the criterion: significant regression, high determination of coefficient (r^2) how a statistics descriptive, residue analyses and biology explication in accord with the statistic model. Both cultivars shown similar vegetative growth, but the cultivar Ouro Negro demonstrating higher total dry matter accumulation,

growth rate and crop growth rate. The relative growth rate was higher for Talismã cultivar. The number of pods per plant (NPP), hundred grain mass (MASS 100) of and grain yield (GY) of Ouro Negro cultivar were higher than Talismã (Table 1).

TABLE 1 - Means of variables population of plats/ha^{-1} (POP), number of pods/plant (NPP), number of seeds/pod (NSP), mass of 100 seeds (MASS 100) and yield, on cultivars of common beans Ouro Negro and Talismã. UFV, Viçosa-MG-Brazil-2004.

	VARIABLES				
CULTIVAR	**POP**	**NPP**	**NSP**	**MASS 100 (g)**	**YIELD (kg ha^{-1})**
Ouro Negro	216,000	10.57	5.12	23.62	2,473
Talismã	206,667	9.06	5.31	20.49	1,918

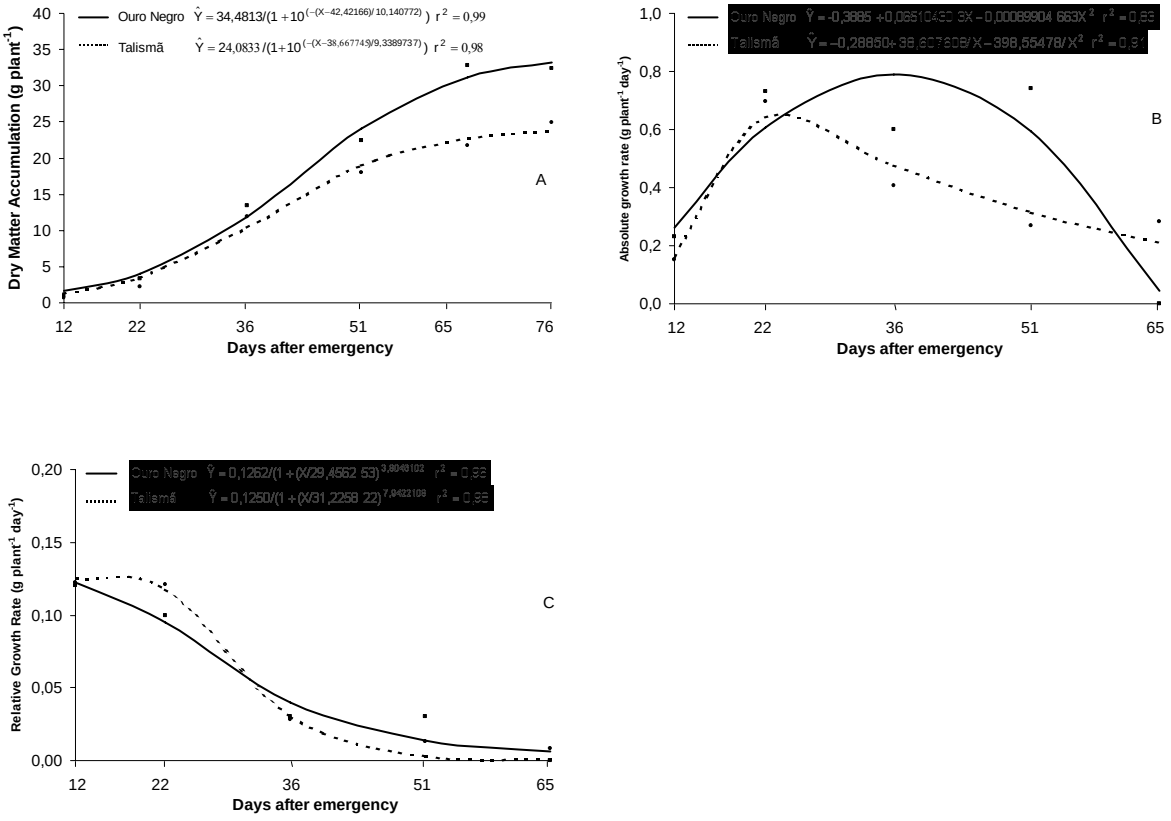

Figure 1. Total dry matter accumulation (A), absolute growth rate (B) and relative growth rate (C) of cultivars Ouro Negro and Talismã, in function of days after emergency.

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Yield of Bean Cultivars in Different Fertilizer Treatments.

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INTRODUCTION: The soil not aways have the nutrients requerement for the plant, it's necessary to take fertilization and correts, to potential yield of crop to will to express. The Recomendation to Use of Correts and Fertilizers to have many regions factors, especific to crop will have installed: level of expected yield, level of tecnology of producer, quality of fertlizers and economic-society aspects. On Estate of Minas Gerais-Brazil (COMISSÃO..., 1999), to crop bean, the recomendation of fertilization has been doing, considering levels of nutrients of soil and levels of tecnology utilized correspondent to expected yield.

MATERIALS AND METHODS: Were evaluating the criteriun of recomendation of fertilization, in three cultivars of common bean (*Phaseolus vulgaris* L.), with base on analyse of soil and level of tecnology, to exist on 5o approximation – Recomendation to Use of Corrects and Fertilizers on Minas Gerais, on Viçosa city, state of Minas Gerais-Brazil, in period November/03 until February of 2004. The soil of area was classified Yellow Red Argissolo Distrófico, clayier textury (EMBRAPA, 1999). The chemical analyse of soil sample (0.0-0.2 m class) show the value: pH (H_2O)= 5,06; P= 9,3 mg dm^{-3}; K= 57 mg dm^{-3}; Ca^{+2}= 1.75 $cmol_c$ dm^{-3}; Mg^{+2}= 0.53 $cmol_c$ dm^{-3}; Al^{+3}= 0.20 $cmol_c$ dm^{-3}; $H^+ + Al^{+3}$= 5.3 $cmol_c$ dm^{-3}; SB= 2.43 $cmol_c$ dm^{-3}; t= 2.63 $cmol_c$ dm^{-3}; T= 7.73 $cmol_c$ dm^{-3}; V= 31.40%; m= 7.6%; M.O.= 3.66 dag kg^{-1}; P-rem= 28.5 mg L^{-1}; Zn^{+2}= 0.73 mg dm^{-3}; Fe^{+2}= 22.1 mg dm^{-3}; Mn^{+2}= 22.9 mg dm^{-3} and Cu^{+2}= 2.36 mg dm^{-3}. It was installed three experiment, witch one with different cultivars: Ouro Negro (black class, Type II/III), Talismã (Carioca class, Type III) and Vermelho (Vermelho class, Type III). The experimental design was the randomized bloks with five replications and five treatments: one treatment without fertilizer and the others expected yield: until 1,200 (Fertilizer 2: 20-70-20 kg ha^{-1} of N, P_2O_5 and K_2O + 20 kg ha^{-1} of N in cover 20 days after the emergency (DAE)); from 1,200 to 1,800 (Fertilizer 3: 20-80-20 kg ha^{-1} of N, P_2O_5 and K_2O + 30 kg ha^{-1} of N in cover 20 days after the emergency (DAE)); 1,800 until 2,500 (Fertilizer 4: 30-90-30 kg ha^{-1} of N, P_2O_5 and K_2O + 40 kg ha^{-1} of N in cover parceled in 20 and 30 DAE); and higher than 2,500 kg ha^{-1} (Fertilizer 5: 40-110-40 kg ha^{-1} of N, P_2O_5 and K_2O + 60 kg ha^{-1} of N in cover, half in 20 and 30 DAE). The treatment were definit with base on analyse of soil (COMISSÃO..., 1999). The soil of every tratment received 1,700 kg ha^{-1} of dolomitic calcareous; every tratments, except the first, were fertilizar in moment of seed with 3 kg ha^{-1} of Zn and 60 g ha^{-1}de Mo (on leaf, 20 DAE). Were utilized ammonium sulfate, simple superphosphorus, chlorine of potassium, zinc sulfate and molibidato of ammonium.

RESULTS AND DISCUSSION: There was efect the treatments on variables: number of seeds/pods (NSP) to cultivar Ouro Negro; number of pods/plant (NPP), NSP and yield to cultivar Talismã. Nothing of variables were affected of significant to treatments to cultivar Vermelho. In period of conduction of experimental, high temperature (average of medium temperature, high and minimum were 22.8; 27.8 and 20.2, respectively), associate the high precipitation (total de 771,4 mm), high humidity relatvy of air (79.8%) and low resistance the boths cultivars to pathogens, was permited the occurrence the disease, Angular Leaf Spot (cv. Ouro Negro) and Rust (cv. Vermelho). Another factor that had influenciaty negativment in yield, "hided" the efect of fertilization is the high preciptation the occurred during every cicly, but principal in period of flowering (R6) until the harvest (R9). The criteriun of recomendation weren't aproprieted, it was more accentuate to Vermelho, followed of Talismã and Ouro Negro. The yield of cultivar Vermelho, was stay a low to waited for, on ervery tratments; in cultivar Talismã, the two

higher leves of yield waited weren't obtained; to Ouro Negro, only the high yield wasn't obtained. The cultivar Talismã showed the high increase, with the lest fertilization. The commum bean is crop that reflect with intensity on yield the change on enviromental condition how nutrients in soil, humidit, temperature and occorrency of insects and diseases on other factors, in this manner to return dificult to agriculturist to make prevision of yield with bases on fertilization criterium.

Table 1- Means of variables population of plats/ha^{-1} (POP), number of pods/plant (NBP), number of seeds/pod (NSP), mass of 100 seeds (MASS 100), yield in funccion of tratments, on cultivars of commun beans Ouro Negro, Talismã and Vermelho. UFV, Viçosa-MG-Brazil-2004.

	VARIABLES				
CULTIVAR OURO NEGRO					
TREATMENT	**POP**	**NBP**	**NSP[2]**	**MASS 100 (g)**	**YIELD (kg ha^{-1})**
WITHOUT FERTILIZER	213,333	9.19	4.83 **B**	24.90	1,995.32
FERT. 2	194,667	10.34	5.48 **A**	23.95	2,248.51
FERT. 3	216,000	10.57	5.12 **A B**	23.62	2,473.06
FERT. 4	182,667	9.62	4.96 **A B**	24.07	2,076.03
FERT. 5	182,667	10.14	5.15 **A B**	23.12	2,250.46
MEAN	197,867	9.97	5.11	23.93	2,208.68
CULTIVAR TALISMÃ					
WITHOUT FERTILIZER	218,667	7.26 **B**	4.99 **B**	20.31	1,555.04 **B**
FERT. 2	234,667	10.23 **A**	4.99 **B**	20.62	2,165.17 **A**
FERT. 3	206,667	9.06 **A B**	5.31 **A**	20.49	1,918.09 **A B**
FERT. 4	214,667	10.21 **A**	4.98 **B**	20.58	2,112.02 **A**
FERT. 5	189,333	10.17 **A**	4.93 **B**	21.02	1,864.44 **A B**
MEAN	212,800	9.38	5.04	20.61	1,922.95
CULTIVAR VERMELHO					
WITHOUT FERTILIZER	259,999	4.71	5.58	18.56	1,133.49
FERT. 2	256,000	4.72	5.61	17.77	1,169.29
FERT. 3	238,667	4.48	5.83	17.55	1,043.60
FERT. 4	273,333	4.98	5.98	17.11	1,144.27
FERT. 5	277,333	4.65	5.82	17.06	1,020.02
MEAN	261,066	4.71	5.77	17.61	1,102.14

Means within a column followed by the same letter are not different, Tukey 5%.

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PATTERN OF NUTRIENTS ABSORPTION BY COMMON BEAN cv. BRS MG TALISMÃ

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Introduction: The nutrient absorption and accumulation by the bean plant makes possible to know the amount of absorbed nutrients and your relative intensities of absorption during the crop cycle. Starting from the absorption and accumulation patterns were obtained basic informations and the most appropriate times for fertilizers applications. In order to charaterize the pattern of nutrient absorption by the new cultivar BRS MG Talismã, recently recommended in Brazil, was carried out one field experiment at winter-spring sowing season, in a typical dark red latossol at experimental area of the Departamento de Agricultura, Universidade Federal de Lavras (UFLA), Lavras – Minas Gerais state, Brazil.

Material and Methods: The experimental design was randomized blocks with three replications and twelve treatments (twelve sampling times, seven days spaced, the starting from the plant emergency). Were used the rows spacing of 0,5 m, the sowing depth of 5 cm and the sowing density of sixteen seeds per meter. The plot was constituted by four 5 m rows. The experiment was conducted under conventional irrigation by aspersion. The bean cultivar was the BRS MG Talismã, a carioca commercial type originating fom the UFLA Bean Genetic Improvement Program that showed growth habit III (semi prostrated), normal cycle and resistance to the 89 race of *Colletotrichum lindemuthianum* and the gold mosaic virus (EMBRAPA, 2002). Each time, twenty plants were sampling for macronutrients and micronutrients levels determinations, separating the aerial part in stems, leaves, flowers, pods and grains. The collected material was dry with circulation of air to 65-70 ^{o}C, even constant weight. The samples were triturated and analyzed at the Laboratories of the Departamento de Ciências do Solo of UFLA.

Results and discussion:
Macronutrients acumulation**:** The maximum accumulation of the primary macronutrients (N, P and K) it happened about 70 DAE-days after emergency (Figure 1). Amoung the secondary macronutrients, the Ca presented larger absorption around 63 DAE and Mg and S showed your higher absorption levels to 84 DAE (Figure 2).
Micronutrients accumulation: The maximum accumulation of the analyzed micronutrients happened between 65 and 77 DAE (Figure 3).

$Y(N)=7,48-0,83x+0,069x^2-0,0006x^3$ $(R^2=92,27)$
$Y(P)=2,16-0,38x+0,02x^2-0,0002x^3$ $(R^2=82,00)$
$Y(K)=5,97-0,97x+0,06x^2-0,0005x^3$ $(R^2=98,32)$

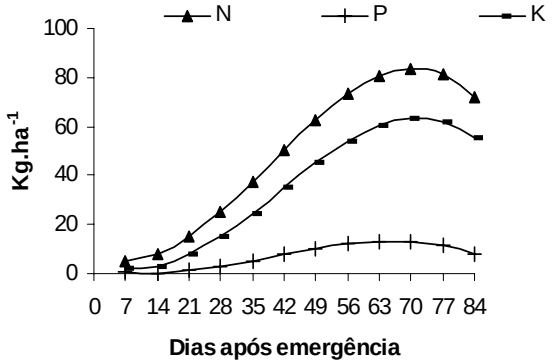

FIGURE 1 – Pattern of primary macronutrients absorption by the bean plant cv. BRS MG Talismã during the crop cycle.

$Y(Ca)=5,84+0,27x+0,05x^2-0,0005x^3$ $(R^2=91,61)$
$Y(Mg)=-0,17-0,004x+0,004x^2-0,00003x^3$ $(R^2=94,45)$
$Y(S)=0,35-0,05x+0,005x^2-0,00003x^3$ $(R^2=95,75)$

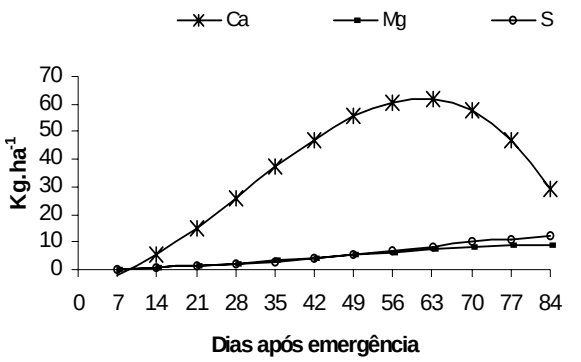

FIGURE 2 – Pattern of secundary macronutrients absorption by the bean plant cv. BRS MG Talismã during the crop cycle.

$Y(B)=18,98-3,23x+0,16x^2-0,0014x^3$ $(R^2=96,48)$
$Y(Fe)=23,05-5,9x+0,36x^2-0,0029x^3$ $(R^2=83,03)$
$Y(Zn)=15,94-2,71x+0,19x^2-0,0016x^3$ $(R^2=95,89)$
$Y(Cu)=4,86-0,7x+0,03x^2-0,0002x^3$ $(R^2=96,84)$
$Y(Mn)=25,96-3,73x+0,2x^2-0,0018x^3$ $(R^2=95,25)$

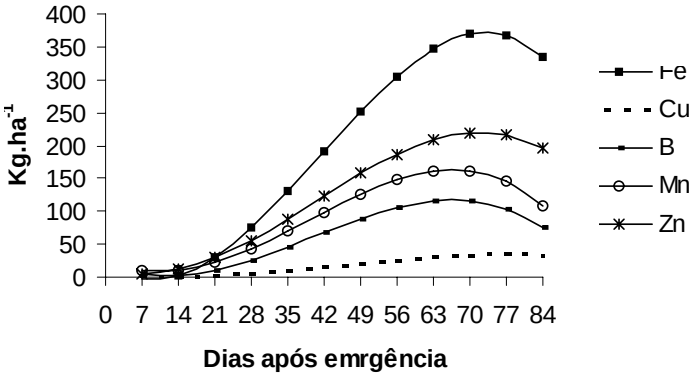

FIGURE 3 – Pattern of micronutrients absorption by the bean plant cv. BRS MG Talismã during the crop cycle.

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MOLYBDENIUM FERTILIZATION IN SNAP BEAN (cv. UEL-2) IN TWO BRAZILIAN OXISOILS

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Introduction

The bean is one of the most important protein and carbohydrates sources for feeding of the Latin America populations. Nitrogen is the nutrient absorbed in larger amount by the culture of bean plant that can export until 200 kg of N $ha^{-1}yield^{-1}$ (Haag et al., 1967). Although it is a leguminous, the bean plant exhibits low efficiency in the biological fixation of nitrogen as it has not been selected for efficient utilization of Rhizobium bacteria for inoculation of this culture (Brito, 2003).

Molybdenum is a micronutrient that participates in the nitrogenase and reductase nitrate enzymatic systems (Marschner, 1995). The deficiency of this nutrient alters the metabolism of the nitrogen, influencing your use for the plants. Some works accomplished in Brazil, with molybdenum fertilization resulted in increases of productivity of the culture when there was reduction of nitrogen fertilization (VIEIRA et al., 1992). This work was carried out with the objective of evaluating the effect of the molybdenum application on agronomic characteristic of the snap bean plant (cv UEL-2) cultivated in two typical oxisoil (sandy and clay) of the north Paraná region, Brazil.

Material and Methods

This experiment was conducted in a green house located at State University of Londrina - PR, in the period of September to November of 2003. Vases with 3 kg of soil collected of the superficial layer of two typical oxysoil (sandy and clay) of the north of Paraná State, Brazil, were used. The liming was accomplished to correct the saturation for bases for 70%. Besides the molybdenum application before of the sowing, all the vases received fertilization corresponding 60 and 30 kg ha^{-1} of P_2O_5 and K_2O, respectively. The snap bean seeds (cv. UEL-2) used in the experiment were previously inoculated with a mixture of the two bacteria: CIAT899 (*Rhizobium tropici*) and IPRF81 (*Rhizobium leguminosarium* bv. *phaseoli*). After emergency two plants by vase, were left. To the 25 days of the emergency, covering nitrogen fertilization was accomplished being applied urea in a equivalent dose to 27 kg ha^{-1} de N. The plants were sprayed with triforine fungicide, for control of mildew (Erysiphe polygoni) to the 25 and 47 days. During the experiment, the soil moisture content was maintained in 70% of the maximum water capacity of the soils. A completely randomized experimental design with five molybdenum doses (0, 30, 60, 90 and 120 g ha^{-1}) with 4 replications was used for each soil type. The obtained data were submitted to variance and regression analyses.

Results and Discussion

For the clay soil, the molybdenum application did not influence any of the studied variables. Significant effects were observed only in the sandy soil. In this case, the height of the plants

increased ($p < 0.05$) lineally with the molybdenum dose (Figure 1a), agreeing with the results observed by Andrade et al., (1998).

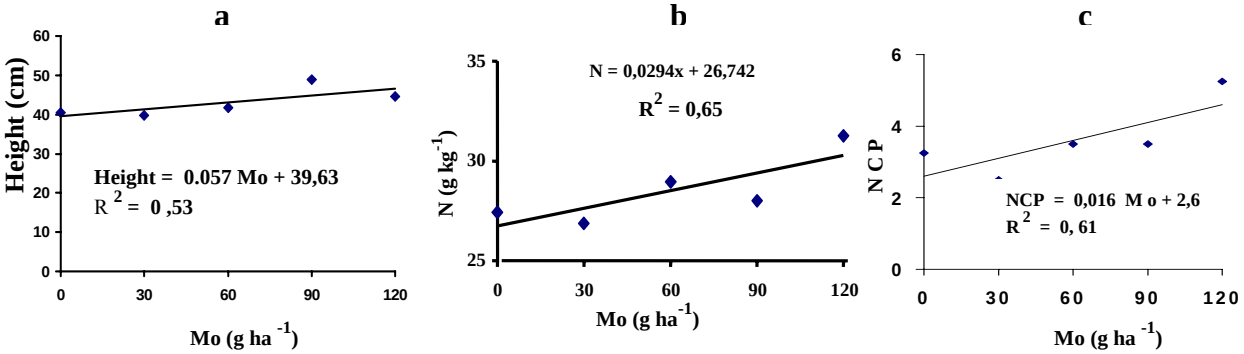

Figure 1. Plant high (**a**) and nitrogen content on dry matter of aerial part (**b**) and umber of commercial pods (NCP) by plant (**c**) in function of the doses Mo in a sand soil.

Nitrogen content in the dry matter of the aerial part (without pods) (Figure 1b) and the number of commercial pods (Figure 1c) increased lineally ($p < 0.05$) in function of the molybdenum doses. The N content varied from 27 to 31 g kg^{-1}. This result was below what it was observed by Vieira et al., (1992), when the covering application of 20 g ha^{-1} of Mo, increased the N leaves content from 28 g kg^{-1} to 55 g kg^{-1} of dry matter.

Conclusions

The agronomic characteristics evaluated on snap beans plants (cv UEL-2) cultivated in clay oxisoil, was not modified by molybdenum fertilization tested.

For the sandy oxisoil, the molybdenum fertilization resulted in increases in the height of the plants, in the number of commercial beans and in the nitrogen content in the dry matter of aerial part (without pods) in snap bean cv. UEL-2.

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BREAKING THE OLD PARADIGM IN UPLAND OXISOLS: NITROGEN APPLICATION FOR HIGHER BEAN YIELD.

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The nitrogen is the most deficient nutrient in tropical soil (CIAT, 1990), followed by phosphorus (Thung, 1990). It has been estimated that 40% of bean production areas is N deficient, a constrain to modern bean production system. The N requirement in Brazil is determined by the foliar analysis and by the response curve and the soil organic matter (SOM) content, both determines the rate of N recommendation. High response was obtained in soils with low SOM and in soils with high SOM content the response of bean to N application is low to nil recommended to eliminate the side dressing. It was also recommended to split N application, where small amount (10 to 20 kg N ha^{-1}) at planting and the rest is applied at 15 to 21 days after germination (DAE). On high SOM the side dressing should be eliminated (Anghinoni et al., 1985). This recommendation was based on evaluations on soils recently opened for agriculture in the early 70's, where base saturation and aluminum toxicity and SOM were limiting. In the last decade, the soil fertility has gradually improved due to yearly high rates of cheap fertilizer application. Liming has neutralized the aluminum toxicity and organic matter on the upper soil layers has been accumulated due to direct planting and no tillage system. The recommendation of split N application of the year 70's is still used to this date, ignoring the progress has been made in cropping system and better soil fertility of the farm land. In irrigated bean production system, N deficiency has been frequently observed at post flowering period in commercial cultivars and yield obtained seldom reached over 3 Mg ha^{-1}, although yield potential of some cultivars is greater than 4 Mg ha^{-1}. Split N application does not satisfy the N supply and side dressing does not deliver N on time at the peak demand of the plant.

Two experiments in split plot design with four repetitions with cv. Carioca was conducted at ST Helena on fertile Oxisol (see Table 1 for soil chemical characteristics) on farm practicing minimum tillage for more than 15 years.

The objective of the first experiment in randomized block design with four repetitions was to verify the optimum rate of N applied at planting time on cv Carioca, varying between 0 to 135 N kg ha^{-1} as Urea applied at 8 cm depth. The basal treatments are 60 K_2O kg ha^{-1} as potassium chloride and 90 P_2O_5 kg ha^{-1} as simple superphosphate.

The second experiment in split plot design and four repetitions has the objective to determine the optimum basal application in combination with optimum time for side dressing. The rate for side dressing was 45 N kg ha^{-1}. The main plot was the N rates applied at planting time (0, 45, 90 and 135 N kg ha^{-1}) and the subplot was the time of side dressing (0, 10, 20 and 30 DAE). The net plot area was 10 m^2. Urea was used as N source and K and P rates were the same as in the experiment one.

Increasing rate of N at planting increased yield significantly and the highest yield was 4116 Mg ha^{-1}, obtained from plot with 90 N kg ha^{-1} applied at planting (Table 2). N rates higher than 90 N kg ha^{-1} did not increase yield. The yield component analysis showed that all yield parameters increased significantly with N application and further increase N rate did not always increase the yield components significantly. Hundred seed weight increased up to the rate of 90 N kg ha^{-1}.

Results from experiment 2 showed that combination of 90 N kg ha^{-1} at planting with side dressing at 10 DAE ^{gave} the highest yield (5455 Mg $ha^{-1)}$. It can be concluded that N is the limiting factor for high bean production and higher yield than 4 mg ha^{-1} can be obtained when adequate N rate was given at planting in combination with side dressing at 10 DAE. These results challenged the common practice of split N application in bean, where low rate was given at planting and higher rate during side dressing at 21 DAE.
It will be verified whether the same result can be obtained in the second year on the same site, so that recommendation can be applied for bean under central pivot irrigation system.

Table 1. Soil chemical characteristics of Oxisol of Santa Helena-GO, 2004

Depth	pH	Ca	Mg	Al	H + Al	P	K	Cu	Zn	Fe	Mn	O.M.
(cm)	$CaCl_2$	$Mmol_c/dm^3$				mg/dm³						g/dm³
0-20	5,1	32	10	3	64	42	1,5	3,0	1,9	12	6,9	33

Table 2. The effect of different rate of N applied at planting time on yield and yield component of CV. Pérola. St. Helena-GO, 2004.

N kg ha^{-1}*	Mean				
	Stand ($10m^2$)	Pods $plant^{-1}$	Seeds pod^{-1}	100 seed wt (g)	Yield (kg ha^{-1})
0	198a	13,8b	4,2b	22,1b	3.098d
45	200a	15,7ab	5,1a	24,5ab	3.834b
90	192ab	16,2ab	4,8ab	26,1a	4.116a
135	181b	17,9a	4,7ab	24,4ab	3.962ab
Mean	-	-	-	-	-
CV(%)	6,1	18,8	18,3	7,8	6,1
LSD (5%)	12,2	3,1	0,89	2,0	242,4

Table 3. The effect of N applications rates at planting time and application time of side dressing on yield of cv. Carioca in St. Helena-GO, 2004.

N* kg ha^{-1}	Side dressing in days after emergence					Mean
	Not Applied	0	10	20	30	
0	2.894	4001	3315	35401	3515	3453d
45	3995	4189	4162	3705	4123	4035c
90	3952	4473	5455	4232	4499	4462a
135	3861	4132	4924	4193	4268	4275ab
Mean	3626c	4199b	4464a	3918c	4101b	

LSD (5%): 205; cv (%): 7.9; Side dressing with 45 N kg ha-1 as urea; * applied before planting.

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PROJECT - SELECTION OF BEAN CULTIVAR (*Phaseolus vulgaris* L.) TO SOIL ACIDITY TOLERANCE

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Exactly with the development of Brazilian agriculture, one of the constraints to the agricultural exploration is soil acidity, as much in traditionally cultivated soil as in new areas in agricultural expansion. Brazilian researchers have been esteemed that 80% of Brazilian soil are acid, presenting raised aluminum concentration and, in lesser scale, iron and manganese. These elements harm the development of crops with consequences in such a way in the top plant how much in root system. In the root, where the plant tissue keeps in direct contact with the soil acidity, the low plant development harms the absorption of the majority of mineral nutrients.

The soil acidity can be amended with lime application, but nor always the liming practice is carried at the time and in way correctly. For this reason, the Center – West region, is harmed, a time that suffers the highest consequences of the process of agriculture expansion. The lime application for soil amendment is a practical routine in national agriculture and that is evolved very deeply, with the machine modernization. However, with the diffusion of no till system in the country in last the 20 years, without the soil revolution, the traditionally productive varieties in areas of high fertility and genetic breeding ones present hardness to adapt in all spread acid soil areas in the country.

The no till system, practical responsible for the sustainable exploration of the Brazilian savanna, does not allow the lime to be carried through sub - superficial layers. Thus, the plants of annual crops, whose cycle of development reaches only from 60 to 120 days, do not reach full development in enough time for the stabilization of soil fertility. Two ways to adapt plants in acid environments are the use tolerant crop to acid environment to wait the necessary time for crop implantation in amended area.

The harmful effect of aluminum in the plants, in general way are similar for all the crops. The intensity of the effect, however, varies with the species, varieties and plant age. The sensible plants to aluminum generally accumulate phosphorus in their tissues not making use of exactly in the metabolic process. The phosphorus is precipitated in the root and therefore it becomes unavailable for the development of the plants. Beyond phosphorus, the calcium concentration is reduced in vegetal tissue. Research carried by Embrapa Rice and Beans has shown that the use of inferior amounts to a one ton of dolomite lime is important to supply plant needs in calcium and magnesium. For neutralization of soil exchangeable aluminum four tons of this same lime has been recommended.

For higher knowledge, a project of selection for acid soil tolerant cultivars is proposed objecting to know, to select and to multiply tolerant lines/cultivars to environmental acidity. Productive cultivars in daily pay launching, created by plant breeding will be submitted to selection test for acid soil. The plants will be developed in savanna condition, in oxisol soil of low fertility that will receive two doses from dolomite lime, 1 and 4 t/ha, receiving fertilization basic in accordance with the official fertilizer recommendation. The plants will be harvested when the first cultivar to launch the first floral button. Plant height, total dry matter production, leaf area, top and root dry matter will be observed to be evaluate as tolerant cultivar to acid soil.

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PROJECT - SELECTION FOR TOLERANT CULTIVAR OF COMMON BEANS (Phaseolus vulgaris L.) TO LOW LEVELS OF SOIL FERTILITY

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The common bean (Phaseolus vulgaris L.) is a demanding plant in phosphorus; a important nutrient for growth plants. Its presence in soil promotes the bean growth and raises the grain production . The beans mainly constitute one of the important alternatives of income and food for the agricultural population of all Brazil, that consumes it as dry grain. It is produced in such a way by small as by great producers that mainly use bred cultivars. In some regions it has been evidenced low levels of productivity, which have been mainly attributed to the creation of productive but demanding cultivars in mineral nutrition.

The great majority of Brazilian soil is acid presenting low fertility with high capacity to phosphorus retention what it leads to the necessity of application of raised doses of phosphates, contributing for cost increases, and to reduce the natural no renewable resources that originate phosphorus soluble fertilizers.

Phosphorus is the nutrient of bigger lack in the Brazilian savanna to the side of calcium and zinc. Researchers of the Embrapa Rice and Beans have verified that the P_2O_5 requirement to be applied in soil for many crops varies between 30 and 120 kg/ha, also used in the selection process of soil low fertility tolerant cultivar, contributing for the increase in the production costs. Adjusted phosphorus doses make possible to get high productivity and economic return.

The removed amounts of phosphorus for beans are generally low; 3.50 kg/ton of grain, mainly when compared with the nitrogen and potassium that are required 100 and 90 kg/ha, respectively. However, despite the apparent decrease concentration in soil solution , the phosphorus concentration as well as its speed of its reestablishment, are not enough to take care of crop need. Adequate phosphorus supply favors the root system growth increasing the absorption of water and nutrients; it increases the plant vigor in no till system; it favors the budding and the fruition and increases the product quality.

In soil, the phosphorus affect the production and grain quality. However, for different soil texture there is a special curves of production. The immobilization of phosphate is higher in clay texts, where are observed greater occurrences of iron and aluminum oxides and low pH values. Crops developed in sandy soils need less phosphorus fertilizer than clay soil due to higher phosphorus adsorption in clay soil.

Many Brazilian researchers show excellent answers of phosphorus in the rise of the income of the bean crop, in function of phosphorus application. For the common bean, this nutrient also has proportionate the great and more frequent answers. Low phosphorus availability in the soil affects negatively the plant growth and grain production. Although the phosphorus is the nutrient better studied in the crop bean, low knowledge, still, regarding the distinguishing parameters of the modern cultivar needs launched in the market.

For higher knowledge about tolerant plant to low fertility soil , a project of cultivar selection is being presented objecting to observe, to select and to multiply tolerant lines/cultivars tolerant to low fertility soil. The plants will be developed in low fertility Latosol of savanna that will have the acidity amended, receiving basic fertilization in accordance with the official fertilizer recommendation. The plants will be harvested when the first blossom of first cultivar presents floral button. As plant parameter will be observed plant height, dry matter production, leaf area, top plant weight part, weight of root system and total plant weight.

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RESPONSE OF BEANS TO RATES OF MOLYBDENUM APPLIED ON FOLIAGE AND PERFORMANCE OF THE SEEDS HARVESTED

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Introduction. Among the micronutrients that are essential for plant growth, molybdenum (Mo) is required in the smallest amounts. It is a constituent of the enzymes nitrate reductase and nitrogenase. Thus, the symptoms associated with deficiency of Mo are closely related to metabolism of N. In many parts of Brazil, response of common beans to Mo application is reported, and when nitrogen is not used as a side dressing, yield has been increased up to 200% due to foliar Mo application. Despite of these advantages of using Mo on common beans, many Brazilian farmers have no access to that technology because they are unaware of it and/or there is no molybdenum fertilizer available on local commerce. One possible solution to this problem is to provide farmers with seeds with high Mo content. The objective of this study was to test in field trial the feasibility of producing bean seeds with high Mo content from plants sprayed with Mo and then verify the effects of sowing these seeds in field without N as a side dressing.

Material and Methods. The field study was divided in two phases. In the first, the effects of high Mo rates (and split) on yield and seed Mo content were studied. In the second, seeds with four 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 in Viçosa, Minas Gerais State. The soil had a pH of 6.1. A randomized complete block design with four replications was used. Each plot had four 5m-long rows. Bean cultivar Ouro Negro was sown in rows spaced 0.5m apart with 15 seeds per meter. Each seed contained 0.028 μg Mo seed^{-1}. The rates (and split) of Mo tested are presented in Table 1. Ammonium molybdate was sprayed on foliage with 225 L ha^{-1} of water. All plants received basal N, P, and K at rates of 24, 37, and 40 kg ha^{-1}, respectively. Urea application (100 kg ha^{-1}) as a side dressing was performed 20 days after emergence (DAE).

Second trial. The trial was installed in winter in Coimbra, Minas Gerais State. The soil had a pH of 6.1. Treatments were four seed sources (0.30±0.068, 1.81±0.320, 2.23±0.914, and 3.01±0.216 μg Mo seed^{-1}), with or without Mo application (factorial 4 x 2). Mo application was made at 23 DAE using sodium molybdate and 225 L ha^{-1} of water. The trial was laid out on a randomized complete block design with five replications. Each plot had five 4m-long rows with 15 seeds per meter. All plants received basal N, P, and K of 24 of 24, 37, and 40 kg ha^{-1}, respectively. Leaves nitrogen status was monitored with a chlorophyll meter Minolta SPAD 502.

Results and Discussion. There were no significant differences in grain yield and 100-seed weight as Mo rates increase from zero (recommended rate) to 1500 g ha^{-1}, but μg of Mo seed^{-1} increased from 0.298 to 3.008 (Table 1). Split the rate of 1000g ha^{-1} of Mo was not advantageous to increase Mo content of seed. In the second trial, there were no significant differences between treatments on leaves N status at 26 and 34 DAE (Table 2). However, when no Mo was applied, N status at 40 DAE was higher in plants from seeds with 3.01 μg Mo seed^{-1} than form seeds with

0.30 and 1.81 μg Mo seed^{-1} (Table 3). Plants from seeds with 1.81 μg Mo seed^{-1} gave the highest yield, which differed significantly from those originated from seeds with 0.30 μg Mo seed^{-1}. Mo applied on foliage did not affect leaves N status, yield, and 100-seed weight. This investigation shows that is possible to produce enriched seeds by foliar application of high Mo rates without yield reduction, and plants raised from these seeds have higher yield potential.

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

Molybdenum treatment	Yield (kg ha^{-1})	100-seed weight (g)	Mo content[1] (μg seed^{-1})
Without Mo	1715*	27.2*	0.298 c**
90 at 20 DAE	2075	28.2	0.790 c
250 at 20 DAE	1703	27.5	1.813 b
500 at 20 DAE	1703	27,5	2.227 ab
750 at 20 DAE	2128	27.1	2.361 ab
500+500 at 20 e 30 DAE	1794	28.4	3.008 a
1.000 at 20 DAE	1921	28.3	2.725 a
500+500+500 at 20, 30 and 40 DAE	1668	27.7	2.926 a
CV(%)	18.9	4.7	17.1

[1] 100-seed weight was used for this calculation.
*Average of four replications. No significant.
**Average of four replications. Means separation by Tukey test at 5%.

Table 2. Effects of Mo content of seed and Mo treatments on chlorophyll readings, yield and 100-seed weight

Mo content of seed (μg seed^{-1})	Mo application[1]	Chlorophyll readings		Yield (kg ha^{-1})	100-seed weight (g)
		26 DAE	34 DAE		
0.30		36.2*	35.4*	1843 b**	22.0*
1.81		37.3	36.6	2127 a	22.4
2.23		37.3	36.0	2003 ab	21.1
3.01		37.5	35.7	2030 ab	21.7
	Yes	37.2^{ns}	36.2^{ns}	1975^{ns}	21.6^{ns}
	No	37.0	35.6	2027	22.0
CV (%)		4.8	7.2	11.0	6.3

[1] Mo applied (90 g/ha) on foliage at 23 DAE with 225 liters/ha of water.
*Average of four replications. No significant.
**Average of four replications. Means separation by Tukey test at 5%.
ns = no significant.

Table 3. Interaction of seed Mo content and foliar Mo treatments on chlorophyll readings at 40 DAE

Mo content of seed (μg seed^{-1})	Mo treatment[1]	
	With	Without
0.30	36.2 a*	33.6 c
1.81	38.0 a	35.6 bc
2.23	35.3 a	37.8 ab
3.01	36.7 a	38.9 a

[1] Mo applied (90 g/ha) on foliage at 23 DAE with 225 liters/ha of water.
* In columns, means separation by Tukey test at 5%.

EFFECT OF BIOLOGICAL TREATMENTS ON GROWTH OF BEAN PLANTS UNDER SALT STRESS

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Salinity is a major environmental constraint to crop productivity throughout the arid and semi arid regions of the world. Between 30% and 40% of the world irrigated agricultural lands are prone to salinity (Foolad and Yin, 1997). *Phaseolus vulgaris* is an important legume for human nutrition in the world, and bean plant growth is sensitive to salinity (Aouani et al., 1998). The deleterious effects of salinity on plant growth are associated with (1) low osmotic potential of soil solution (water stress), (2) nutritional imbalance, (3) specific ion effect (salt stress), or (4) a combination of these factors. In addition to the use of traditional breeding and plant genetic transformations, the use of plant growth-promoting microorganisms may prove useful in developing strategies to facilitate plant growth in saline soils (Mayak et al., 2004). Plant growth-promoting rhizobacteria (PGPR) and fungi can facilitate plant growth indirectly by reducing plant pathogens, or directly via phosphorous solubilization, nitrogen fixation, iron sequesterization by siderophores, phytohormone production (e.g. auxin, cytokinin, or giberellin), and/or enzymatic lowering of plant ethylene levels (Bjorkman et al., 1998; Grichko and Glick, 2001). In the present study, selected biological treatments were evaluated to increase bean growth under saline conditions.

Salinity treatments were established by adding 0, 50 and 100 mM of NaCl to a base complete nutrient solution (Hydro-Sol + $Ca(NO_3)_2$). Electrical conductivity (EC) and osmotic potential of these solutions were 1.91 dS m^{-1} with -0.0004 MPa for 0 mM NaCl, 7.03 dS m^{-1} with -0.23 MPa for 50 mM NaCl, and 11.9 dS m^{-1} with -0.45 MPa for 100 mM NaCl. Snap bean 'Labrador' seeds were sown in plastic pots (10 and 7 cm top and bottom diameter respectively, and 9-cm height, with holes in the bottom), five seeds were sown per pot, with filled a mixture of Arkport sandy loam : vermiculite (1:1, v:v). Moisture content of this soil medium was about 14%. Soil moisture content was increased to 60% of its water holding capacity with all biologicals, except Yield Shield mixed in solutions at recommended dosages by manufacturer before sowing. Yield shield was applied as a seed treatment. All pots were randomized on benches in the greenhouse maintained at 24 ^{0}C day and 21 ^{0}C night. After planting pots were covered with plastic to reduce evaporation. After plant emergence plants were irrigated manually to saturation as needed with 0, 50 or 100 mM saline solutions to maintain the level of salinity. Plants were harvested 21 days after sowing and their fresh weights determined.

All biological treatments used in the study increased plant weight compared to non-treated plants at each NaCl concentration tested (Table 1). The greatest plant weights were obtained with SoilBuilder, Yield Shield and Behold at 0 mM NaCl. No differences were measured between biologicals at 50 or 100 mM NaCl. The present study demonstrated that salinity adversely affected plant growth regardless of biological treatments. However, all biological treatments increased growth compared to the control that was exposed to stress. Earlier researchers have shown that selected biological treatments ameliorated the deleterious effect of salinity on growth of tomato, pepper and canola (Glick et al., 1997; Mayak et al., 2004). Studies have indicated that mobilization or solubilization of nutrients, increasing water use efficiency, stimulation of root

growth, could cause these positive effects by production of phytohormones and enzymatic lowering of plant ethylene concentrations. Different plant growth promoting rhizobacteria or fungi have been used for their beneficial effect on plant growth (Bjorkman et al., 1998; Gosh et al., 2003). Egamberdiyeva and Hoflich (2003) reported PGPR increased the uptake of N, P and K of wheat. Based on these findings, the biological treatments may help alleviate the negative effect of salinity on the growth of bean.

Table. 1. Effect of biological treatments on fresh weight of bean (grams) under salt stress**.**

		NaCl concentration (mM)		
Biologicals	**Source**	**0**	**50**	**100**
Non-Treated		3.9 d	2.2 b	1.1 b
Ag Blend	Advanced Microbial Solutions	4.3 c	2.6 a	1.4 a
Soil Builder	Advanced Microbial Solutions	5.0 a	2.6 a	1.7 a
Yield Shield	Gustafson	4.9 ab	2.8 a	1.7 a
Plant Shield	Bioworks	4.6 bc	2.7 a	1.7 a
Behold	Ecological Laboratories	4.7 abc	2.7 a	1.8 a
Equity	Naturize BioSciences	4.4 c	2.6 a	1.5 a
		< 0.001	<0.0154	<0.001
	Mean	**4.5**	**2.6**	**1.6**

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Identification of Genes Relating to Cold Acclimation in the Genus *Phaseolus*

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INTRODUCTION

Episodic late spring and early fall frosts are a major limitation to dry bean (*Phaseolus vulgaris* L.) production in the northern prairies. In the field, *P. vulgaris* dies at the moment of ice formation (-1.5^{o}C). To avoid spring frost, planting is often delayed until to late May, which limits the length of the growing season. Previous research by Balasubramanian et al. (2004) identified a relative of *P. vulgaris*, *Phaselous angustissimus*, which is capable of withstanding temperatures of -6^{o}C in the field, probably due to a greater ability to cold acclimate. Cold acclimation involves exposure to a low, but non-lethal, temperature for a period of a few days. The acclimation process enables plant species to physiologically withstand sub-zero temperatures (Thomashow, 1999). Previously, our group has determined differences between cold acclimated *P. vulgaris* and *P. angustissimus* in their ability to survive cold temperatures using kill curve experiments, in which cold acclimated plants of each species were removed from a cold chamber at 0.5^{o}C intervals across temperatures ranging from -1^{o}C to -4^{o}C. It was established that the LT50 for acclimatized *P. angustissimus* was 1.5^{o}C lower than *P. vulgaris,* indicating that *P. angustissimus* is capable of modest cold acclimation. The goal of this research is to identify and characterize key genes related to cold acclimation in *Phaseolus* species. To accomplish this, mRNA samples from each species exposed to non-acclimated and acclimated conditions were assayed on a macroarray containing ~2000 cold acclimation cDNAs of *Medicago sativa,* the most studied legume with regards to cold acclimation.

MATERIALS AND METHODS

cDNA preparations and macroarray conditions. mRNA from each species at non-acclimated and acclimated temperatures was extracted using Invitrogen's Plant RNA Extraction Reagent. mRNA was reverse transcribed using a Rediprime II Kit (Amersham, Cat #RPN1633 for 30 reactions) with a ^{33}P labeled dCTP. Macroarray blots were spotted with ~2000 cDNAs from a cDNA library generated from cold acclimated *Medicago sativa*. Each cDNA clone was spotted in replicates of three. The ~2000 cDNAs were hybridized overnight at 65^{o}C with radiolabelled cDNA from each *Phaseolus* species at each condition and exposed to Fuji Imaging Plates overnight. ArrayGuage software was used to analyze exposed plates.

RESULTS AND DISCUSSION

P. vulgaris was more transcriptionally responsive (+/- 4X change in expression) to cold acclimation (195 up-regulated genes and 278 down-regulated genes) compared to *P. angustissimus* (54 up-regulated genes and 48 down-regulated genes). Furthermore, of the 195 up-regulated *P. vulgaris* genes, 133 were unchanged in *P. angustissimus*, and of the 279 down-regulated *P. vulgaris* genes, 178 were unchanged in *P. angustissimus*. Correspondingly, 27 of the

54 up-regulated *P. angustissimus* genes were non-responsive in *P. vulgaris*, and 15 of the 48 down-regulated *P. angustissimus* genes did not respond in *P. vulgaris* (data not shown). Most strikingly, 8 of the 27 distinctly up-regulated genes in cold acclimated *P. angustissimus* were actually significantly down-regulated in *P. vulgaris* during cold acclimation. On the other hand, no genes which were up-regulated in *P. vulgaris* were down-regulated in *P. angustissimus* in the same manner. These 8 genes correspond to well documented proteins, including a kinase, two cytochrome P450s, a dehydrin, and a basic-helix-loop-helix transcription factor (Table 1). These types of proteins have been shown to be significantly up-regulated in other species that cold acclimate (Seki et al., 2002). These results suggest that not only is *P. angustissimus* better able to survive cooler temperatures, but that it employs a different transcriptional strategy than *P. vulgaris* when subjected to cold acclimation temperature incubation.

We intend to confirm these gene expression results using northern blots and/or real time reverse transcription experiments. Selected differentially expressed genes will be cloned from both parents and used to study the inheritance of these genes and frost tolerance phenotype in the progeny of crosses between *P. angustissimus* and *P. vulgaris.*

Table 1. Functional categories of 8 genes up-regulated in cold acclimated *P. angustissimus* but down-regulated in *P. vulgaris*.

Functional Category	No.	Description
Cryoprotection	1	dehydrin-like protein
Oxygen Binding	2	Cytochrome P450
Photosynthesis	1	putative pyruvate dehydrogenase E1 alpha subunit
Signal Transduction	1	protein kinase family protein
Transcription Mediation	1	helix-loop-helix-like protein
Unknown	2	unknown

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EFFECT OF POST EMERGENCE HERBICIDES ON DIFFERENT DEVELOPMENT STAGES OF DRY BEAN

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Introduction

Dry beans are produced in irrigated areas at central valley of Chile, and emergence on new weed flushes after each irrigation needs to delay some herbicide application to decrease weed density after bean flowering. Traditionally, post emergence herbicides to control broadleaf weeds (bentazon or fomesafen) or grass weeds (clethodim, fluazifop-p-butil), are recommended to spray while weeds are actively growing, when bean plant have 1-3 trifoliate leaves (AFIPA; 2002). However, this maintains low weed density during a short time, and later weed emergence obligates to repeat or delay the application time, inclusively until flowering. The objective of this experiment was to evaluate the effect of post emergence herbicides on three development stages of two bean cultivar at the central valley of Chile.

Materials and Methods

An experiment was carried out during 2003-2004 season at the central valley of Chile, 36º 52´ S. L., 71º 55´ W. L. Two bean cultivar, Torcaza-INIA and Curi-INIA, tortola and black market class cultivars, respectively, were planted on November 8, 2003. The herbicides treatments were sprayed at three development stages: 1-3 pairs of trifoliate leaves, just before flowering (bud stage) and flowering. Weeds were hand removed every 20 days to allow only herbicide effect. The herbicides were sprayed with 200 l/ha of water using a CO_2 sprayer at 0.21 MPa, and the treatments are in Table 1. The experiment, for each variety, was conducted in a strip-block design with four replications. Main plots, bean development stages, were 2.5 x 16 meters and herbicides treatments were 2.5 x 2.0 meters.

Table 1. Herbicides treatments sprayed on three development stages on two bean varieties. Chillán 2003-2004.

Treatments	Dosis i.a. [(1)](l or kg/ha
1. Clethodim [(2)]	0.192
2. Clethodim [(2)]	0.384
3. Fluazifop-p- butyl [(2)]	0.175
4. Fluazifop-p- butyl [(2)]	0.350
5. Bentazon	0.96
6. Bentazon	1.92
7. Fomesafen [(2)]	0.25
8. Fomesafen [(2)]	0.50

[(1)] a. i.: active ingredient
[(2)] Plus no ionic surfactant

Results

Delayed herbicide spraying until flowering decreased pod per plant, grain per pod and yield of Torcaza-INIA, when compared to early application, in spite of lack of weeds. The number of grain per plant decreases when spraying was delayed until flowering. This meant about 18% of less yield due to effect of herbicide application time. There was no effect on grain weight. On the other side, delayed herbicides application until bud stage, did not affect yield or yield components (Table 2).

Table 2. Effect of herbicides spraying time on three development stages of bean Torcaza- INIA on yield and yield components. 2003-2004.

Development stages	Seeds/ plant	Pods / plant	Seeds / pod	100-seeds weight g	Yield (kg/ha)
3 pairs trifoliate leaves	71 a	16..9 a	4.3 a	50.2 a	3260 a
Bud stage	67 a	16.4 a	4.2 ab	49.7 a	3090 a
Flowering	58 b	15.2 b	4.0 b	49.5 a	2670 b
C.V.(%)	10.9	8.1	6.3	6.9	12.6

[1] Means within a column followed by the same letter are not different, LSD (0.05).

Curi-INIA, had similar behavior, although there was effect only on pod per plant among the yield components, while grains per pod and grain weight were not affected by herbicides or application time. However, the effect on pod per plant was enough to decrease the number of grain per plant. This meant about 23% of lower yield when herbicides were spraying during flowering respect to 3 trifoliate leaves (Table 3).

Table 3. Effect of herbicides spraying time on three development stage of bean Curi INIA on yield and yield components. 2003-2004.

Development stages	Seeds/ plant	Pods/ plant	Seeds/ pod	100-seeds weight g	Yield (kg/ha)
3 pairs trifoliate leaves	119 a	23.2 a	5.2 a	21.1 a	2520 a
Bud stage	115 a	22.7 a	5.1 a	21.1 a	2420 ab
Flowering	100 b	20.1 b	5.2 a	213 a	1940 b
C.V. (%)	10.1	9.1	3.1	5.0	14.4

[1] Means within a column followed by the same letter are not different, LSD (0.05).

Increased rates of herbicides decreased yield of Torcaza-INIA, especially when used both broadleaf herbicides, while Curi-INIA was not (Table 4). It is possible that growth habit of Torcaza-INIA type III, had been more affected than Curi-INIA, type I, due to longer time of flowering.

Table 4.Effect of herbicide treatments on dry bean yield of two cultivars, average three development stages. 2003-2004.

Herbicides	Rates a.i. (l – kg/ha)	Yield (kg/ha)	
		Torcaza-INIA	Curi-INIA
1. Clethodim	0.192	3160 a	2380 a
2. Clethodim	0.384	2970 ab	2330 a
3. Fluazifop-p- butyl	0.175	3170 a	2250 a
4. Fluazifop-p- butyl	0.350	2940 ab	2300 a
5. Bentazon	0,96	3130 a	2340 a
6. Bentazon	1,92	2780 b	2340 a
7. Fomesafen	0,25	3080 a	2250 a
8. Fomesafen	0,50	2810 b	2240 a
C.V.(%)		12.7	13.7

[1] Means within a column followed by the same letter are not different, LSD (0.05).

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PERFORMANCE OF ANTHRACNOSE RESISTANT "CARIOCA-TYPE" BEAN LINES FROM THE CROSS PÉROLA X OURO NEGRO

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Several measures are used by the farmers to control the diseases affecting the common bean. However, the use of resistant cultivars is by far the most recommended method mainly because it is cost effective and environment friendly. The "carioca-type" common bean varieties are prevalent among the ones recommended for cultivation in Brazil (MAPA, 2004). More than two million hectares are cultivated annually with this type of bean. These "carioca type" cultivars possess excellent agronomic qualities, but they are susceptible to the main pathogens affecting the common bean. This is the case cultivar Pérola, one of the most extensive grown varieties in the State of Minas Gerais (Southeastern Brazil). This cultivar is susceptible to the main races of *Colletotrichum lindemuthianum*, causal agent of the anthracnose disease, identified in the State of Minas Gerais and in other parts of Brazil. The black-seeded cultivar Ouro Negro, also recommended for cultivation in the State of Minas Gerais, harbors resistance gene(s) which confer resistance to several *C. lindemuthianum* races. Artificial crosses were used to transfer the resistance gene(s) from Ouro Negro to Pérola. Several lineages with "carioca" grain pattern, resistant *C. lindemuthianum* were selected. In this work, $F_{7:8}$ lineages were evaluated, under field conditions for their agronomic performance.

A total of 81 genotypes (75 lineages and 6 controls) were tested in Viçosa and Coimbra, state of Minas Gerais. A triple square lattice design was used. The rows were 2.0 m long and spaced by 0.5 m from each other. As controls, the two genitors were used (Ouro Negro and Pérola), in addition to cv. Talismã, lines Rudá-P (pyramided with anthracnose, rust and angular leaf spot (ALS) resistance genes), and Rudá-derived lines VC4 and Vi 4899. In the experiments, productivity, grain aspect and ALS severity were determined. Resistance to this disease is an important characteristic for the State of Minas Gerais. To evaluate ALS severity a 1-to-9 symptom scale was used (CIAT, 1987). To evaluate grain aspect, a 1-to-5 scale was used. Grade 1 is attributed to non-flat, medium sized, light beige "carioca-type" grains with light brown stripes, and with no halo. Grade 5 is attributed to grains which do not present typical "carioca" pattern (Marques Jr. et al., 1997). The statistical analyses were conducted with the aid of the statistical package GENES (Cruz, 2001).

Significant effect was observed for the source of variation "lineages" in both individual and combined analyses. Considering the two places of evaluation, the mean productivity of the lineages was of 3,658 kg/ha, varying from 2,701 to 4,317 kg/ha. Productivity of 23 lineages was statistically equal to that of cv. Pérola (3,467 kg/ha). These are promising lineages as cv. Pérola is a highly productive cultivar compared to other "carioca-type" varieties in Brazil. Thirty-three lineages presented grades smaller than 2.2 for grain aspect, statistically comparable to the grade of cv. Pérola (1.5). The severity grades for ALS varied from 2.7 to 6.0. Cultivar Ouro Negro was the most susceptible among the genotypes tested (grade 5.7), while cultivars Pérola, Vi 4899 and Rudá-P behaved presented moderate resistance, with grades lower or equal to 4. Sixty-seven

lineages were compared to cv. Pérola, in relation to the resistance to ALS. Six lineages were more resistant, with grades varying from 2.7 to 3.3. Considering the results obtained in the field evaluations, it was possible to select ten lineages with "carioca-type" grains (grades lower than 2.3), productivity statistically equal to that of cv. Pérola and resistant to anthracnose (*C. lindemuthianum* races 73, 81 and 89), and to ALS (Table 1). Some of these lineages were included in the regional evaluation assay with great potential to be recommended as commercial cultivars for the State of Minas Gerais, Brazil.

Table 1. Grain productivity (kg/ha), grain aspect and angular leaf spot reaction of the 10 best $F_{7:8}$ lineages from the cross Pérola x Ouro Negro, tested in Viçosa and Coimbra, state of Minas Gerais, Brazil, during the "dry season" of 2004.

	Productivity (kg/ha)			Grain aspect	Angular leaf spot
Cultivar	Viçosa	Coimbra	Mean		
OP-196	5,149[1/]	3,484 a b[1/]	4,317 a[1/]	2.3[1/]	3.7 b[1/]
OP-145	4,616	4,016 a	4,316 a	1.3 a	4.0 b
OP-195	4,542 a	3,801 a b	4,172 a	2.2	4.0 b
OP-58	4,504 a	3,661 a b	4,083 a	1.5 a	2.7
OP-188	4,503 a	3,625 a b	4,064 a	2.2	3.7 b
OP-166	4,449 a	3,659 a b	4,054 a	2.2	4.0 b
OP-60	4,437 a	4,011 a	4,224 a	1.8 a	4.0 b
OP-192	4,178 a	3,981 a	4,080 a	2.0 a	4.0 b
OP-29	4,176 a	3,867 a b	4,022 a	1.8 a	3.3 b
OP-186	4,061 a	4,096 a	4,079 a	2.3	3.0 b
Pérola*	3,702 a	3,231 a	3,467 a	1.5 a	4.0 b
Ouro Negro*	2,891 b	2,923 b	2,907 b	5.0 b	5.7 a
Mean	3,909.50	3,388.25	3,658	2.20	4.30
CV(%)	9.74	12.33	9.18	11.85	12.73

[1/] Averages followed by the same letters in the same column, do not differ to each other (Dunnett, $P<0.05$)
* Controls (genitors).

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Graphical Method in Studies of Adaptability and Stability of Cultivars

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This study proposes a novel methodology that uses, principally, a graphical method to make the data visualization and interpretation of experiments conducted in various environments easier.

The graphic method is established based on the standardization of the means of the cultivars evaluated in experiments conducted in various environments by the expression $z_{ij} = \left(x_{ij} - \overline{x}_{.j}\right)/ s_{.j}$, where z_{ij} is the value of the standardized variable corresponding to cultivar i in environment j where $i = 1,...,n$ and $j = 1,...,a$; x_{ij} is the mean of cultivar i in environment j; $\overline{x}_{.j}$ is the mean of environment j; $s_{.j}$ is the phenotypic standard deviation of the cultivar means in environment j.

As the standardized variable z_{ij} assumes positive and negative values, the graphical visualization is facilitated by adding a constant so the z_{ij} values are always positive. This way the mean of the z_{ij} values for cultivar i in a environments ($\overline{Z}_{i.}$) is a measure for the adaptation of cultivar i while the coefficient of variation of z_{ij} for cultivar i in the different environments (CV_{Z_i}) is a measure for the stability of cultivar i. The standardized values (z_{ij}) are used to construct a diagram for each cultivar. The dimensions of the axes (environments) are equivalent to the values of z_{ij} of cultivar i in environment j.

Example of Application

For an exemplification we used data of an evaluation of 25 common bean lines in 11 environments in Brazil, in 2002 and 2003. Figure 1 presents the estimates of the means ($\overline{Z}_{i.}$) and of the coefficients of variation (CV_{Z_i}) obtained by the graphic method.

The constant three was added in the present case to make all z_{ij} values positive. This way, values below this constant indicate a performance below the mean of the environment. The Scott Knott test at a significance level of 5% classified the lines in two groups, of which the more adapted one presented $\overline{Z}_{i.}$ values above 3.26 (Ouro Negro) (Figure 1).

According to the values of coefficients of variation the most unstable lines were CIV-453, CIV-76 and Carioca, and the most stable ones OP-S-64, Ouro Negro and CIV-151 (Figure 1). It is worth emphasizing that OP-S-64 associated a high adaptability with a high stability which evidently is what every breeder wishes. This fact can easily be visualized in Figure 2A *or* (Figure 2A). Note that this line presented a performance above the mean in most environments, only lower in environment 7, forming a nearly perfect circumscribed circle, “full ball”. Cultivar Carioca on the other hand presented less absolute adaptation in nearly all evaluated environments and low stability, “slack ball”, which is also quickly visualized in Figure 2B *or* (Figure 2B).

The graphic method therefore allows that the breeder’s decisions *are* taken graphically or statistically based on the inference drawn on the mean $\left(\overline{Z}_{i.}\right)$ and coefficient of variation $\left(CV_{Z_i}\right)$ of the standardized values.

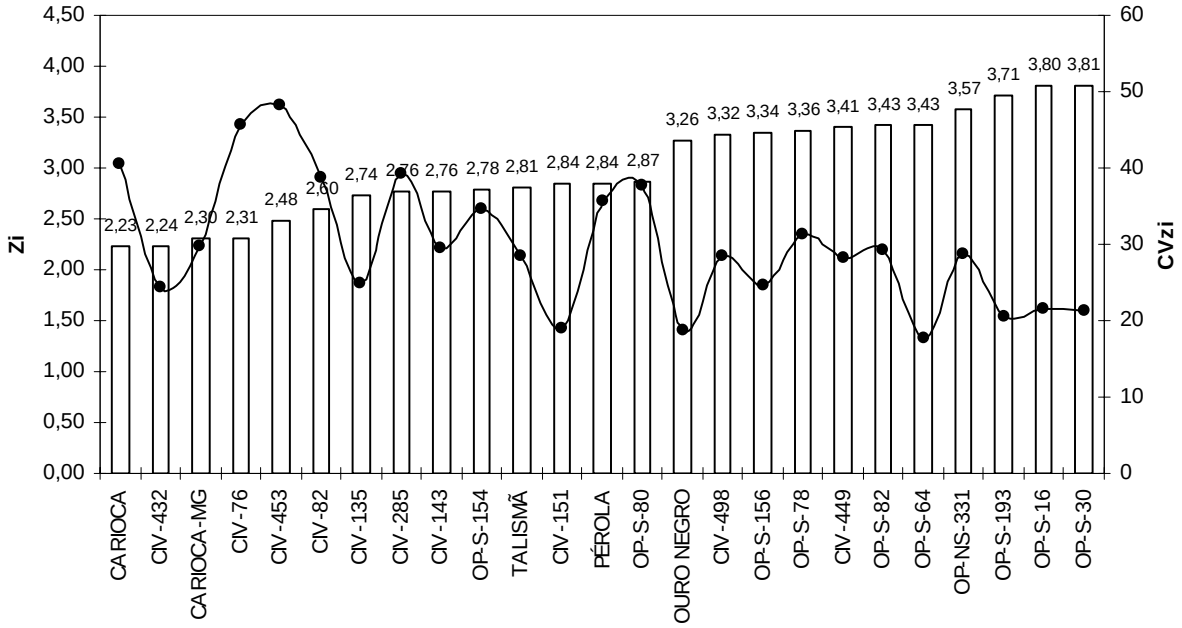

Figure 1. Estimates of the means ($\overline{Z}_{i.}$) and coefficients of variation (CV_{Z_i}) by the graphic method for 25 common bean lines evaluated in 11 environments in Brazil, in 2002 and 2003.

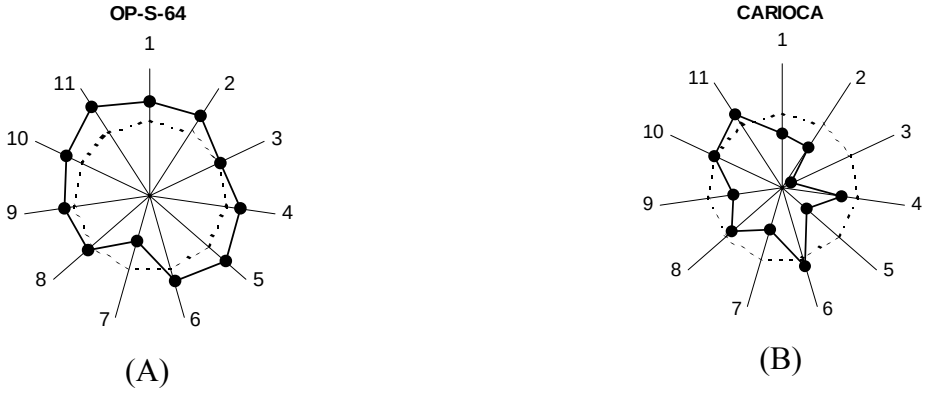

Figure 2: Representation of the performance of the common bean lines OP-S-64 (A) and Carioca (B) using the graphic method. The dotted line represents the mean of the environment, the value of the constant (three) associated to variable *Z*, and the axes of each one of the evaluated environments.

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Diallel Analysis of the Combining Ability of Common Bean (*Phaseolus vulgaris* L.) Cultivars

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Introduction

The obtaining of cultivars with better productive performance must be one of the primary objectives of common bean genetic breeding programs, so that, a viable alternative is to wide of the genetic base of this species, through crosses. Diallel analysis procedure has, without question, great importance, especially in breeders programs because it allows wide recombination of the genomes with greater possibilities of identifying superior recombinants in segregant generations (Ayele, 1994; Cruz, 2001). The present study had as objective to estimate the general and specific combining abilities and discriminate the superior parents and hybrid combinations as a first step in developing a breeding program for species to increase productivity.

Material and Methods

The common bean cultivars Talismã, Uirapuru, FT Soberano, BRS Campeiro, IAC Tibatã and Juriti were chosen because of their divergent morphological and agronomic characteristics, and used as parents in a complete diallel without reciprocals. The populations consisting of six parents and 15 F_1, totaling 21 treatments were assessed in the greenhouse at the Nucleus for Research Applied to Agriculture (Nupagri), at the Department of Agronomy, UEM, Maringá, PR in 2003 and at the experimental area of the Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina S.A. (Epagri). The experimental design was a randomized complete block with three replications. Experimental unit was composed by two rows of plants spaced of 0.5 m and with 4.0 m of length, totaling an useful plot area of 3.0 m^2.

Results and Discussion

Table 1 shows the partitioning of the sum of the squares attributed to the genotype effects as well as the means of the squares of the effects for the four characteristics. The values of the mean squares for general combining ability (GCA) for all the characteristics evaluated were highly significant at the 1% and 5% levels of probability, by the F test.

The significant GCA and SCA differences observed among the cultivars indicated that additive and non-additive genetic effects were involved in the control of these characteristics. Therefore, new cultivars could be obtained from these parents. The mean squares for GCA were greater than those corresponding to SCA for the assessed characteristics.

Estimates of the effects of GCA (g_i) of the parents for the grain yield and yield components assessed, revealed that BRS Campeiro, FT Soberano, and Talismã cultivars presented larger estimate g_i values, expressed respectively by 120.172**,** 55.800, and 15.779, for grain yield.

Most of combinations presented positive values of relative heterosis (H_{MP}), with emphasis for the combination between FT Soberano x BRS Campeiro, whose magnitude was of 123.76%.

In conclusion, the cultivars BRS Campeiro, FT Soberano, and Talismã are indicated for obtaining yield increase, which may be used in intrapopulation breeding programs. Among the 15 hybrids evaluated, the most promising combinations were IPR Uirapuru x IAC Tibatã, IPR Uirapuru x FT Soberano, BRS Campeiro x IPR Juriti and BRS Campeiro x IAC Tibatã, with respective magnitudes of 389.15, 374.26, 219.60, and 198.06. These hybrids presented high estimates of specific combining ability, the parents assessed could be used in interpopulational breeding program.

Table 1. Mean squares from diallel analysis of F_1 generation and parents

SV	DF	Mean Squares[1/] NPP	MNSP	MSW	GY
Treatments	20				
GCA	5	14.572**	0.528**	19.610**	196855.73*
SCA	15	15.817**	1.093**	21.488*	209416.69*
Error	40	14.157**	0.340**	18.984*	192668.75*
		3.620	0.093	4.855	85369.21
Mean square of effects					
GCA					
SCA		0.508	0.0416	0.693	5168.64
Error		3.512	0.082	4.710	35766.51
		3.620	0.093	0.022	85369.21

[/]NPP = mean number of pods per plant; MNSP = mean number of seeds per pod; MSW = mean seed weight; GY = grain yield; DF = degree of freedom; SV = source of variation; GCA = general combining ability; SCA = specific combining ability.

** = significant at 1% level; * = significant at 5% level.

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DRY MATTER PARTITIONING IN COMMON BEAN SOLE CROP AND INTERCROP WITH SUNFLOWER

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Introduction. The production of common bean intercropped with maize is one of the farming systems more used by the small farms, nevertheless, when is associated with this maize exists a reduction in the production of biomass with respect to sole crop (Kandel *et al*. 1997). The objective of this study was to determine whether the production and dry matter partitioning of common bean are reduced when they are sown with sunflower.

Material and Methods. The study was conducted in Montecillo, Méx., in summer, 2002. There were six treatmens: three intercrops of common bean (*Phaseolus vulgaris* L.) cvs. Canario, Bayomex (determinate type) and Michoacan (indeterminate type) and one of sunflower (cv. Victoria), and three sole crops of common bean. Pure stands and intercrops were sown at population density of 8.3 (bean) and 4.2 (sunflower) plants m^{-2} on 25 may, 2002 in a dry clay soil with pH 7.8 and content of organic matter and total nitrogen of 3.8 % and 47 kg ha^{-1} respectively. All experiment was fertilized with 100-100-0 NPK. The experimental design was randomized blocks with factorial arrangement, with 4 replicates. At physiological maturity we evaluated total biomass and dry matter partitioning.

Results and Discussion. The analysis of variance (ANOVA), it did not show to significant changes due to the factor sowing system (S), nor for the interaction you cultivars * sowing systems (C*S). Differences between bean cultvars (C) were observed. At physiological maturity the production of total biomass of the cv. Michoacan in sole crop (1336 g m^{-2}) and intercrop with sunflower (1346 g m^{-2}) was upper to the remaining treatments, this as a result of a greater allocation of dry matter in leaf, stem, pod and seed..

In all cultivars, the greater dry matter partitioning happened in the stem. In cultivars of determinate type (Canario and Bayomex) the greater proportion of dry matter after the stem it corresponded to pericarp, to the leaves and the seed (figure 1). Opposite tendencies were found

by Escalante and Kohashi (1980), in where report that the greater partition of biomass in the common bean cv. Michoacan 12-A-3 was in the leaves, seed, stem and pericarp. With respect to Michoacan (indeterminate type) in figure 1, it is observed that the major dry matter partitioning after the stem , it happened in the seed (which was reflected in a greater harvest index), pericarp and leaf, similar results were found by Martinez and Kohashi (1990) in bean cv. Negro 150.

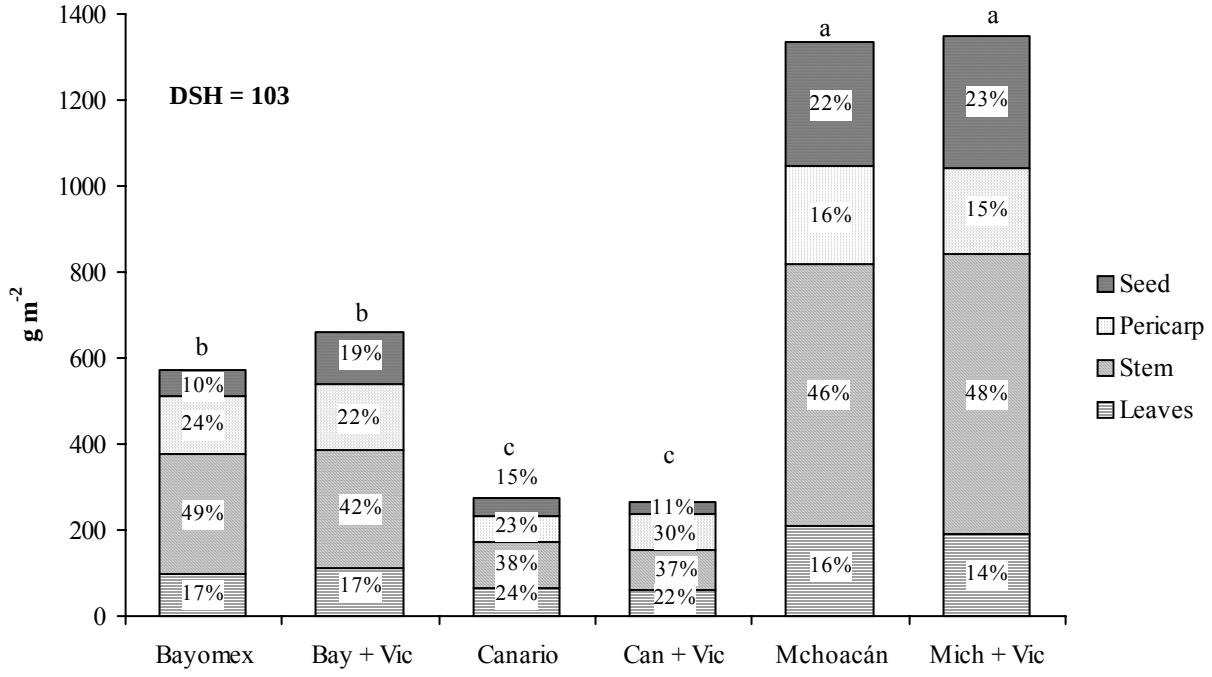

Figure 1. Biomass, allocation and dry matter partitioning in common bean sole crop an intercrop. Montecillo, Méx. Summer 2002.

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Small-Scale Bean Thresher and Seed Cleaner

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At Washington State University Vancouver Research and Extension Center, we have developed some small-scale technologies to thresh and clean dry bean seeds and grains. Our program provides small-scale growers with cost effective and appropriate technologies that enable them to produce and market dry beans. For a full description of the bean thresher and seed cleaner, see our web page, http://sustainableseedsystems.wsu.edu/nicheMarket/smallScaleThreshing.html.

Bean Thresher. To make a small-scale bean thresher, we followed and slightly modified the design of Allen Dong and Roger Edberg, I-Tech, PO Box 413, Veneta OR 97487, which is posted on the U.C. Davis website, http://agronomy.ucdavis.edu/LTRAS/itech/thresh.html#shred. This design is public domain and has no copyright. The thresher is a modified chipper-mulcher and some of the key elements include: thresher hammers that are bolted together so they beat plant material but do not shred or grind it; 3/4 to 3/8-inch mesh in the bottom that keeps the plant material within the thresher until beans have been threshed from pods; a 1.5 HP/1725 RPM motor that operates the thresher and needs to have its own cooling fan; 9-inch and 2-inch pulleys that generate a thresher speed of about 500 RPM.

To use the thresher, harvest beans from the field before pods begin to shatter, however, plants must be sufficiently dry to thresh. Plants that are too moist will bind-up the thresher mechanism. Dry plants outside for 3 days if weather is sunny and dry. Turn the plants 1-2 times each day to increase drying. If weather is cool or moist, dry beans inside using a box fan. Beans are sufficiently dry if the pods begin to shatter, but are too dry if beans split. Place either whole plants or pods only, as you choose, into the thresher chute, located at the top of the machine. Beans, crushed pods, stems and leaves will fall out the bottom of the thresher into the collection box. Beans tend to fall to the bottom of the collection box while plant debris tends to be on top. Scoop off the plant debris and discard. It takes approximately 5 minutes to thresh 100 plants with this technique.

Seed Cleaner. Our bean cleaner design is based on a Clipper Seed Cleaner, which was commonly used throughout North America in the late 1800s and early 1900s. This cleaner removes chaff, light seeds and other light debris. In our design, a large squirrel cage type fan (ours is from a greenhouse blower) provides the airflow, powered by a light duty electric motor with 1750-rpm CCW rotation. The motor pulley is 8-inches while the opposing pulley is 2-inches. The rotation direction of the motor needs to be set to blow debris out the back of the cleaner. Airflow is very high, and is excellent for beans, although there would be a need to slow down the fan or block its flow for other seeds. The chute is 36-inches in height, and sides are cut from ¾-inch plywood using an elongated version of the Clipper air chute as the template. The top and bottom of the chute are made with Luan, a thin pressboard material with a smooth coating on one side, but a thin flexible material such as sheet metal or plastic may be used. The Luan is stapled to the top and bottom of the air chute and facilitates the movement of beans and debris. We constructed a slanted shelf at the top of the cleaner where beans are poured into the cleaner. The opening into the cleaner is 1.5-inches and is small enough that debris is not blown

back. Beans fall down the chute into a collection box located under the motor while debris is blown out the back at the top.

To use the seed cleaner, place the beans along with the fine plant debris into the top chute of the cleaner. The beans quickly fall into a collection box at the bottom, while the remnants are blown out the back of the cleaner. Remove by hand small rocks and dirt clods that fall into the collection box with the beans.

Figure 1. Bean thresher made from a modified chipper-mulcher at WSU Vancouver REU.

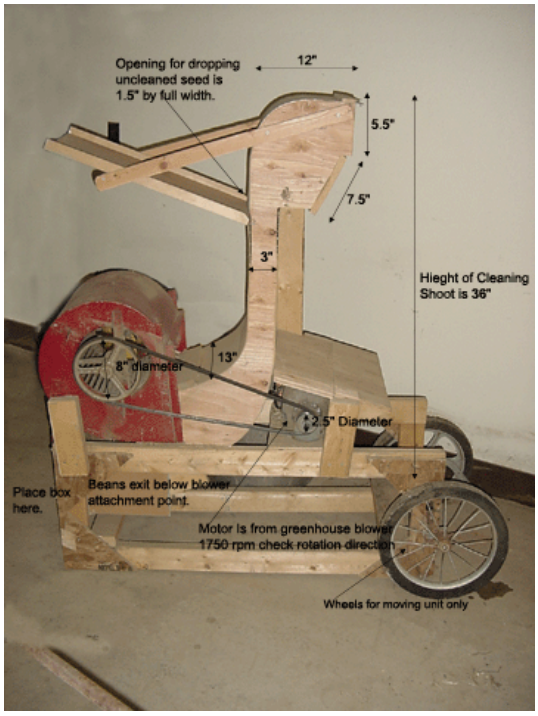

Figure 2. Seed cleaner constructed at WSU Vancouver REU.

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RELEASE OF PARTIAL WHITE MOLD RESISTANT PINTO BEAN GERMPLASM LINES USPT-WM-1 AND USPT-WM-2

The Agricultural Research Service, U.S. Department of Agriculture, the North Dakota State Agricultural Experiment Station, and the Agricultural Experiment Station of Michigan State University announce the release of USPT-WM-1 and USPT-WM-2 pinto bean (*Phaseolus vulgaris* L.) germplasm lines with partial resistance to white mold caused by the fungal pathogen *Sclerotinia sclerotiorum* Lib. deBary. Scientists participating in the development of this germplasm were Phil Miklas (USDA-ARS, Prosser, WA), Ken Grafton (North Dakota State University), Darrin Hauf (North Dakota State University), and James Kelly (Michigan State University). White mold is rated the number one economic disease problem of dry bean production in the U.S., and is a major problem in pinto beans, which are highly susceptible to the disease. The disease is endemic in all production regions of the U.S., and is most problematic under moist conditions resulting from rains or excess irrigation during flowering and mid-pod fill stages from late July through August. The resistance in USPT-WM-1 and USPT-WM-2 is conferred in part by two QTL that derive from ICA Bunsi navy bean (synonymous with Ex Rico 23 in Canada).

USPT-WM-1 and USPT-WM-2, previously tested as AN-37 and AN-69, respectively, derive from a recombinant inbred population from the cross 'Aztec'/ND88-106-04. Aztec is a semi-upright pinto bean cultivar from Michigan State University that is susceptible to white mold. ND88-106-04, from the cross N85007/ICA Bunsi, is an upright navy bean breeding line from North Dakota State University with resistance to white mold putatively derived from ICA Bunsi. USPT-WM-1 and USPT-WM-2 are F_5 derived bulk lines from separate F_2 plants that underwent generation advance by random single-seed descent method for four generations ($F_{2:3:4:5}$) from F_2 to F_5.

Initially, $F_{5:7}$ bulks of USPT-WM-1 and USPT-WM-2 were selected based on superior partial resistance to white mold and agronomic characteristics across four white mold field environments in ND and WA in 2001 and 2002. Across environments, mean disease score based on a scale from 1 to 9, where 1 is no visible infection and 9 is a completely susceptible reaction, was 3.7 and 4.0 for USPT-WM-1 and USPT-WM-2, respectively, compared to 6.8 for Aztec and 5.0 for ICA Bunsi. The lines also exhibited upright Type IIb/IIIa growth habits with disease avoidance characteristics including open canopy scores of 2.4 and 3.8, respectively, based on a 1 to 5 scale where 1 is a completely open and 5 a completely closed canopy, compared to scores of

2.7 for Aztec and 3.7 for ICA Bunsi; taller canopy heights of 49 and 50 cm, respectively, compared to 41 and 44 cm for Aztec and ICA Bunsi; reduced lodging scores of 3.9 and 5.0, where 1 is no lodging and 9 completely lodged, compared to 6.2 and 6.3 for Aztec and ICA Bunsi; and slightly later maturity of 94 and 97 days compared to 90 and 96 days for Aztec and ICA Bunsi. Both lines exhibit stay-green stem trait with scores of 2.6 and 3.8 based on 1 to 5 scale, where 1 = 0 to 20% and 5 = 80 to 100% stay-green stem, compared to 1.8 and 3.8 scores for Aztec and Bunsi, respectively. Seed size based on weight of 100 seeds was 33.6 and 34.7 g for USPT-WM-1 and USPT-WM-2, respectively, and 33 g for Aztec. Yield was 2908 and 2667 lbs/A compared to 2552 lbs/A for Aztec.

In 2003, both lines were tested in the international Bean White Mold Nursery administered by Jim Steadman, University of Nebraska. Average mean ranking for resistance among 13 entries across 12 separate greenhouse and field tests was 5.1 for USPT-WM-1 and 7.5 for USPT-WM-2 compared to 6.2 for ICA Bunsi. In a 2004 white mold nursery conducted in Michigan, USPT-WM-1 and USPT-WM-2 had the second and third highest yields, 4370 and 4250 lbs/A, respectively, of 64 entries. 'Buster' pinto at 3600 lbs/A was the next closest pinto bean in yield. The weight of 100 seeds in this trial was 35 and 38 g compared to 34 g for Buster. Harvest maturity of the lines was 1 and 4 days later than the 92 days for Buster. Desirability scores were 5.0 and 6.5 for USPT-WM-1 and USPT-WM-2, respectively, compared to 5.0 for Buster, based on a 1 to 7 scale where 1 is undesirable and 7 highly desirable plant growth appearance. The desirability score for USPT-WM-2 was the highest observed among the 64 lines and cultivars tested. The mean white mold disease score based on percentage infection was 41 and 30% compared to 37% for ICA Bunsi and 56% for Buster.

Seed appearance for both lines is in the pinto bean market class, but the background color is darker than commercial cultivars, which may be due in part to the *I* gene for resistance to *Bean common mosaic virus* that is present in both cultivars being derived from the navy bean parent ND88-106-04. The *I* gene and its tight association with the *B* locus, depending upon source, is known to cause seed darkening in pinto, red, and pink bean market classes. Both lines were susceptible to bean rust Race 53 in greenhouse pathogen tests conducted at North Dakota State University and exhibited moderate susceptibility to *Beet curly top virus* in Washington.

USPT-WM-1 and USPT-WM-2 will be most useful for incorporating resistance to white mold primarily in the pinto bean market class, but also in the medium-seeded great northern, pink, and small red market classes as well. Seed will be maintained by USDA-ARS at Prosser, WA, and provided in small quantities upon written request. We ask that appropriate recognition of source be given when this germplasm contributes to the development of a new cultivar or germplasm line.

_____ _____

Director, North Dakota Agricultural Experiment Station Date
North Dakota State University

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Director, Michigan Agricultural Experiment Station Date
Michigan State University

_____ _____

Administrator, Agricultural Research Service Date
U.S. Department of Agriculture
Authorship: Miklas, Grafton, Hauf, and Kelly

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and
IDAHO AGRICULTURAL EXPERIMENT STATION
MOSCOW, IDAHO 83844

RELEASE OF COMMON BACTERIAL BLIGHT RESISTANT DARK RED KIDNEY BEAN GERMPLASM LINE USDK-CBB-15

The Agricultural Research Service, U.S. Department of Agriculture, and the Idaho Agricultural Experiment Station announce the release of USDK-CBB-15 dark red kidney (*Phaseolus vulgaris* L.) germplasm line with a high level of resistance to common bacterial blight caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*). Scientists participating in the development of this germplasm were Phil Miklas (USDA-ARS, Prosser, WA), James Smith (USDA-ARS, Stoneville, MS), and Shree Singh (University of Idaho). Common bacterial blight is a major seed-borne disease of dry and snap beans worldwide. The disease is endemic to the U.S. bean production regions east of the continental divide and problematic in Colorado, Michigan, Minnesota, Nebraska, New York, North Dakota, and Wisconsin. Genetic resistance in the host provides the most effective control of this disease, and planting certified disease-free seed is critical. USDK-CBB-15 possesses two major QTL and perhaps other minor genes that confer a high level of resistance to *Xap*. Marker-assisted selection using the SAP6 and SU91 markers tightly linked with QTL derived from great northern landrace cultivar Montana No.5 and breeding line XAN 159, respectively, enabled us to expedite development of USDK-CBB-15 for combating this entrenched disease problem in the U.S.

USDK-CBB-15 (previously tested as PS99-009F-5-15-1) derives from a "modified" backcrossing scheme (dark red kidney*4/XAN 159); modified, because a different dark red kidney parent was used for each backcross, and the initial cross underwent two generations of pedigree selection. Thus, USDK-CBB-15 is a modified $BC_3F_{1:4}$ bulk from the cross K97305/3/ SVM-2242//I9566-21-4-2/'Montcalm'. K97305 is an advanced dark red kidney breeding line from Michigan State University with high yield potential. SVM-2242 is an early maturing dark red kidney breeding line from Sacramento Valley Milling. I9566-21-4-2 is an F_3 derived line from the cross Montcalm/XAN 159 selected for presence of SAP6 and SU91 markers and resistance to common bacterial blight in greenhouse leaf inoculation assays. XAN 159 with the pedigree UI-114/PI319441//PI319443/3/'Masterpiece' is an advanced breeding line from CIAT with resistance to common bacterial blight derived via interspecific hybridization with tepary bean (*P. acutifolius*). XAN 159 is the source of a major resistance QTL linked with the SU91 SCAR marker also developed at CIAT. Montcalm with the pedigree GN No.1/'Dark Red Kidney' is a dark red kidney cultivar from Michigan State University with moderate resistance to common bacterial blight conferred by a major QTL linked with the SAP6 SCAR marker (developed by USDA-ARS, Prosser, WA) that was derived from Montana No.5 via Great Northern No.1.

Marker-assisted selection was employed each backcross to identify BC_nF_1 plants with the SAP6 and SU91 markers for subsequent backcrossing with the susceptible dark red kidney parents. From the last backcross a BC_3F_1 plant (PS99-009F) with both markers was selfed to produce an

F_2 progeny which was planted in the field in Prosser, WA, and screened for seed type. F_3 progenies from F_2 single plant selections (PS99-009F-5) were tested for reaction to common bacterial blight in leaf inoculations tests conducted at the USDA-ARS Tropical Agriculture Research Station at Mayaguez, PR. An individual F_3 plant (PS99-009F-5-15) with high level of resistance and confirmed to possess SAP6 and SU91 markers was selfed to produce an F_4 progeny which was screened for bacterial blight reaction at Mayaguez. An F_4 plant (PS99-009F-5-15-1) with high level of resistance was selected to produce USDK-CBB-15 that was subsequently increased for three generations and evaluated in multiple greenhouse tests for reaction to common bacterial blight and examined in the field for yield and maturity.

USDK-CBB-15, in a greenhouse leaf inoculation test conducted at Kimberly, ID, in January 2004, had a mean disease score of 2 based on a 1 to 9 scale where 1 is no visible infection and 9 is completely susceptible. In comparison, the common bacterial blight resistant dark red kidney bean line USDK-CBB-10, released by USDA-ARS, Prosser, WA, in 2001, which possesses SAP6 QTL had a mean disease score of 7. In a repeated test in December 2004, USDK-CBB-15 scored 3.6 compared to 8.6 for USDK-CBB-10 and 8.7 for Montcalm. USDK-CBB-15 possesses both the SAP6 and SU91 markers linked with major QTL for resistance derived from Montana No.5 (via Montcalm) and tepary bean (via XAN 159), respectively. Thus, USDK-CBB-15 exhibits a much higher level of resistance to common bacterial blight than USDK-CBB-10 and Montcalm.

USDK-CBB-15 exhibits a Type I determinate bush growth habit typical of kidney bean. Yield was 108% of 'Red Hawk' dark red kidney at Othello,WA, in 2004. Average weight of 100 seeds was 52 g, same as Red Hawk. USDK-CBB-15 matured in 98 d, two days later than Red Hawk. Seed appearance was rated commercially acceptable for the dark red kidney market class. USDK-CBB-15 also exhibits a hypersensitive resistance response to the NL-3 strain of *Bean common mosaic necrosis virus* (BCMNV) in Prosser greenhouse tests, which infers presence of the *I* gene for resistance to *Bean common mosaic virus* (BCMV).

USDK-CBB-15 will be most useful for incorporating resistance to common bacterial blight in the dark red kidney market class, but also other large-seeded market classes of Andean origin as well. Seed will be maintained by USDA-ARS at Prosser, WA, and provided in small quantities upon written request. We ask that appropriate recognition of source be given when this germplasm contributes to the development of a new cultivar or germplasm line.

_____ _____

Director, Idaho Agricultural Experiment Station Date
University of Idaho

_____ _____

Administrator, Agricultural Research Service Date
U.S. Department of Agriculture
Authorship: Miklas, Smith, Singh

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UNIVERSITY OF NEBRASKA AGRICULTURAL RESEARCH DIVISION
LINCOLN, NEBRASKA 68503

RELEASE OF BELMINEB-RMR-8, -9, -10, -11, -12, and -13, ERECT, SHORT VINE, RUST AND MOSAIC RESISTANT GREAT NORTHERN BEAN GERMPLASM LINES

The Agricultural Research Service, U.S. Department of Agriculture, The Michigan Agricultural Experiment Station, and University of Nebraska Agricultural Research Division announce the release of six rust and mosaic resistant, high yielding, upright short vine, type II, white seeded, great northern dry bean germplasm lines, BelMiNeb (BMN)-Rust and Mosaic Resistant (RMR)-8, -9, -10, -11, -12, and -13.

BMN-RMR-8, -9, -10, -11, -12 and -13 are the first and only great northern bean lines to combine four genes for resistance to the bean rust pathogen, *Uromyces appendiculatus*, with two genes for resistance to the viruses bean common mosaic (BCMV) and bean common mosaic necrosis (BCMNV). All six lines are homozygous for their rust and mosaic resistance genes which provide resistance to all 90 races of the bean rust pathogen that have been identified and maintained at Beltsville, MD, and to all known strains of BCMV and BCMNV. BMN-RMR-8, -9, -10, -11, -12 and -13 are the first released great northern bean lines to combine the *Ur-6* with *Ur-3*, *Ur-4*, and *Ur-11* rust resistance genes. They also combine the *bc-3* and *I* mosaic resistance genes. These lines were developed and selected by Dr. M. A. Pastor-Corrales, Vegetable Laboratory, Plant Sciences Institute, USDA, ARS, Beltsville, Maryland; Dr. J. R. Stavely, formerly of the Molecular Plant Pathology Laboratory; Dr. James D. Kelly, Crop and Soil Sciences Department, Michigan State University, East Lansing, Michigan; Dr. James Steadman, Department of Plant Pathology, the late Dr. Dermot P. Coyne, and Dale T. Lindgren, Department of Agronomy and Horticulture, University of Nebraska, Lincoln, Nebraska.

The most important source of rust resistance in BMN-RMR-8, -9, -10, -11, -12, and -13 is the *Ur-11* gene that is effective against 89 of the 90 races of *U. appendiculatus* maintained at Beltsville. U. S. Department of Agriculture plant introduction (PI) 181996 and PI 190078 are sources of the *Ur-11*. Both of these PIs have black seeds, indeterminate growth habit, and are photoperiod sensitive, flowering only in long night/short day situations. They were introduced from Guatemala in 1949 and 1950, respectively, and their comprehensive rust resistance was identified at Beltsville in the late eighties. The single race of *U. appendiculatus*, known as race 108, for which *Ur-11* is not effective, is controlled by *Ur-3* and *Ur-4*. The *Ur-3* rust resistance gene is also effective against 44 races maintained at Beltsville. The *Ur-3* gene has remained effective against rust pathogen races/pathotypes in dry beans in the United States since its

introduction into dry bean cultivars 12 years ago. This gene is also effective in South Africa where rust is the most devastating disease of dry beans. Published research from Beltsville has shown *Ur-3* to be linked to *Ur-11* in repulsion. This linkage was broken to recombine *Ur-3* with *Ur-11* in the previously released pinto (BelDakMi-RMR-14, -15, -16, -17, and -18) and great northern (BelMiNeb-RMR-7) germplasm lines. The *Ur-4* gene that also controls race 108 is effective against 29 races of *U. appendiculatus* that are maintained at Beltsville. In addition, all six lines being released contain the independent *Ur-6* gene that is effective against 22 races. The *Ur-3* and *Ur-6* genes are epistatic to *Ur-11* for all the races controlled by them and *Ur-11*. Michigan pinto breeding lines P94207 and P94232 are the sources of the *Ur-3* and *Ur-6* genes and the type II growth habit. Line P94207 was released as 'Kodiak', with *Ur-3, Ur-6*, *bc-1*^{2}, and *I* in 1998. The source of the *Ur-4* gene in BMN-RMR-8, -9, -10, -11, -12, and -13 is the great northern line BelMiNeb(BMN)-RMR-3 into which *Ur-4* had been introgressed from the navy line BelMiDak(BMD)-RR-2. The original source of *Ur-4* in BMD-RR-2 was the snap bean Early Gallatin. The *Ur-4* rust resistance gene is present in many snap bean cultivars. Pinto line P 94232 and great northern line G 94567 were the source of *bc-3*. Several lines and cultivars in the pedigree of BMN-RMR-8, -9, -10, -11, -12, and -13, were the sources of the *I* gene for resistance to BCMV. The combination of dominant *I* and recessive *bc-3* genes with distinctly different mechanisms of resistance offer complete, and probably durable resistance, to all known strains of BCMV and BCMNV.

BMN-RMR-8, -9-10, -11, -12, and -13 are derived from a series of F_1, F_2, and F_3 crosses, backcrosses and selections containing desired rust and mosaic resistance genes. 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 initial cross was followed by subsequent selections for great northern seed type and desired rust and mosaic disease resistance genes. 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, the great northern parent 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.

The rust resistance of BMN-RMR-8, -9, -10, -11, -12, and -13 was confirmed by inoculation under greenhouse conditions with available races of *U. appendiculatus*. The rust resistance of progenitor plants of these releases was confirmed by results from inoculations with eight selected races of the bean rust pathogen that produce well proven and characteristic reactions in bean plants that have the *Ur-3*, *Ur-4*, *Ur-6*, and *Ur-11* genes. Mosaic resistance was confirmed from inoculation with BCMV strains NL4 and or US 5, and BCMNV strain NL3. Presence of the *I* gene was reconfirmed by using molecular markers tightly linked to the I gene. These lines were selected from 272 rust resistant great northern lines evaluated in September 2000 at the Saginaw Valley Bean and Sugar Beet Research Farm, Saginaw, Michigan, for erect plant habit (type II); early maturity, good pod-to-ground clearance; desirable seed size, color, and shape; and high yield. BMN-RMR-8, -9, -10, -11, -12, and -13 consist of bulked F_6 and F_7 greenhouse seeds from all F_5 plants from the remnant seeds from the same seed lot used for field evaluations. The rust resistance of these lines is expressed as symptomless to faint chlorotic reaction to races controlled by *Ur-6*, faint nonsporulating chlorotic to well defined, small necrotic reactions to all

races controlled by *Ur-3*, but not by *Ur-6*, as faint chlorotic spots to all races controlled by *Ur-4 and Ur-11*, and as tiny (less than 0.3 mm in diameter) uredinia to the races controlled by Ur-11, but not controlled by *Ur-3*, *Ur-4*, and *Ur-6*. Resistance to BCMV and BCMNV is expressed as symptomless, apparently immune reaction.

BelMiNeb-RMR-8 (tested in the field as 5-4051) produced under field conditions erect plants with moderately early maturity, high yield, good pod-to-ground clearance, and large, attractive, dull, white, great northern seeds that averaged 33.9 grams/100 seeds. The seed weight compared well with the great northern cultivars Alpine (31.2 grams), Weihing (34.5 grams), and Starlight (36.6 grams). BMN-RMR-8 was rated

BelMiNeb-RMR-9 (tested in the field as 5-4059) produced under field conditions erect plants with moderately early maturity, high yield, good pod-to-ground clearance, and white, large great northern seeds that averaged 34.3 grams/100 seeds.

BelMiNeb-RMR-10 (tested in the field as 6-1911) produced under field conditions erect plants with moderately early maturity, high yield, good pod-to-ground clearance, and white, large great northern seeds that averaged 33.3 grams/100 seeds.

BelMiNeb-RMR-11 (tested in the field as 6-2267) produced under field conditions erect plants with moderately early maturity, high yield, good pod-to-ground clearance, and white, large great northern seeds that averaged 36.7 grams/100 seeds.

BelMiNeb-RMR-12 (tested in the field as 6-1772) produced under field conditions erect plants with moderately early maturity, high yield, good pod-to-ground clearance, and white, large great northern seeds that averaged 35.7 grams/100 seeds.

BelMiNeb-RMR-13 (tested in the field as 6-2298) produced under field conditions erect plants with moderately early maturity, high yield, good pod-to-ground clearance, and white, large great northern seeds that averaged 36.3 grams/100 seeds.

BMN-RMR-8, -9-10, -11, -12, and –13 are homozygous for the indicated rust and mosaic resistance genes and many other characteristics, but because they are being released as bulked F_6 and F_7 seeds from numerous F_5 and F_6 plants they still have some variability and are likely to be still segregating for some characteristics. It is recommended that those who obtain seeds should plant them individually, save the seeds from each plant, and select from the subsequent generations for desired characteristics. A limited quantity of seed is available from Dr. M. A. Pastor-Corrales, Vegetable Laboratory, Room 240, Building 010 A, BARC-West, ARS, USDA, 10300 Baltimore Avenue, Beltsville, MD 20705-2350. Seeds of these lines will be deposited in the National Plant Germplasm System to be available for research development of new cultivars or germplasm. When any of these lines contributes to a new cultivar, it is requested that recognition be given to the source.

Director, Michigan Agricultural Experiment Station	Date
Director, University of Nebraska, Agricultural Research Division	Date
Deputy Administrator, Crop Production and Protection Agricultural Research Service, USDA	Date

Release of CSU FW-1 and CSU FW-2 Fusarium Wilt Resistant Pinto Germplasm Lines

M.A. Brick, J.B. Ogg, H.F. Schwartz, J.J. Johnson, and F. Judson, Colorado State University; and S. P. Singh, University of Idaho.

The Colorado Agricultural Experiment Station announces the release of two pinto bean (*Phaseolus vulgaris* L.) germplasm lines CSU FW-1 and CSU FW-2. CSU FW-1 tested as CO 96737 and CSU FW-2 tested as CO 83810 were developed at Fort Collins, CO for resistance to Fusaruim wilt (FW) caused by *Fusarium oxysporum* Schlechtend:Fr. f. sp. *phaseoli* (Kendrick and Snyder) (*Fop*). CSU FW-1 is an $F_{5:8}$ line derived from the cross BelDakMi RR-3/CO 07010-2. BelDakMi RR-3 was released by Dr. Rennie Stavely, USDA/ARSBeltsville, MD and carries the *Ur-11* and *Ur-6* alleles that confer resistance to 64 races of rust caused by (*Uromyces appendiculatus* [Pers.] Unger var. *appendiculatus*]. CO 07010-2 providing Fusarium wilt resistance, was derived from the cross NW 410/Roza //BAC 125. NW 410 and Roza are pinto and pink cultivars respectively. BAC 125 is an indeterminate upright growth habit Type II small white-seeded experimental line developed at the International Center for Tropical Agriculture (CIAT). BAC 125 possesses tolerance to common bacterial blight and the *I* resistance gene for *Bean common mosaic virus* (BCMV). Average severity index for CSU FW-1 for reaction to *Fop* race 4 was 3.0, compared to 9.0 for the susceptible pinto 'UI 114'. CSU FW-1 is also resistant to the predominant strains of bean rust in the High Plains, however the specific allele composition is not known. CSU FW-1 has been tested for yield in the Midwest Regional Performance Nursery, the Western Regional Bean Trials, and by the Crops Testing Program at Colorado State University (Johnson et al. 2002, 2003). Yield for CSU FW-1 and 'Bill Z' were 2097 and 2351 kg ha^{-1}, respectively across 11 test environments in Colorado. Seed weights were 40.6 and 36.8 g 100 $seed^{-1}$, respectively. CSU FW-1 is susceptible to BCMV.

CSU FW-2 is an $F_{3:5}$ line derived from the cross Bill Z//BelDAkMi RR-3/CO 07010-2. Pinto 'Bill Z' was released by Colorado State University in 1985 (Wood et al., 1989). Average severity index for CSU FW-2 for reaction to *Fop* race 4 was 1.0, compared to 9.0 for UI 114. CSU FW-2 is resistant to the predominant strains of bean rust in the High Plains, however the specific allele composition is not known. Average seed yield for CSU FW-2 and Bill Z were 1221 and 1908 kg ha^{-1}, respectively over two test environments at Fort Collins, CO. Seed weights were 38.0 and 35.4g 100 $seed^{-1}$, respectively. CSU FW-2 has not been tested for reaction to BCMV.

These germplasm lines combine mid-season maturity, semi-upright growth habit, and moderate yield potential and will provide FW resistance genes for development of cultivars of pinto and other market classes.. Limited seed quantities of CSU FW-1 and CSU FW-2 may be obtained from Mark Brick, Department of Soil and Crop Sciences, Colorado State University, Fort Collins, CO upon written request.

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and

THE UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURE RESEARCH SERVICE
WASHINGTON, D.C. 20250

RELEASE OF 'QUINCY' PINTO DRY EDIBLE BEAN

The Agriculture Research Center of Washington State University and the Agricultural Research Service, U.S. Department of Agriculture jointly announce the release of 'Quincy' a new pinto dry bean (*Phaseolus vulgaris* L.). This new release was developed to provide a superior virus resistant pinto cultivar for the Northwestern states bean growing areas. This will be the first pinto cultivar released by WSU/USDA - ARS to possess dominant *I* gene resistance to seed borne bean common mosaic virus (BCMV) and *bc-2*^{2} gene, which combination provides complete resistance to all known strain of BCMV worldwide. Scientists participating in the development of this variety were A.N. Hang (Washington State University), M.J. Silbernagel (retired, USDA-ARS), and P.N. Miklas (USDA-ARS-Prosser).

Quincy pinto ($F_{6:9}$), breeding line USPT-73, was derived from a cross RR 'Othello'//'Othello'/A-55 made in 1991. RR Othello is a rust resistance pinto selected from Othello released in 1986 by D.W. Burke. A-55 is a black-seeded, upright type II-A plant growth habit developed by S.P. Singh in Columbia. Quincy pinto has *Ibc-2*^{2} gene resistance to BCMV and complete resistance to curly top virus (CTV). Quincy is a type 2 to 3 plant growth habit depending upon the weather conditions of each year. Quincy is taller than Othello and about 4 to 7 days later than Othello in maturity. It is a medium to late maturity pinto. Quincy plant is taller than Othello and is also more upright with short vine than Othello. Quincy yielded 21% and 48% higher than Othello

and Burke, respectively, under stress conditions of inadequate fertilizer and soil moisture and heavy root rot pressure soil (mainly *Fusarium solani).* Quincy is susceptible to bean rust caused by *Uromyces appendiculatus* (pers.:Pers) Unger. Quincy (previously tested as LB2008 and USPT-73) has higher yield than Othello in the National Cooperative dry bean nurseries and comparable to other pintos grown in Colorado. At Othello, Washington Quincy and Othello averaged 3,813 kg ha^{-1} compared to 3,905 kg ha^{-1} in 7 years from 1996 to 2003, respectively. Seed of Quincy is slightly larger than Othello 43.7 vs 39.6 g per 100 seeds. Quincy is an acceptable canner in trials conducted by USDA-ARS and the Michigan Agricultural Experiment Station in 1997 - 1998 and at New York Agricultural Experiment Station in 2002 and 2003.

Quincy has been released as a non exclusive public variety without Plant Variety protection. Breeder and Foundation seed will be maintained by Washington State Crop Improvement Association, Inc. Department of Crop and Soil Sciences, WSU Seed House, Pullman, WA 99164-6420.

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Director, Washington Agricultural Research Center Date

_____ _____

Administrator, Agricultural Research Service Date
U.S. Department of Agriculture

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RELEASE OF DARK RED KIDNEY DRY BEAN 'FIERO'

The Agricultural Research Center of Washington State University, the Idaho Agricultural Experiment Station and the Agricultural Research Service, U.S. Department of Agriculture, jointly announce the release of a new dark red kidney dry bean 'Fiero'. This cultivar is a high yielding, upright bush, mid-season maturity, and disease resistant dark red kidney dry bean adapted to the U.S. Pacific Northwest.

Fiero is an F_6 derived F_9 line from the cross 'Montcalm'/K-59. Montcalm is a very popular commercial cultivar release by Michigan State University that has long been the dark red kidney standard; however, its yields are generally low. Germplasm line K-59 is a curly top virus (CTV) resistant light red kidney germplasm developed by D.W. Burke. Both parents have dominant *I* gene resistance to bean common mosaic virus (BCMV) and an intermediate level of resistance to halo blight. The stems, leaves and pods of K-59 are resistant to Race 2 of the halo blight bacterium [*Pseudomonas syringa* pv *phaseolicola* (Burkholder) Young et al.]. Fiero was selected from individual plants possessing desirable disease resistance, seed quality and architectural traits. Fiero has complete resistance to CTV and *I* resistance to BCMV. Fiero also has tolerance to halo blight. Fiero has an upright determinant bush growth habit (Type I growth habit) like Montcalm and resistance to lodging.

Tested across 40-location years in the Cooperative Dry Bean Nursery (CDBN) in 1997 and 1998, Fiero was the highest yielding dark red kidney bean, 17% yield higher than Montcalm. Fiero matures in 100 d, 1 to 2 d later than Montcalm. Seed size is slightly larger than Montcalm, 57 vs. 55 g 100^{-1} seeds. Fiero had acceptable canning quality for overall appearance, color and shape for a dark red kidney, in tests conducted by USDA-ARS/Michigan State Agricultural Experiment Station and New York State University.

Fiero has been released as a non exclusive public variety without Plant Variety Protection. Breeder and foundation seed will be maintained by Washington State Crop Improvement Association, Inc. Department of Crop and Soil Sciences, WSU Seedhouse, Pullman, WA 99164-6420.

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Director, Washington Agricultural Research Center Date

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Director, Idaho Agricultural Experiment Station Date

_____ _____
Administrator, Agricultural Research Service Date

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THE UNITED STATES DEPARTMENT OF AGRICULTURE
WASHINGTON, D.C. 20250

RELEASE OF LIGHT RED KIDNEY DRY BEAN 'BLUSH'

The Washington Agricultural Research Center and the Idaho Experiment Station and the Agricultural Research Service, U.S. Department of Agriculture, jointly announce the release of a new light red kidney dry bean 'Blush'. This cultivar is a large seeded, upright, mid-season maturity, and disease resistant light red kidney dry bean adapted to the U.S. Pacific Northwest. Blush is an F_{10} derived F_{13} line from the cross 84BR-1122/K-42. Breeding line 84BR-1122 was a root rot [*Fusarium solani* (Mart.) Sacc. f. sp. *phaseolin* (Burkholder) W.C. Snyder & H.N. Hans] tolerant bush snap bean developed by USDA-ARS at Prosser, WA. K-42 is a light red kidney breeding line released by Burke et al. K-42 has shown resistance to halo blight [caused by *Pseudomonas syringa* pv. *phaseolicola* (Burkholder) Young et al.]. Blush was selected for individual plants possessing desirable disease resistance, seed quality and architectural traits.

Blush has complete resistance to curly top virus (CTV) and *I, bc*-1 gene resistance to bean common mosaic virus (BCMV). Blush also has some root rot tolerance from 84BR-1122. Blush has an upright growth habit (Type I) like 'Kardinal' and is resistant to lodging. Blush was yield tested as USWA-33, in the advanced yield trials in Othello for 3 years since 1995 and yield was comparable to Kardinal. Bush also has yield tested across 44-location years in 1997 and 1998 in the National Cooperative Dry Bean Nurseries (CDBN) where it has yielded 9% more than

Kardinal, 7% more than California Early Light Red Kidney (CELRK) and 7% more than 'Chinook 2000'. Blush matures in 93 d, which is 3 d later than Kardinal and Chinook 2000, and 7 d later than CELRK. Seed of Blush is larger than Kardinal, 56 vs. 52 g 100^{-1} seeds. Blush had acceptable canning quality in test conducted by USDA-ARS and the Michigan Agricultural Experiment Station, performing better than CERLK for color and shape.

Blush has been released as a non-exclusive public variety without Plant Variety Protection. Breeder and foundation seed will be maintained by Washington State Crop Improvement Association, Inc. Department of Crop and Soil Sciences, WSU Seedhouse, Pullman, WA 99164-6420.

_____ _____

Director, Washington Agricultural Research Center Date

_____ _____

Director, Idaho Agricultural Experiment Station Date

_____ _____

Administrator, Agricultural Research Service Date

'BRS SUPREMO': A BLACK COMMON BEAN CULTIVAR WITH ERECT PLANT TYPE RECOMMENDED FOR THE CENTRAL WEST AND SOUTH BRAZIL

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During the 2003/2004 growing season it was produced, in Brazil, about 2,7 million tons of common bean in an area of 2,7 million hectares meaning a national average productivity of 1.000 $kg.ha^{-1}$. Although the average productivity has been growing, the average per capita consumption has been decreasing representing an annual consumption of only 12,7 $kg.inhabitant^{-1}$.
In Brazil, the black beans national production does not meet the internal demand consumption, which occurs mostly in the states located in the south and in Rio de Janeiro and Espírito Santo. To meet this demand it is necessary an annual import of about 100 thousand tons. The common bean genetic improvement program, at Embrapa Rice and Beans, is focused on cultivars that are more productive, more resistant to diseases and have an erect plant type enabling mechanical harvest and representing a better quality product to the final consumer and a higher revenue to the farmers. With this philosophy it has been released the black bean cultivar BRS Supreme to the States of Santa Catarina, Paraná, Goiás and Federal District. Besides of the above qualities, this cultivar is resistant to several rust pathotypes, to bean common mosaic virus and to four pathotypes of the causal agent of anthracnose.
BRS Supremo is a black bean originated from the single cross between W22-34 and VAN 163, performed at Embrapa Rice and Beans in 1988. The bulk method was used in the F_2 generation. In the F_3 and F_4, after inoculation with the pathotype 89 of *Colletotrichum lindemuthianum*, the modified mass selection was performed and susceptible plants were eliminated. One pod per plant was collected from the remaining resistant plants to reconstitute the population. In the F_5 and F_7 plants were selected by the bulk method and in the generations F_6 and F_8 it was used the modified mass selection. In F_8, after inoculation with the pathotype 95 of *Colletotrichum lindemuthianum* the susceptible plants were eliminated and the remained plants were harvested individually originating the F_9 lines from where the AN 9310960 line was selected based on grain yield, erect plant type and disease resistance. In 1999 this line was evaluated together with additional 31 lines and two controls in the National Trial, conducted under six different environments, in the States of Goiás (1), Mato Grosso do Sul (2), Minas Gerais (1), Rio de Janeiro (1) and Espírito Santo (1). The joint analysis of the grain yield data and other agronomic characteristics provided the elements to promote AN 9310960 to the Regional Trial with the pre-commercial name of CNFP 7762. The line was then evaluated in a field trial for cultivar release with twelve lines and two controls in a randomized complete block design with four replications in 30 different environments in the States of Goiás (13), Federal District (2), Paraná (7) and Santa Catarina (8).
In the last 30 field trials conducted during "wet" and "dry" seasons in the States of Santa Catarina and Paraná and in field trials during de "wet" and "winter" seasons in the State of Goiás and Federal District, the line CNFP 7762 presented an average grain yield 2% superior than the

cultivars IPR 88 - Uirapuru and BRS Valente in the States of Santa Catarina and Paraná and the cultivars Diamante Negro and BRS Valente in the State of Goiás and Federal District (Table 1).

Table 1. Yield of cultivar BRS Supremo during "wet" and "dry" seasons in Santa Catarina and Paraná States and in "wet" and "winter" seasons in Goiás State and Federal District, obtained from 2001 to 2004 and compared to yields of two controls.

Region	State	Season	BRS Supremo (kg.ha^{-1})	Mean for Control[1] (kg.ha^{-1})	Relative yield (%)	Number of environments
South	SC/PR	"wet"	2464	2438	101	10
		"dry"	2499	2263	110	5
Center-West	GO/DF	"wet"	2322	2355	99	11
		"winter"	2401	2285	105	4
Media			2410	2358	102	

[1]IPR 88 - Uirapuru and BRS Valente in Santa Catarian and Paraná, and Diamante Negro and BRS Valente in Goiás and Federal District.

Cultivar BRS Supremo presents grain size and color uniformity, excellent cooking qualities and a chocolate brown broth (Table 2).

Table 2. Industrial and technological grain qualities of the black bean cultivar BRS Supremo.

Cultivar	Cooking time (minute)	Soluble solids (%)	Protein (%)	100 grain weight (g)
BRS Supremo	31,0	12,1	23,3	24,6
BRS Valente	28,1	10,9	19,2	21,5
Diamante Negro	34,0	11,2	20,0	21,3

Cultivar BRS Supremo, under artificial inoculation, was resistant to bean common mosaic virus and to the pathotypes 55 (lambda), 89 (alfa-Brazil), 95 (Kappa) and 453 (zeta) of *Colletotrichum lindemuthianum*. In field trials it was resistant to several rust pathotypes, moderate resistant to angular leaf spot and susceptible to bean golden mosaic virus and common bacterial blight.
BRS Supremo presents an erect growth habit with high yield potential in any crop system tested and under different soil and environment conditions. It has also good resistance to lodging, with a growing cycle of 83 days from emergency to physiological maturation.
BRS Supremo is a new option for bean growers involved with black bean grain type production, for the "wet" and "dry" seasons in the State of Santa Catarina and Paraná and "wet" and "winter" seasons in the State of Goiás and Federal District.
Genetic seed stocks are maintained by Embrapa Rice and Beans and basic seed is available at Embrapa Technology Transfer.

Institutions involved in the cultivar evaluation:
Embrapa Arroz e Feijão; Embrapa Cerrados; Embrapa Soja; Embrapa Negócios Tecnológicos - Ponta Grossa; Agência Goiana de Desenvolvimento Rural e Fundiário; Universidade de Rio Verde/Fesurv; Avena S/C Ltda; Cooperativa Regional Agropecuária de Campos Novos; C. Vale Cooperativa Agroindustrial; Escola Agrotécnica Federal de Concórdia; Cooperativa dos Produtores de Sementes de Laranjeiras do Sul Ltda; Sementes Campo Verde; Universidade Estadual de Londrina; Cooperativa Agrícola Mista de Prudentópolis; Detec Assessoria Técnica S/C Ltda; Anastácio Ceregatti Sanchez Ltda. (Holambra Agrícola II); Cooperativa Regional Agropecuária de Taquarituba.

‘BRS HORIZONTE’: NEW BEAN VARIETY WITH CARIOCA COMMERCIAL GRAIN TYPE FOR THE SOUTH AND CENTER WEST REGIONS OF BRAZIL

Leonardo Cunha Melo[1], Luis Cláudio de Faria[1], Carlos Agustín Rava[1], Maria José Del Peloso[1], Joaquim Geraldo Cáprio da Costa[1], José Luiz Cabrera Díaz[1], Josias Correa de Faria[1], Heloisa Torres da Silva[1], Aloisio Sartorato[1], Priscila Zaczuk Bassinello[1] and Francisco José Pfeilsticker Zimmermann[1]

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Common bean is one of the most important Brazilian crops, with a great social importance as the main daily protein source.

Due to the national bean demand for the carioca commercial grain type, the common bean breeding program at Embrapa Rice and Beans has put a great effort for developing new bean cultivars with this grain type associated to erect plant type, resistance to diseases and lodging. An example of such effort is the development of the dry bean cultivar BRS Horizonte by Embrapa Rice and Beans and the International Center for Tropical Agriculture - CIAT, as partners, and it releases to the growers of South and Center West regions of Brazil. Although BRS Horizonte does not show any yield superiority when compared to cultivars Pérola, Eté and Iapar 81 used as a control, it shows some advantages by its erect growth habit, resistance to five pathotypes of the anthracnose causal organism and to the bean common mosaic virus, earliness and good resistance to lodging.

BRS Horizonte is originated from the cross EMP 250 /4/ A769 /// A 429 / XAN 252 // Pinto VI 114, performed at International Center for Tropical Agriculture, Cali, Colombia and named as line FEB 208. In 1999 this line was evaluated together with 37 other bean lines plus three controls, in a National Bean Trial, in seven different environments in the States of Goiás (1), Mato Grosso do Sul (2), Minas Gerais (3) and Espírito Santo (1). The joint analysis of the grain yield data and other agronomic characteristics provided the elements to promote the line FEB 208 to the Regional Trial with the pre-commercial name of CNFC 8202. This line was then evaluated in a field trial for cultivar release with eighteen other bean lines and two controls in a randomized complete block design (each plot consisted of four rows of 4 m) with four replications in 33 different environments in the States of Goiás (14), Federal District (4), Paraná (7) and Santa Catarina (8).

In the 33 field trial for cultivar release conducted during "wet" and "dry" seasons in Santa Catarina and Paraná, and in the "wet" and "winter" seasons in Goiás and Federal District, BRS Horizonte presented an average grain yield of 2,362 $kg.ha^{-1}$, not differing statistically from the average yield of the varieties Pérola, Eté and Iapar 81 used as a control (Table 1).

Table 1. Average yield of the dry bean variety BRS Horizonte in the State of Santa Catarina and Paraná during the "wet" and "dry" seasons and in the State of Goiás and Federal District during the "wet" and "winter" seasons, compared to the average yield of two controls, from 2001 to 2003.

Region	State	Season	BRS Horizonte ($kg.ha^{-1}$)	Mean for Control[1] ($kg.ha^{-1}$)	Relative yield (%)	Number of environment
South	SC/PR	"wet"	2323	2279	102	10
		"dry"	2262	2330	97	5
Center West	GO/DF	"wet"	2239	2272	99	9
		"winter"	2771	3022	92	8
Mean			2362	2418	98	

[1]Iapar 81 and Pérola in Santa Catarina and Paraná, and Eté and Pérola in Goiás and Federal District.

Besides the carioca grain type, BRS Horizonte also presents excellent cooking qualities and a higher protein content when compared to the controls (Table 2).

Table 2. Technological grain quality of the carioca bean cultivar BRS Horizonte.

Variety	Cooking time (minute)	Protein (%)	100 grain weight (g)
BRS Horizonte	33	26,0	27,7
Pérola	29	21,3	26,6
Iapar 81	29	22,5	25,1

Cultivar BRS Horizonte, under artificial inoculation, was resistant to bean common mosaic virus and to the pathotypes 55 (lambda), 89 and 89 AS (alfa-Brazil), 95 (Kappa) and 453 (zeta) of *Colletotrichum lindemuthianum*. In field trials it presented an intermediary reaction to rust, and was susceptible to angular leaf spot and common bacterial blight.

BRS Horizonte presents a prominent erect growth habit and good resistance to lodging enabling it for mechanical harvest. It, also, has a growing cycle of 75 to 85 days from emergency to physiological maturation depending on environment conditions. Normally it is harvested earlier than the variety Pérola used as a control.

By all mentioned advantages BRS Horizonte is another option for bean growers involved with carioca bean grain type production, for the South States of Santa Catarina and Paraná and the Center West State of Goiás and the Federal District.

Genetic seed stocks are maintained by Embrapa Rice and Beans and basic seed is available at Embrapa Technology Transfer.

Institutions involved in the cultivar evaluation:

Embrapa Arroz e Feijão; Embrapa Cerrados; Embrapa Soja; Embrapa Negócios Tecnológicos-Escritório de Ponta Grossa; Agência Goiana de Desenvolvimento Rural e Fundiário ; Universidade de Rio Verde/Fesurv; Avena S/C Ltda; Cooperativa Regional Agropecuária de Campos Novos; C. Vale Cooperativa Agroindustrial; Escola Agrotécnica Federal de Concórdia; Cooperativa dos Produtores de Sementes de Laranjeiras do Sul Ltda; Sementes Campo Verde; Universidade Estadual de Londrina; Cooperativa Agrícola Mista de Prudentópolis; Detec Assessoria Técnica S/C Ltda; Anastácio Ceregatti Sanchez Ltda. (Holambra Agrícola II); Cooperativa Regional Agropecuária de Taquarituba.

'BRS PITANGA**'**: **NEW DRY BEAN VARIETY OF THE SMALL PURPLE COMMERCIAL GROUP**

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The Brazilian bean production from 1994 to 2003 oscillated between 1,97 and 2,77 million tons.year^{-1}. Last year it reached 3,0 million tons, practically attending the entire Brazilian population needs. Beans, in Brazil, is part of the daily basic diet of its population and involves a large production area cultivated mainly by small farmers. The Brazilian people is regionally demanding regarding the kind of grain for consumption such as color, shape and size. As a function of this exigent market the bean improvement genetic program at Embrapa Rice and Beans is developing new genotypes of the small purple commercial bean group with high yield, earliness, erect plant growth habit and disease resistance. The new BRS Pitanga cultivar, of the small purple commercial group, is the result of this effort and was released to the growers of the State of Goiás and Federal District. This cultivar has an erect plant growth habit and is resistant to four pathotypes of the anthracnose causal agent, to rust and to the bean common mosaic virus. BRS Pitanga was originated from the single cross between FEB 163 and AN512879, performed at Embrapa Rice and Beans. The bulk method was used from the F_2 to F_4 generations, with selection for commercial purple grain type. In the F_5 generation plants were selected for commercial purple grain type and harvested individually. From the F_6 families it was selected the line LM 95105718, for its productivity, erect plant growth habit and resistance to diseases. In 1997, this line was evaluated together with an additional 27 bean lines and two controls, in the National Trials, under 8 different environments in the States of Goiás (2), Mato Grosso (1), Mato Grosso do Sul (2), Minas Gerais (1), Bahia (1) and Espírito Santo (1). The joint analysis of the grain yield data and other agronomic characteristics provided the elements to promote LM 95105718 to the Regional Trial with the pre-commercial name of CNFR 7866. In the growing season of 1999/2000 this variety was evaluated with other 8 bean lines and two controls in a randomized complete block design with four replications (each plot consisted of 4 rows of 4m) in 10 different environments in the States of Goiás (9) and Federal District (1).
In 10 Regional Trials, conducted during the "dry" and "winter" seasons in the State of Goiás and Federal District, line CNFR 7866 presented the same average yield as the control cultivars (Table 1).

Table 1. Average yield of BRS Pitanga compared to the average yield of the best two control cultivars in the Regional Trials of 1999/2000.

Region	Season	BRS Pitanga (kg.ha^{-1})	Mean for Control[1] (kg.ha^{-1})	Relative Yield (%)	Number of environment
GO e DF	"dry"	1.541	1.632	94,4	3
	"winter"	2.282	2.261	101,0	7
Mean	-	2.059	2.072	99,4	---

[1]Roxo 90 and Safira.

BRS Pitanga presents grain size and color uniformity, a very important characteristic for the purple grain group, excellent cooking qualities and an excellent aspect after cooking (Table 2).

Table 2. Industrial and technological grain qualities of the purple bean BRS Pitanga compared to the variety Roxo 90.

Variety	Cooking time (minute)	Soluble solids (%)	Protein content (%)	100 grain weight (g)
BRS Pitanga	21,0	9,3	21,5	20,3
Roxo 90	26,0	9,5	-	23,1

Cultivar BRS Pitanga, under artificial inoculation, was resistant to bean common mosaic virus and to the pathotypes 55 (lambda), 89 (alfa-Brazil), 95 (Kappa) and 453 (zeta) of *Colletotrichum lindemuthianum*, the causal agent of anthracnose. In field trials it was resistant to rust, moderately resistant to angular leaf spot and susceptible to common bacterial blight.
BRS Pitanga presents an erect growth habit in all tested environments. It has also good resistance to lodging for the entire growing cycle (mean of 83 days from emergency to physiological maturity).
BRS Pitanga is a new option for bean growers involved with the small purple grain type bean production, that presents excellent cooking qualities, erect plant growth habit, resistance to lodging and diseases. This cultivar was released for the State of Goiás and Federal District. Genetic seed stocks are maintained by Embrapa Rice and Beans and basic seed is available at Embrapa Technology Transfer.

Institutions involved in the cultivar evaluation:
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BEAN IMPROVEMENT COOPERATIVE

- 2004 Financial Statement -

Balance on Hand: January 1, 2004		$9,291.00
INCOME		
2004 Dues		3,894.00
2004 Dues CD		445.00
Back Issues		112.00
Bank Interest		50.00
	TOTAL INCOME	4,501.00
EXPENSES		
Postage, Office Supplies and Copy Charges		2,136.00
Printing Volume 47		2,946.00
BIC Meeting Supplies		600.00
Bank Charges		9.00
	TOTAL EXPENSES	5,691.00
Balance on Hand: December 31, 2004		$8,101.00