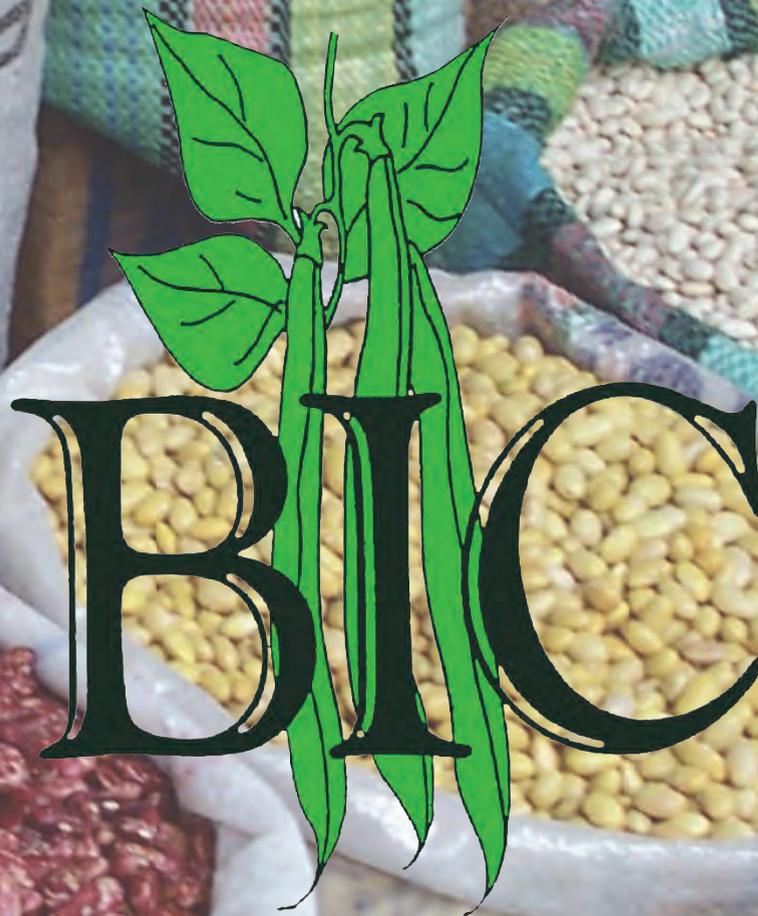




50

**ANNUAL REPORT OF THE
BEAN IMPROVEMENT
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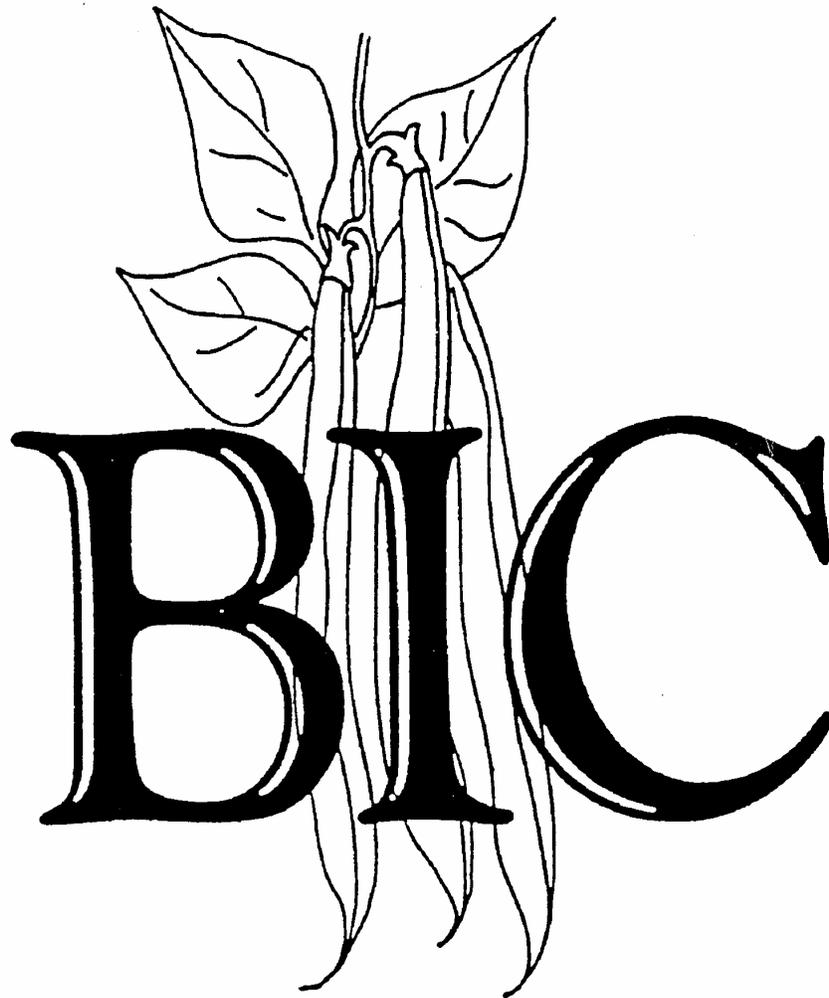


**A VOLUNTARY AND INFORMAL ORGANIZATION
TO EFFECT THE EXCHANGE OF INFORMATION AND MATERIALS**

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Coordinating Committee

Jim Beaver
Jim Kelly (President)
Phil Miklas
Soon Jai Park
Howard F. Schwartz (Ex officio)

Antonio de Ron
Ken Kmiecik
Jim Myers
Ron Riley
Ron Shellenberger

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

Dr. James D. Kelly, BIC President
Department of Crop & Soil Sciences
Michigan State University
East Lansing, MI 48824
U. S. A.

Tele: 517-355-0271 extension 1181 // FAX: 517-353-3955

Email: kellyj@msu.edu

<http://www.css.msu.edu/bic>
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TABLE OF CONTENTS

Page

L Annual Report of the Bean Improvement Cooperative	i
BIC Committee Membership - 1957 to 2007	ii
BIC Awards Committee Membership - 1957 to 2007	ii
BIC Genetics Committee Membership – 2005 to 2007	ii
BIC Meritorious Service and Distinguished Achievement Award Recipients - 1957 to 2005	iii
BIC Awards Nomination Request for 2007	v
BIC/NAPIA Meeting in 2007	vi
New Bean Breeder	vi
Tentative Agenda BIC/NAPIA Meeting in 2007	vii
In Memory of Bob Henson	viii

RESEARCH PAPERS FOR 2007

[BOWMAN-BIRK INHIBITOR \(BBI\) GENES IN WILD PHASEOLUS SPECIES: BIOCHEMICAL AND MOLECULAR ANALYSES](#)

I. Galasso, L. Lioi, M.C. Amenduni, B. Campion, R. Bollini, F. Sparvoli, A.R. Piergiovanni.....	1
EVIDENCE OF GENE FLOW AMONG ARGENTINEAN WILD BEANS AND LANDRACES USING MORPHOAGRONOMIC AND MOLECULAR DATA	
M. Z. Galván, M. C. Menéndez-Sevillano, A. Lanteri and P. A. Balatti.....	3
GRAFTING AS TOOL IN BEAN INTERSPECIES BREEDING	
Valarmathi Gurusamy, Bert Vandenberg and Kirstin Bett.....	5
INTERSPECIFIC HYBRID DERIVED-LINES DEVELOPED BY HERBERT LAMPRECHT: A SOURCE OF DISEASE RESISTANCE FOR COMMON BEAN	
J. Erron Haggard and James R. Myers.....	7
TOLERANCE TO SUB-ZERO TEMPERATURES IN PHASEOLUS ACUTIFOLIUS AND DEVELOPMENT OF INTERSPECIES HYBRIDS WITH P. VULGARIS	
J. Martínez-Rojo, V. Gurusamy, A. Vandenberg and K. E. Bett.....	9
COMPARATIVE PHOTOSYNTHETIC RESPONSES OF WILD AND CULTIVATED P. ACUTIFOLIUS AND P. VULGARIS TO SALINITY	
Jeannette S. Bayuelo-Jiménez and Noé Jasso Plata.....	11
RELATIVE GROWTH OF WILD AND CULTIVATED PHASEOLUS ACUTIFOLIUS AND P. VULGARIS UNDER SALT STRESS	
Jeannette S. Bayuelo-Jiménez and Noé Jasso Plata.....	13
EXPRESSION OF SOME MODEL PLANT EMBRYOGENESIS GENES IN PHASEOLUS OVULES	
Souleymane Silué, Jean-Marie Jacquemin and Jean-Pierre Baudoin.....	15
MICROSATELLITE DIVERSITY OF MESOAMERICAN COMMON BEANS (PHASEOLUS VULGARIS L.)	
Lucy M. Díaz and Matthew W. Blair.....	17
MORPHO-AGRONOMICAL CHARACTERIZATION OF DOMESTICATED COMMON BEAN CORE COLLECTION FROM MÉXICO	
M. L. Patricia Vargas-Vázquez, José S. Muruaga-Martínez, Rigoberto Rosales-Serna, Patricia Pérez-Herrera, Homar R. Gill-Langarica, Miguel Á. Martínez-Damián and Netzahualcoyotl Mayek-Pérez.....	19
GENETIC ANALYSIS OF CULTIVATED Phaseolus vulgaris L. CORE COLLECTION OF INIFAP-MEXICO	
Homar R. Gill-Langarica, M. L. Patricia Vargas-Vázquez, José S. Muruaga-Martínez, Rigoberto Rosales-Serna and Netzahualcoyotl Mayek-Pérez.....	21
SEED YIELD OF BLACK SEEDED LINES INTROGRESSED WITH WILD PHASEOLUS VULGARIS	
Jorge A. Acosta-Gallegos, Bismarck Aguilar Garzón, Raúl Rodríguez-Guerra, Manuel Mendoza Hernández, Horacio Guzman-Maldonado and James D. Kelly.....	23
INHERITANCE OF PLANT HEIGHT, INTERNODE LENGTH AND BRANCH NUMBER IN CLIMBING COMMON BEAN POPULATIONS	
Oscar E. Checa, Hernán Ceballos, Matthew W. Blair.....	25

<u>CHARACTERIZATION OF COMMON BEAN (PHASEOLUS VULGARIS L.) VARIETIES FROM SERBIA</u>	
Zorica T. Nikolić, Mirjana A. Vasić, Mirjana B. Milošević, Milka Lj. Vujaković and Jelica M. Gvozdanović-Varga	27
<u>GENETIC PROGRESS AFTER FIVE CYCLES OF PHENOTYPIC RECURRENT SELECTION FOR EARLY FLOWERING OF CARIOCA COMMON BEAN</u>	
Flávia Barbosa Silva, Magno Antonio Patto Ramalho, Ângela de Fátima Barbosa Abreu	29
<u>EFFECT OF SOAKING AND COOKING ON THE OLIGOSACCHARIDES AND LECTINS IN RED KIDNEY BEANS (PHASEOLUS VULGARIS L.)</u>	
G. Nyombaire, M. Siddiq and K. Dolan	31
<u>A NEW MINIMALLY PROCESSED RED KIDNEY BEAN (Phaseolus vulgaris L.) SUGAR-COATED SNACK</u>	
G. Nyombaire, M. Siddiq, K. Dolan and J.B. Harte	33
<u>THE USE OF A LOW-COST EXTRUDER TO INACTIVATE PHYTOHEMAGGLUTININS IN LIGHT RED KIDNEY BEANS</u>	
G. Nyombaire, K. Dolan and M. Bennink	35
<u>FUNCTIONAL CHARACTERISTICS OF EXTRUDED PINTO AND NAVY BEAN FLOUR (Phaseolus vulgaris L.)</u>	
G. Nyombaire, E. Birmingham, J.B. Harte, S.Venkatesh, M. Siddiq and K.D. Dolan	37
<u>ABSORBANCE OF THE SOAK WATER TO PREDICT CANNING QUALITY</u>	
Shawna Bushey and George Hosfield	39
<u>A TEST TO PREDICT COLOR LOSS IN BLACK BEAN DURING THERMAL PROCESSING</u>	
Shawna M Bushey and George L Hosfield	41
<u>SEED IRON AND ZINC LEVELS IN A COLLECTION OF COLOMBIAN RELEASED VARIETIES GROWN AT TWO LEVELS OF PHOSPHORUS FERTILIZATION</u>	
M.W. Blair, C. Astudillo, A. Hoyos, Y. Viera, O. Mosquera	43
<u>GENE EXPRESSION ANALYSIS OF GENES INVOLVED IN THE PHYTIC ACID PATHWAY OF COMMON BEAN (P. VULGARIS L.) USING REAL-TIME PCR</u>	
M. Fileppi, I. Galasso, B. Campion, R. Bollini and, F. Sparvoli	45
<u>BREEDING FOR HIGH SEED-ZN AS A VALUE ADDED TRAIT IN NAVY BEAN</u>	
R. Gelin, K. Grafton, G.A. Rojas-Cifuentes and A.J. Vander Wal	47
<u>ALPHA-GALACTOSIDASE ACTIVITY IN COMMON BEANS</u>	
Carmen Jacinto-Hernández, Ramón Garza García and Irma Bernal-Lugo	49
<u>POLYPHENOL OXIDASE IN BEAN CULTIVARS WITH DIFFERENT</u>	
Carmen Jacinto-Hernández, Ramón Garza-García, Albino Campos Escudero and Irma Bernal-Lugo	51
<u>POLYPHENOLIC PROFILES OF THREE BEANS VARIETIES</u>	
Devanand L. Luthria, Long-Ze Lin, James M. Harnly and Marcial A. Pastor-Corrales	53
<u>NICHE MARKET SHELL BEAN VARIETY TRIAL</u>	
Devanand L. Luthria, Long-Ze Lin, James M. Harnly and Marcial A. Pastor-Corrales	55
<u>MODIFIED ATMOSPHERE PACKAGING OF GREEN-SHELLED COMMON BEANS</u>	
Nalini Pandya, James Beaver and Carol Harper	57
<u>NEW BULGARIA VARIETIES OF GARDEN BEAN (PHASEOLUS VULGARIS L.) WITH IMPROVED QUALITY</u>	
S. Sofkova and G. Pevicharova	59
<u>EVALUATION OF PLASTID PIGMENTS CONTENT IN GARDEN BEAN (PHASEOLUS VULGARIS L.) PODS</u>	
S. Sofkova and V. Petkova	61
<u>ASSOCIATION BETWEEN BIOCHEMICAL DESCRIPTORS AND THE POD FIBROUSNESS IN THE CHARACTERIZATION OF SNAPBEAN GERMPASM FOR LATIN AMERICAN FRESH CONSUMPTION</u>	
A. Tofiño, C. Ocampo and M. Blair	63
<u>GROWTH AND YIELD OF SNAP BEAN (PHASEOLUS VULGARIS L.) WITH APPLICATION OF GROWTH REGULATORS</u>	
Jesús Rubén Torres-García, J. Alberto Escalante-Estrada, Teresa Rodríguez-González, David Martínez-Moreno and Carlos Ramírez-Ayala	65
<u>VARIABILITY STUDY OF 89 SNAP BEAN GENOTYPES USING THE AFLP MOLECULAR TECHNIQUE</u>	
A.P. Tofiño, J.F. Calderón, J.D. Palacio and M. Blair	67
<u>PHASEOLIN: VARIABILITY AND REFERENCE MATERIALS IN WILD AND CULTIVATED COMMON BEAN</u>	
O. Toro, C. H. Ocampo and D. G. Debouck	69

<u>MOLECULAR CHARACTERIZATION OF COMMON BEAN CULTIVARS BY PHASEOLIN AND RAPD MARKERS</u>	
M.C. Gonçalves-Vidigal, M.R. Costa, P.S. Vidigal Filho, A. Gonela and A. L. Sansigolo	71
<u>GENETIC DIVERSITY OF COMMON BEAN GERMPASM COLLECTION OF THE NUPAGRI BY RAPD MARKERS</u>	
M.C. Gonçalves-Vidigal, P.S. Vidigal Filho, J.C. Molina, A. Gonela, G.F. Lacanallo and V. L. Souza.....	73
<u>FUNCTIONAL MARKERS OF COMMON BEAN FROM DISEASE RESISTANCE GENE ORTHOLOGS</u>	
Zahirul I. Talukder, Phillip N. Miklas, Thomas P. Gonnella and Khwaja G. Hossain.....	75
<u>DEVELOPMENT AND GENETIC MAPPING OF A SCAR MARKER FOR THE BEAN GOLDEN YELLOW MOSAIC GEMINIVIRUS RESISTANCE GENE BGM-1</u>	
M.W. Blair, L.M. Rodriguez, F. Pedraza, F. Morales, S. Beebe, P. Miklas and J. Beaver.....	77
<u>INTROGRESSION OF CMV RESISTANCE INTO SNAP BEAN</u>	
Michell E. Sass, Thomas German and James Nienhuis.....	79
<u>BIOASSAYS TO DIAGNOSE SELECTED BEAN POTYVIRUSES</u>	
J. W. Shail Jr., A. G. Taylor and R. Provvidenti	81
<u>REPRODUCTION OF SOYBEAN CYST NEMATODE ON DRY BEAN CULTIVARS</u>	
S. Poromarto and B.D. Nelson	83
<u>VARIABILITY IN COLLETOTRICHUM LINDEMUTHIANUM FROM ECUADOR AND GUATEMALA</u>	
Halima Awale, Esteban Falconi, Julio Cesar Villatoro and James D. Kelly	85
<u>INHERITANCE OF RACE-SPECIFIC RESISTANCE TO ANTHRACNOSE IN THE DIFFERENTIAL CULTIVAR AB136</u>	
A. Campa, E. Pérez Vega, R. Giraldez and J.J. Ferreira	87
<u>ANTHRACNOSE RESISTANCE IN STEM AND LEAVES OF COMMON BEAN IS CONFERRED BY ASSOCIATION OF DOMINANT AND RECESSIVE GENES</u>	
Ana Lilia Alzate-Marin, Ivan Schuster, Maurilio Alves Moreira and Everaldo Gonçalves De Barros	89
<u>TRANSFER OF ANGULAR LEAF SPOT RESISTANCE GENE FROM ‘CORNELL 49-242’ TO THE CARIOCA CULTIVAR RUDÁ</u>	
Jeziel D. Damasceno, Demerson A. Sanglard, Bruno P. Balbi, Everaldo G. de Barros and Maurilio A. Moreira.....	91
<u>GENETIC DIVERSITY IN PHAEOSARIOPSIS GRISEOLA BASED ON ISSR MOLECULAR MARKERS</u>	
Geraldina E. Fermoselle , Sebastian A. Stenglein and Pedro A. Balatti.....	93
<u>VIRULENCE OF 6 ISOLATES OF PHAEOSARIOPSIS GRISEOLA (SACC) [FERR] UPON 41 COMMON BEAN LANDRACES IN TANZANIA</u>	
N.C. Fivawo and S. N. Msolla	95
<u>IMPACT OF ANGULAR LEAF SPOT ON GRAIN YIELD OF COMMON BEAN LINES</u>	
Magno Antonio Patto Ramalho, Ângela de Fátima Barbosa Abreu and João Bosco dos Santos.....	97
<u>INTENSITY OF ANGULAR LEAF SPOT AND ANTHRACNOSE AND ANTHRACNOSE TRANSMISSION BY SEEDS OF BEANS CULTIVATED IN THREE CROPPING SYSTEMS</u>	
Trazilbo J. de Paula Jr., Rogério F. Vieira, Hudson Teixeira and Clibas Vieira.....	99
<u>INTROGRESSION OF ANGULAR LEAF SPOT RESISTANCE GENES IN COMMON BEAN ISOLINES</u>	
Demerson A. Sanglard, Jeziel D. Damasceno, Bruno P. Balbi, Everaldo G. de Barros and Maurilio A. Moreira.....	101
<u>COMMON BEAN GENOTYPES RESISTANT TO ANGULAR LEAF SPOT, RUST AND ANTHRACNOSE</u>	
Aloísio Sartorato	103
<u>THE DILEMMA OF RECURRENT BACKCROSSING FOR INTROGRESSING COMMON BACTERIAL BLIGHT RESISTANCE IN COMMON BEAN</u>	
Robert W. Duncan, Shree P. Singh and Robert L. Gilbertson	105
<u>TWO CYCLES OF RECURRENT SELECTION FOR PYRAMIDING COMMON BACTERIAL BLIGHT RESISTANCE IN COMMON BEAN</u>	
Robert W. Duncan, Shree P. Singh and Robert L. Gilbertson	107
<u>IMPROVING RESISTANCE TO CBB IN ANDEAN COMMON BEAN USING MAS</u>	
S. Fernandez, A. Ibeas, C. Asensio and M.C. Asensio-S.-Manzanera	109
<u>PHYSICAL MAPPING OF A MAJOR QTL CONDITIONING COMMON BACTERIAL BLIGHT ON CHROMOSOME 1 IN COMMON BEAN</u>	
Shuyu Liu, Kangfu Yu, Margaret Haffner, and Soon J. Park.....	111
<u>SEED QUALITY OF DRY BEAN PRODUCED AT A SEMIARID LOCATION IN THE HIGHLANDS OF MEXICO</u>	

R. Navarrete-Maya, D. Brena-Gómez, J. A. Acosta-Gallegos, J. Navarrete-Maya, I. Cuellar-Robles, and F. J. Ibarra-Pérez	113
<u>TOWARDS THE IDENTIFICATION OF COMMON BACTERIAL BLIGHT RESISTANCE GENES IN PHASEOLUS VULGARIS</u>	
G.E. Perry, Y. Reinprecht, J. Chan and K.P.P.Pauls	115
<u>FOLIAGE, POD AND INTERNAL SEED INFECTION OF SELECTED COMMON BEAN LINES WHEN INOCULATED WITH TWO STRAINS OF XANTHOMONAS AXONOPODIS PV. PHASEOLI</u>	
Mildred Zapata, James Beaver and Tim Porch.....	117
<u>RUST RESISTANCE GENE PRESENT IN COMMON BEAN CULTIVAR OURO NEGRO (Ur-ON) DOES NOT CORRESPOND TO Ur-3+</u>	
Thiago Lívio Pessoa Oliveira de Souza, Suelen Nogueira Dessaune, Demerson Arruda Sanglard, Maurilio Alves Moreira and Everaldo Gonçalves de Barros	119
<u>IDENTIFICATION OF POTENTIAL CANDIDATE GENETIC MARKERS IN PHASEOLUS VULGARIS FOR RESISTANCE TO PHAKOPSORA PACHYRHIZI</u>	
C. M. Bellato, R. D. Frederick, J. Bowers, D. Zhang and M. Pastor-Corrales.....	121
<u>EVALUATION OF COMMON BEAN CULTIVARS FROM THE UNITED STATES FOR THEIR REACTION TO SOYBEAN RUST UNDER FIELD CONDITIONS IN BRAZIL AND SOUTH AFRICA</u>	
M. A. Pastor-Corrales, A. Sartorato, M. M. Liebenberg, M. J. del Peloso, P. A. Arraes Pereira, J. Nunes Junior, H. Dinis Campos	123
<u>ASIAN SOYBEAN RUST ON COMMON BEAN AND OTHER LEGUMES</u>	
MM Liebenberg, AJ Liebenberg and ZA Pretorius.....	125
<u>REACTION OF ONTARIO DRY BEANS TO ASIAN SOYBEAN RUST (PHAKOPSORA PACHYRHIZI) IN PRELIMINARY TRIALS IN SOUTH AFRICA</u>	
Soon J. Park, Merion M. Liebenberg and Liezl C. van Jaarsveld	127
<u>CORRELATIONS OF WHITE MOLD RESISTANCE IN SNAP BEAN (PHASEOLUS VULGARIS L.) AMONG FIELD EVALUATIONS, OXALIC ACID TEST, AND STRAW TEST</u>	
Yong Suk Chung, Michell E. Sass, and James Nienhuis.....	129
<u>BULGARIAN LANDRACES AND LINES OF COMMON BEAN (PHASEOLUS VULGARIS L.) WITH PHYSIOLOGICAL RESISTANCE TO WHITE MOLD</u>	
Dimitar Genchev and Ivan Kiryakov	131
<u>IDENTIFICATION OF PARTIAL RESISTANCE TO SCLEROTINIA SCLEROTIUM IN COMMON BEAN AT MULTIPLE LOCATIONS IN 2006</u>	
L.K. Otto-Hanson and J.R. Steadman	133
<u>DEVELOPING WHITE MOLD RESISTANT INTERSPECIFIC BREEDING LINES FROM THE SECONDARY GENE POOL OF COMMON BEAN</u>	
Shree P. Singh, Henry Terán, Howard F. Schwartz, Kristen Otto, and Margarita Lema	135
<u>WHITE MOLD FUNGICIDE TRIALS ON DRY BEANS IN MICHIGAN</u>	
G. Varner and K.A. Terpstra	137
<u>IDENTIFICATION OF ROOT ROT RESISTANCE SOURCES IN COMMON BEAN</u>	
Esteban Falconí, Ángel Murillo I., José Pinzón, Eduardo Peralta I and George Abawi	139
<u>TILLAGE, HUMIDITY AND FERTILIZATION EFFECTS ON BEAN YIELD, CHARCOAL ROT INCIDENCE AND PATHOGENICITY OF <i>Macrophomina phaseolina</i></u>	
Krystal Lira-Méndez, Arturo Díaz-Franco and Netzahualcoyotl Mayek-Pérez.....	141
<u>NATURAL INCIDENCE OF <i>Phytophthora phaseoli</i> IN DRY BEAN GROWN IN THE HIGHLANDS OF MEXICO</u>	
R. Navarrete-Maya, J.A. Acosta-Gallegos, E.I. Cuéllar-Robles, J.A. Ocampo H. and J. Navarrete-Maya.....	143
<u>ROOT ROT RESISTANT SNAP BEANS ADAPTED TO ORGANIC PRODUCTION</u>	
James Nienhuis and Michell Sass	145
<u>SUSCEPTIBILITY OF GREEN MANURE SPECIES TO TWO SOILBORNE PATHOGENS OF COMMON BEAN</u>	
T.J Paula Jr., H. Teixeira, R.F. Vieira, R.C. Lima, T.B. Silveira and M.S. Lehner.....	147
<u>ROOT GRAVITROPISM OF GENOTYPES OF COMMON BEANS USED FOR BREEDING IN BRAZIL</u>	
Rogério F. Vieira and Jonathan P. Lynch.....	149

<u>SIMPLE METHOD TO EVALUATE ROOT HAIRS OF A LARGE NUMBER OF COMMON BEAN GENOTYPES</u>	
Rogério F. Vieira; Celestina N. Jochua and Jonathan P. Lynch.....	151
<u>BREEDING COMMON BEANS FOR DROUGHT TOLERANCE IN SOUTHERN ETHIOPIA</u>	
Asrat Asfaw, Fistum Alemayehu and Fekadu Gurum.....	153
<u>COMMON BEAN IMPROVEMENT FOR HIGHLAND ADAPTATION IN SOUTHERN ETHIOPIA</u>	
Asrat Asfaw, Fistum Alemayehu, Fekadu Gurum and Yasin Goa.....	155
<u>LEAF AREA OF TWO BEAN VARIETIES UNDER SOIL WATER DEFICIT</u>	
Antonio García-Esteva ¹ ; Josué Kohashi-Shibata ² and José Luis Cervantes-Flores.....	157
<u>INHERITANCE OF DROUGHT TOLERANCE TRAITS IN ANDEAN X ANDEAN AND ANDEAN X MESOAMERICAN F2 POPULATIONS.</u>	
Godwill S. Makunde, Stephen Beebe, Matthew W. Blair, Rowland Chirwa and Davies Lungu	159
<u>INDICATORS OF DRY BEAN PERFORMANCE UNDER RAINFED CONDITIONS AT THE HIGHLANDS OF MEXICO</u>	
J. S. Padilla-Ramírez, E. S. Osuna-Ceja, E. Acosta-Díaz and J. A. Acosta-Gallegos	161
<u>SOURCES OF VARIATION OF COMMON BEAN FOR DROUGHT TOLERANCE</u>	
A.P. Rodiño, M. Riveiro, M. Santalla and A.M. De Ron.....	163
<u>PATTERN OF MACRONUTRIENTS ABSORPTION BY COMMON BEAN cvs. BRS MG TALISMÃ AND OURO NEGRO AT CONVENTIONAL AND NO TILL SYSTEMS</u>	
M.J.B. Andrade, N.M.B.Vieira, J.G. Carvalho, J. Alves Junior, A.R.Moraes, and P.M. Rezende	165
<u>PATTERN OF MICRONUTRIENTS ABSORPTION BY COMMON BEAN cvs. BRS MG TALISMÃ AND OURO NEGRO AT CONVENTIONAL AND NO TILLING SYSTEMS</u>	
N.M.B.Vieira, M. J. B. Andrade, J. G. Carvalho, J. Alves Junior, A.R.Moraes and P. M. Rezende.....	167
<u>BEAN (Phaseolus vulgaris L.) YIELD REDUCTION BASED ON WEED INTENSITY COMPETITION</u>	
J. Alberto Escalante-Estrada and Ma. Teresa Rodríguez-González	169
<u>EFFECTS OF INDENTATION, TIDE RIDGING AND TILLAGE SYSTEMS ON SEED YIELD OF DRY BEAN IN THE SEMIARID HIGHLAND OF MEXICO</u>	
E. S. Osuna-Ceja, J.S. Padilla-Ramírez, M.A. Martínez-Gamiño, E. Martinez Meza and J.A. Acosta-Gallegos.....	171
<u>OXADIARGYL: A PRE EMERGENCE HERBICIDE TO CONTROL BLACK NIGHTSHADE (SOLANUM NIGRUM) IN DRY BEAN</u>	
Alberto Pedreros and Juan Tay	173
<u>SNAP BEAN (Phaseolus vulgaris L.) YIELD AND WEED CONTROL WITH SUNFLOWER (Helianthus annuus L.) RESIDUE</u>	
Nicolás Salinas-Ramírez, J. Alberto Escalante-Estrada, Teresa Rodríguez-González and Eliseo Sosa-Montes	175
<u>EFFECTS OF WASTE CASSAVA PLANT ON EMERGENCE OF SNAP BEAN (UEL-2) SEEDLINGS</u>	
O.R. Brito, F.Q. Assunção, E. Miglioranza, R.M. Brito	177
<u>COMMON BEAN CULTURE NITROGEN FERTILIZATION UNDER NO-TILLAGE SYSTEM</u>	
O. Brito, R., Kuhn, O.J., Richard and A. Miglioranza E.	179
<u>NODULATION AND YIELD OF BUSH AND CLIMBING BEANS INOCULATED WITH RHIZOBIA STRAINS</u>	
Paul Kimani, G.K Gicharu, N. Mburugu, H. Boga and R. Cheruiyot.....	181
<u>THE EFFECT OF NITROGEN USE ON GROWTH AND DEVELOPMENT OF COMMON BEAN AND SUNFLOWER INTERCROP</u>	
Edgar J. Morales-Rosales and J. Alberto Escalante-Estrada.....	183
<u>LEGUME PIPE – A TIME SENSITIVE RESOURCE FOR THE AMERICAN BEAN INDUSTRY</u>	
H. F. Schwartz.....	185
<u>COMMON BEAN FARMER’S CRITERIA IN CULTIVAR CHOICE</u>	
Camila Bonemann Chollet and Irajá Ferreira Antunes	187
<u>LOCAL AGRICULTURAL RESEARCH COMMITTEE (LARC): A PARTICIPATORY STRATEGY TO IMPROVE DRY BEAN PRODUCTION IN ECUADOR</u>	
N. Mazón, E. Peralta and D. Ruonavaara.....	189
<u>GENOTYPE EVALUATION OF BLACK BEAN GROUP IN MARINGÁ, PARANÁ STATE, BRAZIL</u>	
Carlos Alberto de Bastos Andrade, Pedro Soares Vidigal Filho and Tanea Aparecida Crispim Splendor	191
<u>GENOTYPE EVALUATION OF COLORED BEAN GROUP IN MARINGÁ, PARANÁ STATE, BRAZIL</u>	
Carlos Alberto de Bastos Andrade, Tanea Aparecida Crispim Splendor and Pedro Soares Vidigal Filho	193
<u>BEAN VARIETIES FOR THE HUMID TROPICAL LOWLANDS</u>	
Paul Kimani, Jean-Paul Lodi Lama, Matondo Nsebua and Lunze Lubanga	195

Arnulfo Pajarito-Ravelero and Rigoberto Rosales-Serna.....	201
<u>BRS EXPEDITO, A NEW BLACK SEEDED COMMON BEAN CULTIVAR</u>	
Irajá Ferreira Antunes, Expedito Paulo Silveira, Heloisa Torres da Silva.....	203
<u>BRSMG MAJESTOSO: ANOTHER CARIOCA GRAIN TYPE BEAN CULTIVAR FOR THE STATE OF MINAS GERAIS</u>	
Ângela de Fátima Barbosa Abreu ³ , Magno Antonio Patto Ramalho, José Eustáquio de Souza Carneiro, Maria José Del Peloso, Trazilbo José de Paula Júnior, Luís Cláudio de Faria, Leonardo Cunha Melo, Everaldo Gonçalves de Barros, Maurílio Alves Moreira, Israel Alexandre Pereira Filho, Maurício Martins, João Bosco dos Santos, Carlos Agustín Rava, Joaquim Geraldo Cáprio da Costa and Aloísio Sartorato.....	205
<u>BRS COMETA: A CARIOCA GRAIN TYPE COMMON BEAN CULTIVAR WITH ERECT GROWTH HABIT</u>	
Luis Cláudio de Faria, Maria José Del Peloso, Leonardo Cunha Melo, Joaquim Geraldo Cáprio da Costa, Carlos Agustín Rava, José Luiz Cabrera Díaz, Josias Correa de Faria, Heloísa Torres da Silva, Aloísio Sartorato, Priscila Zaczuk Bassinello and José Benedito de Freitas Trovo	207

GENETIC STOCKS AND RELEASE NOTES

<u>RELEASE OF ABC-WEIHING COMMON BACTERIAL BLIGHT, RUST AND MOSAIC RESISTANT, SEMI-UPRIGHT, HIGH SEED QUALITY GREAT NORTHERN BEAN GERMPLASM LINE</u>	
N. Mutlu, C.A. Urrea, P.N. Miklas, J.R. Steadman, M.A. Pastor-Corrales, D.T. Lindgren, J. Reiser, A.K. Vidaver and D.P. Coyne.....	209
<u>RELEASE OF GREAT NORTHERN ‘HUNGERFORD’ DRY BEAN</u>	
Shree P. Singh, Henry Terán, Marie Dennis, Margarita Lema, Richard Hayes and Craig Robinson	211
<u>RELEASE OF PINTO ‘KIMBERLY’ DRY BEAN</u>	
Shree P. Singh, Henry Terán, Marie Dennis, Margarita Lema, Richard Hayes and Craig Robinson	213
<u>RELEASE OF GREAT NORTHERN ‘SAWTOOTH’ DRY BEAN</u>	
Shree P. Singh, Henry Terán, Marie Dennis, Margarita Lema, Richard Hayes and Craig Robinson	215
<u>RELEASE OF PINTO ‘SHOSHONE’ DRY BEAN</u>	
Shree P. Singh, Henry Terán, Marie Dennis, Margarita Lema, Richard Hayes and Craig Robinson	217
<u>RELEASE OF COMMON BEAN GERMPLASM LINE HR67</u>	
Soon J. Park*, Kangfu Yu, Shuyu Liu and Terry Rupert	219
<u>SUBJECT MATTER INDEX</u>	221
<u>FINANCIAL STATEMENT</u>	222
<u>MEMBERSHIP DIRECTORY</u>	223

Cover: Dry beans (*Phaseolus vulgaris*, *Vigna*, *Vicia*) for sale in the market in Ibarra, Ecuador. Photo courtesy of J. Beaver.

THE 50TH ANNUAL REPORT OF THE BEAN IMPROVEMENT COOPERATIVE

The Bean Improvement Cooperative (BIC) is commemorating 50-years of expanding the frontiers of knowledge related to bean improvement. We will celebrate this momentous occasion at the Twenty-fourth Biennial Meeting to be held from October 28 through November 1, 2007 at the Concourse Hotel and Conference Center near the campus of the University of Wisconsin, Madison, Wisconsin. Madison was the site of the first BIC meeting in 1959. In addition, the associated meetings with our colleagues in the North American Pulse Improvement Association (NAPIA), Crop Germplasm Committee, BIC Genetics Committee and the Regional W-1150 Committee are scheduled. Please refer to the information provided by the local organizing committee in the current report, and look for additional information on the BIC web site www.css.msu.edu/bic. A call for abstracts will be mailed directly to all BIC members by the local organizing committee later this year. We want to make this a memorable meeting, so please share this information with interested colleagues who might like to attend these meetings and/or join the BIC.

As we enter our fiftieth year of existence, it is interesting to look to the past. The incisive vision of our founders Drs. Frazier and Zaumeyer who recognized the need for a research organization that would encourage interaction between public and private sector scientists, provide a bridge to the international community and recognize different end users of our commodity. Membership started around 60 and swelled to over 400 at one time, but membership is now under 300. The BIC 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 **BIC Meritorious Service Award** and the **BIC Achievement Award**, and forward your nominations to the Awards Committee Chairperson, Howard Schwartz by August 1, 2007. A current membership list of BIC Committees and those who have received awards throughout the history of the BIC is provided in the current issue to assist you in nominating colleagues for these awards.

We will continue to recognize our founding members through 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 or related research. 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 from Oregon State and Dr. Bill Zaumeyer an equally distinguished bean pathologist with the USDA-ARS. The Awards Committee in agreement with the BIC President and the Local Meeting Committee Chair will choose the successful recipient of the Lectureship for 2007. The Lectureship will be awarded at the meeting in Madison and nominations should be sent to Howard Schwartz by August 1, 2007.

Looking forward to celebrating our Fiftieth Anniversary and seeing you all in Madison, Wisconsin in October,

Dr. James D. Kelly, BIC President

BIC COMMITTEE MEMBERSHIP - 1957 TO 2007

Coordinating Committee (approximate year of appointment):

1957	Dean, Enzie, Frazier* (BIC Coordinator/President), McCabe, Zaumeyer
1960	Anderson, Atkin, Dean, Enzie, Frazier , McCabe, Zaumeyer
1962	Anderson, Atkin, Dean, Frazier , Pierce, Polzak, Zaumeyer
1968	Anderson, Coyne , Dean, Jorgensen, Polzak, Zaumeyer
1971	Briggs, Coyne , Dean, Jorgensen, Polzak, Zaumeyer
1972	Burke, Coyne , Dean, Jorgensen, Kiely, Polzak, Zaumeyer
1974	Ballantyne, Bravo, Burke, Coyne , Dickson, Emery, Evans, Kiely, Saettler, Zaumeyer
1977	Ballantyne, Bliss, Coyne, Dickson , Emery, Evans, Graham, Meiners, Morris, Saettler, Zaumeyer
1978	Atkin, Ballantyne, Bliss, Coyne, Dickson , Graham, Meiners, Morris, Saettler, Sprague
1979	Atkin, Bliss, Dickson , Graham, Hagedorn, Meiners, Morris, Sprague, Wallace
1980	Atkin, Bliss, Dickson , Hagedorn, Morris, Sprague, Steadman, Temple, Wallace
1982	Atkin, Coyne, Dickson , Hagedorn, Sprague, Steadman, Temple, Wallace, Wyatt
1983	Coyne, Dickson , Hagedorn, Saettler, Silbernagel, Steadman, Temple, Wallace, Wyatt
1985	Coyne, Dickson , Mok, Saettler, Silbernagel, Steadman, Temple, Wallace, Wyatt
1986	Coyne, Dickson , Mok, Saettler, Schoonhoven, Schwartz, Silbernagel, Steadman, Wallace
1988	Brick, Dickson, Emery, Magnuson, Roos, Schwartz , Singh, Steadman, Uebersax
1992	Dickson, Emery, Grafton, Magnuson, Schwartz , Singh, Stavely, Steadman, Uebersax
1994	Antonius, Dickson, Grafton, Magnuson, Park, Schwartz , Singh, Stavely, Uebersax
1996	Antonius, Grafton, Park, Schwartz , Singh, Stavely, Myers, Kotch, Miklas, Riley
1998	Antonius, Beaver, Kelly , Kotch, Miklas, Myers, Park, Riley, Schwartz (ex officio), Singh, Vandenberg
2001	Antonius, Beaver, Kelly , Kotch, Miklas, Myers, Park, Riley, de Ron, Schwartz (ex officio), Vandenberg
2003	Beaver, Kelly , Kmiecik, Kurowski, Miklas, Myers, Park, Riley, de Ron, Schwartz (ex officio), Vandenberg
2007	Beaver, Kelly , Kmiecik, Miklas, Myers, Park, Riley, de Ron, Schwartz (ex officio), Shellenberger, Vandenberg

Awards Committee:

1971	Baggett, Briggs, Burke, Dean, Wallace	1985	Emery, Hagedorn, Sandsted, Schwartz
1973	Burke, Dean, Mauth, Zaumeyer	1987	Emery, Hagedorn, Sandsted
1975	Ballantyne, Frazier, Mauth	1989	Coyne, Silbernagel, Wallace
1977	Ballantyne, Curme, Frazier, Schuster	1995	Coyne, Dickson, Stavely
1979	Ballantyne, Schuster, Silbernagel, Temple	1997	Coyne, Schwartz, Stavely
1981	Abawi, Bliss, Monis, Silbernagel	2001	Hosfield, Magnuson, Schwartz
1983	Adams, Bliss, Burke, Dean, Morris	2004	Hosfield, Schwartz, Singh
		2007	Hosfield, Schwartz, Singh

Genetics Committee:

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

2007: Tim Porch (Chair), James S. Beaver, Matthew W. Blair, Paul Gepts, Phil McClean, Phil Miklas, Carlos Urrea, 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. Tim Porch, USDA ARS SAA TARS, 2200 P.A. Campos Ave., Suite 201, Mayaguez PR 00680: ph. (787) 831-3435, ext. 254; fax. (787) 831-3386; and e-mail; mavtp@ars-grin.gov**

RECIPIENTS OF BIC MERITORIOUS SERVICE & ACHIEVEMENT AWARDS

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 Stavelly- 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**, 1998-2007)
 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
- 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
- 2005 Perry B. Cregan – USDA-ARS, Beltsville, Geneticist, Soybean Genomics, [Frazier - Zaumeyer Distinguished Lectureship]
 Jorge A. Acosta Gallegos, INIFAP, Mexico, Plant Breeder
 Phillip N. Miklas, USDA-ARS, Prosser, Plant Geneticist
 David M. Webster, Seminis Seeds, Plant Breeder
 A. ‘Bert’ Vandenberg, University of Saskatchewan, Plant Breeder [Achievement Award]

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 Fiftieth Anniversary of BIC Biennial Meeting in Madison Wisconsin, from October 28 to November 1, 2007. Please help us select worthy recipients.

BIC AWARD NOMINATION

Return by August 1, 2007 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

Two 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]

2007 BIC/NAPIA MEETINGS

Madison, Wisconsin

The BIC/NAPIA biennial meeting and associated meetings will be held Oct. 29 through November 2, 2007 at the Concourse Hotel and Conference Center, 1 West Dayton Street, Madison, Wisconsin. The Concourse is located in the heart of downtown Madison one block from the Capitol and six blocks from the campus of the University of Wisconsin - Madison. Lodging will be available at a reduced rate at the Concourse Hotel. The hosts for the 50th Anniversary meeting of BIC are the: University of Wisconsin - Madison, Harris Moran Seed Company, Syngenta, and Seminis Vegetable Seeds.

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.

City of Madison: <http://www.visitmadison.com/>
Concourse Hotel: <http://www.concoursehotel.com>
University of Wisconsin: <http://www.wisc.edu/>

Airlines with flights into the Dane Co. Regional Airport include: Northwest, Delta, American, United, and Midwest Express. The Concourse has complementary shuttle service.

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, **James D. Kelly** (kellyj@msu.edu) or members of the local organizing committee: **Roxanne Mainz** (roxanne.mainz@syngenta.com), **Ken Kmiecik** (Ken.Kmiecik@seminis.com), or **Rob Gehin** (r.gehin@harrismoran.com)

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 2007 Biennial Meeting of the BIC and associated meetings. The deadline for receiving abstracts is **Friday September 28, 2007**. 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. Details on nominating colleagues are provided elsewhere in this report. Additionally the local committee request input in identifying a suitable individual to deliver the Frazier-Zaumeyer Lecture, or suggesting a topic for the BIC workshop on Wednesday Oct 31st.

New Bean Breeder at the North Dakota State University

Dr. Juan Manuel Osorno is the new dry bean breeder at North Dakota State University. Juan recently completed his Ph.D. at North Dakota State University in plant breeding and genetics and assumed his new responsibilities in January, 2007. Juan has considerable experience in bean breeding, having received his M.S. degree from the University of Puerto Rico - Mayaguez with Dr. Jim Beaver and working for several years at CIAT with Dr. Shree Singh.

TENTATIVE AGENDA FOR THE 2007 BIC/NAPIA MEETING

Monday Oct 29, 2007	Large meeting room	
BIC Meeting		
BIC Registration	7:00	9:00
Breakfast Buffet	8:00	9:00
Welcome & Introductions	8:45	9:00
Frazier-Zaumeyer Lecture	9:15	10:15
Break	10:15	10:45
Oral Session	10:45	12:15
Lunch on your own	12:15	1:30
Oral Session	1:30	3:30
Break		
Poster Session (1)	3:30	5:30
Rooms open for independent meetings		

Tuesday Oct, 30, 2007	Large meeting room	
BIC Meeting		
Breakfast on your own		
<i>Rooms open for independent meetings</i>		
Oral Session	9:00	10:00
Break	10:00	10:30
Oral Session	10:30	12:15
Lunch on your own	12:15	1:30
Oral Session	1:30	3:30
Break		
Poster Session (2)	3:30	5:30
Awards Banquet	6:30	9:30

Wednesday Oct. 31, 2007	Large meeting room	
BIC Meeting		
Breakfast on your own		
<i>Rooms open for independent meetings</i>		
BIC Workshop	9:00	10:00
Break	10:00	10:30
BIC Workshop	10:30	12:15
Lunch on your own	12:15	1:30
BIC Business meeting	1:30	2:30
Phaseolus CGC	2:30	3:30
BIC Genetics Committee	3:30	5:00

Thursday Nov 1, 2007	Smaller meeting room	
BIC W1150 / NAPIA		
W1150 Meeting	8:00	10:00
Break	10:00	10:30
W1150 Meeting	10:30	12:15
Lunch on your own	12:15	1:30
NAPIA meeting		

Friday Nov 2, 2007	Smaller meeting room	
NAPIA Meeting		
NAPIA meeting		
NAPIA Awards Luncheon		
NAPIA meeting		

Sunday Oct. 28, 2007
BIC registration 7:00 PM until 10:00 PM

Thursday Nov. 1, 2007
NAPIA registration 8:00 AM until 12:00 PM

IN MEMORY OF BOB HENSON

The bean community lost a dear friend and colleague when Dr. Bob Henson, Associate Agronomist at the NDSU Carrington Research & Extension Center, Carrington, ND, passed away unexpectedly on July 3, 2006. He and a graduate student from North Dakota State University were attending the International Ascochyta Conference in Le Tronchet, France, where they were presenting results of their field research.

Dr. Henson was born on March 27, 1947 in Duluth, MN. He lived with his family in Minnesota and graduated from Buffalo High School. He participated in high school sports, was a standout football player in college, and later an enthusiastic soccer player among friends at the international locations where he worked. Bob was an outgoing person who was equally comfortable among field workers and government officials, often offering his candid remarks to all. He traveled extensively and made numerous friends at each stop. To travel with him was an adventure in itself.

Bob was an international scholar and contributor to the betterment of global agriculture. He received a B.A. degree in Chemistry from Macalester College, and Master of Agriculture and Ph.D. in Agronomy with emphasis on Plant Physiology from the University of Minnesota. His research topic for the Ph.D. degree at the University of Minnesota involved studying symbiotic nitrogen fixation in soybean and alfalfa. This led to his research on improving BNF in common bean. From 1983 through 1989, Bob was the project manager of the University of Wisconsin-CNPAF Nitrogen Fixation Project sponsored by the USAID/Bean Cowpea CRSP at Goiania, Goias, Brazil. There and later in Ecuador and Bolivia, Bob was a major participant in collaborative grain legume research projects sponsored by the International Atomic Energy Agency to utilize non-radioactive isotopes for measuring nitrogen fixation under field conditions. He was author or co-author of numerous publications in refereed and popular journals, and a co-developer of the dry bean cultivar 'Ouro Negro' (Henson et al., 1993. Registration of 'Ouro Negro', a high-yielding, high-nitrogen fixing common bean variety. *Crop Sci.* 33:644).

Dr. Henson spent much of his early career in international agriculture and spoke several languages. As a member of the Peace Corps, he directed small-farmer agricultural projects in the Philippines. Following his work in Brazil, he was a World Bank Consultant in Mexico, a dry bean physiologist at INIAP, Sta. Catalina, Quito, Ecuador, and an advisor to the IBTA National Grain Legume Program, San Benito, Cochabamba, Bolivia.

Dr. Henson returned to the U.S. in 1996 as an Assistant Scientist at the University of Minnesota West Central Experiment Station in Morris, MN. Since 1998, Bob was an Agronomist at the North Dakota State University Carrington Research & Extension Center where he conducted field research and coordinated programs on oilseeds, pulses, small grains, and alternative crops, being especially active with the sunflower industry.

In addition to the Bean Improvement Coop, Bob was a member of the Tri-Societies, the North American Pulse Improvement Assn. B.O.D., and International Soil Science Soc., among others, as well as the Phi Kappa Phi and Gamma Sigma Delta Honor Society of Agriculture.

The time Bob spent in Brazil was especially significant not only for his scientific contributions to bean research, but even more-so personally. It was there he met his wife, Soraia. They especially enjoyed time spent with family and friends at their lake house near Ortonville, Minnesota. In Carrington, he was active in church and community affairs and with their childrens' sports, FFA and school activities. He is survived by his wife Soraia Braga Henson, sons, Robert and Peter, and daughter Gabriela. They continue to reside at 525 Joal Drive, Carrington, ND 58421.

BOWMAN-BIRK INHIBITOR (BBI) GENES IN WILD PHASEOLUS SPECIES: BIOCHEMICAL AND MOLECULAR ANALYSES

I. Galasso¹, L. Lioi², M.C. Amenduni², B. Campion³, R. Bollini¹, F. Sparvoli¹, A.R. Piergiovanni²

¹ Istituto di Biologia e Biotecnologia Agraria, IBBA-CNR, Milano (Italy)

² Istituto di Genetica Vegetale, IGV-CNR, Bari (Italy)

³ Istituto Sperimentale per l'Orticultura, CRA, Montanaso Lombardo, Lodi (Italy)

Introduction – The Bowman-Birk inhibitor (BBI) family is the most widespread group of serine proteinase inhibitors in common bean as well as in other legumes. BBI are double-headed inhibitors with low molecular mass (6–9 kDa) and high cysteine content. They have two distinct binding loops with the ability to inhibit two molecules of the same enzyme or two different proteases such as trypsin and chymotrypsin. Recent works have shown that soybean BBI, besides being storage protein and recognized agent for seed protection, is also effective in preventing or suppressing carcinogenic processes in both *in vitro* and *in vivo* models (Armstrong et al., 2003). The trypsin inhibitor activity (TIA) in cultivated and wild common bean seeds was recently evaluated and the corresponding genes were isolated by PCR, cloned and sequenced (Piergiovanni and Galasso, 2004; Piergiovanni et al., 2005). The analysis of the primary gene structure from several common bean genotypes showed that polymorphisms were present both outside and within the enzyme binding loops. Moreover, our studies evidenced that the variant P'2-Arg at TI site characterized by reduced trypsin inhibition constant was more frequent in the Mesoamerican than in the Andean populations. This finding is in agreement with the assay of antitryptic activity that showed a generally lower inhibition activity in the Mesoamerican accessions than in the Andean populations (Piergiovanni et al., 2005). Although in the seeds of cultivated common bean quantitative variation in TIA has been well documented, scarce information is available in literature about the level of trypsin inhibitors in seeds of *Phaseolus* wild species and no data are available about these protein-coding genes. The study of BBI family in different *Phaseolus* wild species may lead to the identification of new variants potentially active against proteinases from different insect pests or to new variants at chymotrypsin inhibitory site, having anti-carcinogenic properties.

Materials and methods – Twenty-four accessions belonging to twelve wild *Phaseolus* species obtained from CIAT, IPK, USDA, and National Botanic Garden of Belgium were investigated in this study (Table 1). Trypsin inhibitor activity was measured on defatted meal spectrophotometrically according to the literature (Della Gatta et al. 1988). BBI genes were amplified by PCR using two specific primers designed on the gene sequence encoding for the BBI. The amplified product of about 300 bp was gel-purified and cloned in pGEM[®]T vector. Several clones for each sample were sequenced. For Southern hybridisation, 3 µg of genomic DNA were digested with *Eco*RI, and transferred onto a nylon membrane. One BBI clone from common bean was labelled with α -[32P]-dCTP and used as probe for filter hybridisation.

Results and discussion - With the exception of *P. leptostachyus*, where no BBI genes were amplified by PCR, all the remaining species analyzed showed two or more BBI genes. Southern blot hybridisation confirms this result. Indeed, no hybridisation was detected in *P. leptostachyus* while four to six hybridisation fragments were observed in *P. coccineus*, *P. glabellus*, and *P. maculatus*. In these taxa an internal *Eco*RI restriction site is present in the isolated BBI gene, suggesting that at least two of the fragments can be the result of the cleavage of a single gene. Analysis on the primary structure of the gene isolated from all the wild *Phaseolus* species showed that these genes are strictly conserved and nucleotide identities among the different species ranged between 90-99%. Among the analysed species only *P. coccineus* and *P.*

costaricensis, similarly to *P. vulgaris*, showed two types of trypsin binding loop that differed each other for the residue at P'2 position: the most active P'2-Ile and the weaker P'2-Arg. This finding confirms that *P. costaricensis* is more closely related to the domesticated *Phaseolus* group than the other wild species.

As shown in Table 1, the TI activity detected in seeds of several wild *Phaseolus* species covers a very wide range. Significant intraspecific variation was observed for those species for which more than one accession was analyzed. The highest value was detected in seeds of *P. oligospermus* (62.4 TIU/mg). *Phaseolus lunatus* accessions showed a higher average of TI contents compared to those typically observed in common bean. Very low TI contents were detected in all five *P. leptostachyus* accessions (Table 1) suggesting that the detected activity could not be due to proteinaceous inhibitors but to non-protein compounds present in coats and known to be also able to inhibit the trypsin enzyme.

Among the wild species investigated in this study potentially interesting appear to be the *P. oligospermus* due to its very high TI level that could be related to a high anti-carcinogenic activity and *P. leptostachyus* due to the absence of BBI gene a property useful to investigate on the influence of this molecule on the nutritional value of seeds. More studies are planned on these two work hypotheses.

Table 1 Trypsin inhibitory activity in seeds of some wild *Phaseolus* species.

Species	Accession code	TIU/mg sb	Species	Accession code	TIU/mg sb
<i>P. acutifolius</i>	NI558	9.6	<i>P. lunatus</i> (m)	G25314	50.4
<i>P. coccineus</i>	NI16	32.3	<i>P. lunatus</i> (wm)	G25843	39.7
<i>P. costaricensis</i>	G40604	46.3	<i>P. lunatus</i> (wa)	G26461	54.6
<i>P. filiformis</i>	G40513	14.4	<i>P. maculatus</i>	NI1237	45.0
<i>P. glabellus</i>	G40585	42.0	<i>P. microcarpus</i>	NI709	13.5
<i>P. leptostachyus</i>	NI1036	5.8	<i>P. oligospermus</i>	G40691	62.4
<i>P. leptostachyus</i>	G40603	5.5	<i>P. parvulus</i>	PI535366	13.3
<i>P. leptostachyus</i>	NI710	5.2	<i>P. vulgaris</i> (wm)	G11051	9.6
<i>P. leptostachyus</i>	NI1043	4.8	<i>P. vulgaris</i> (wm)	G12949	10.0
<i>P. leptostachyus</i>	NI1569	4.6	<i>P. vulgaris</i> (wm)	NI1406B	23.7
<i>P. lunatus</i> (m)	PHA8071	40.2	<i>P. vulgaris</i> (wm)	G12862	18.4
<i>P. lunatus</i> (a)	PHA8110	29.4	<i>P. vulgaris</i> (wa)	G23585	20.4

a- Andean gene-pool; m-Mesoamerican gene-pool; w- wild; TIU/mg sb - trypsin inhibitor unit for mg of substance; Research supported in part by project MiPAF-PROM

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EVIDENCE OF GENE FLOW AMONG ARGENTINEAN WILD BEANS AND LANDRACES USING MORPHOAGRONOMIC AND MOLECULAR DATA

M. Z. Galván, M. C. Menéndez-Sevillano¹, A. Lanteri² & P. A. Balatti

Instituto de Fisiología Vegetal- Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, CC327 La Plata, (1900) Argentina; ²Instituto Nacional de Tecnología Agropecuaria (INTA) EEA Cerrillos Salta, Argentina; ³Cátedra de Taxonomía, Fac. de Cs. Naturales y Museo, Universidad Nacional de La Plata.

Argentina is ranked fourth among countries exporting common bean around the world. Beans are cultivated mainly in northwestern Argentina, an area that also represents the southern most limit of the Andean diversification center of the common bean. Based on morphological data the presence of wild-weedy-cultivated complexes was reported in this region (Menéndez Sevillano, 2002; De Ron *et al.*, 2004), suggesting that gene flow is occurring between sympatric populations. Diversity in cultivated beans as revealed by molecular analysis is low (Galván *et al.*, 2001; Galván *et al.*, 2003), therefore it is important to identify sources of diversity, such as wild populations and bean landraces, to use them in breeding programs. The purpose of this work was to characterize wild populations and landraces from Argentina by means of morphoagronomic and molecular data.

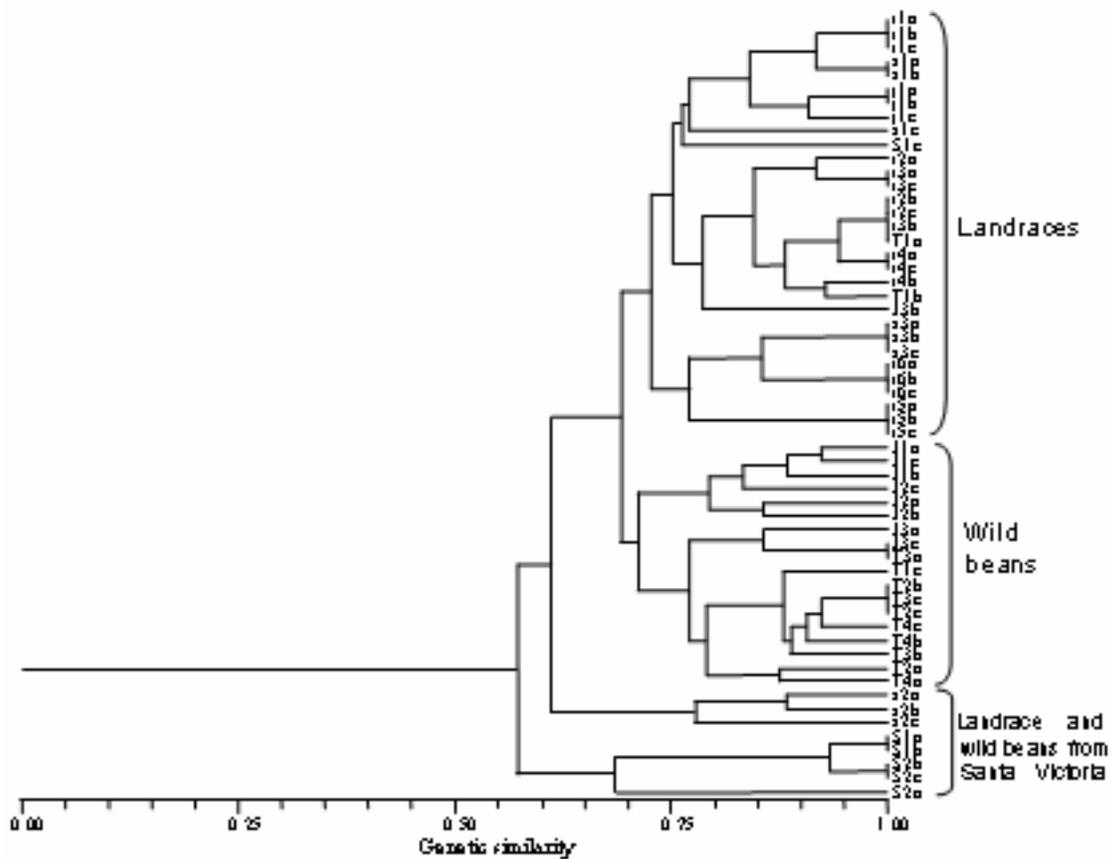
Ten quantitative traits and ten ISSR primers were assayed among nine wild populations of common bean (*Phaseolus vulgaris* var *aborigineus*) and ten landraces from Northern Argentina. Cluster Analysis and Principal Coordinates Analysis of the molecular data grouped wild beans and landraces in two highly similar clusters, also showing a great variability within each cluster (Figure 1). In addition, it was observed a geographical structured genetic variability of wild beans indicated by the Mantel correlation test. On the other hand, genetic variability among landraces suggested homogeneous selection exerted by farmers in different sites.

Wild beans and landraces from one of the sites analyzed (Santa Victoria, Salta; 22°15'S Latitude and 24° 58'W Longitude) showed high genetic similarity values considering the Jaccard index. One landrace from this site generated unique ISSR patterns and also showed segregation of wild characteristics such as small seeds, dehiscent pods and mottled seed pattern. These results suggested the existence of gene flow between wild and domesticated beans in this area. Therefore, the analysis of the variability and fertility of the hybrids generated would be of a great interest in order to use these beans to introgress characters from wild beans to domesticated cultivars, to broaden the genetic base of commercial beans.

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Figure 1. Phenogram of 9 wild accessions and 10 landraces of common bean from Argentina analyzed by means of ISSR markers using Jaccard similarity index (Cophenetic Correlation Coefficient: 0.77).



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GRAFTING AS TOOL IN BEAN INTERSPECIES BREEDING

Valarmathi Gurusamy, Bert Vandenberg and Kirstin Bett
Dept. of Plant Sciences, University of Saskatchewan
51 Campus Dr. Saskatoon, SK S7N 5A8 Canada

INTRODUCTION:

Attempts to introgress genes from *P. angustissimus* into common bean either through direct backcrossing or through bridge crossing using *P. acutifolius* as bridging species is hindered by many genetic and physiological problems. Major hurdles include complete pollen sterility in the F₁, very early embryo abortion upon backcrossing, recalcitrant rescued backcross embryos and problems in multiplication and maintenance of F₁ hybrids through stem cuttings. Screening of these wide hybrids for specific traits or for various procedures such as colchicine dipping etc. is only possible through vegetative propagation. Stem cuttings is a common method of mass multiplication. Unfortunately rooting the stem cuttings of hybrids produced between *P. acutifolius* (W6 15578 and PI 430219) with *P. angustissimus* (PI 535272) and *P. acutifolius* (PI 430219) with *P. vulgaris* has not been successful. In general stem cuttings of dry bean varieties establish in 2 weeks with 60-75% success rate. Several techniques for rooting, including layering, and manipulation of concentrations of rooting hormones and substrates in soil as well as in water were unsuccessful. Thus grafting techniques were tried. Preliminary evaluation with either of the parents as the rootstock were unsuccessful (data not included) with drying out of the rootstock and scion observed in 24-48 hours. This might be due to the thin stem of both *P. angustissimus* and *P. acutifolius*. Thus the objective was to assess the possibility of grafting onto a species like *P. vulgaris* which has a larger stem girth, for multiplication and maintenance of interspecies F₁ hybrids.

MATERIALS & METHODS:

Two cultivars of *P. vulgaris*: “ICA Pijao” and “NY5-1” were used as root stocks. Five scion donors, including four F₁ hybrids: 1) *P. acutifolius* W6 15578 (*P. ac* 78) / *P. angustissimus* PI 535272 (*Pa*), 2) *P. acutifolius* PI 430219 (*P. ac* 19) / *Pa*, 3) *P. vulgaris* ICA Pijao (Pijao) / *Pa*, 4) NY5-1 / *P. ac* 78; and ICA Pijao and NY5-1 as controls were used. For each scion, ten seeds of each root stock were sown in 4” pots (1 seed /pot) filled with SunShine Mix #3 (SunGrow Horticulture Canada Inc., AB, Canada). Interspecies hybrids were trimmed and maintained to encourage abundant shoot growth. All donor plants were grown in large pots filled with Sunshine Mix #3. All plants were grown and maintained in growth cabinets set at 23/18°C (day/night). Cool white fluorescent bulbs provided a day length of 12 hours at an average light intensity of 394 $\mu\text{mol m}^{-2}\text{s}^{-1}$ with ambient humidity. Root stocks were grown for 5-7 days. Just prior to first leaf emergence, the root stock was excised below the cotyledons. A vertical slit was made with a sharp blade. Shoot tips with 1-2 internodes were clipped from the donor plant and a “v” shaped cut was made at the end. The scion was immediately inserted into the root stock slit and the graft union was wrapped with Parafilm strips to protect from drying. The pot carrying the graft was placed on a larger pot and covered with a Sun Polybag (Sigma) and secured using a rubber band to maintain humidity. Bags were removed once the grafts were established with at

least one new internode. The experiment was conducted with 2 replications. Observations on percent establishment and days to flowering were recorded.

RESULTS & DISCUSSION:

Results revealed that there were significant differences among the treatments (% establishment and days to flowering); although no difference was observed between the two root stocks based on percent establishment of the various grafts (Table 1). Percent establishment of interspecies hybrids onto a different third species (treatments 3,4,10 and 11) was not significantly different from those hybrids grafted on to one of the parents or the same species (treatments 1, 2, 7 and 9). Success rate in grafting the interspecies F_1 hybrids onto a different species was not influenced by the third species. However, grafting of NY5-1 scion onto either *P. vulgaris* rootstock resulted in poor establishment of grafts and early flowering compared to all other grafts. That might be due to the determinant plant type of this donor, which led to difficulties in obtaining a multiple shoot tips with vegetative growth devoid of reproductive buds during the experimental timeframe.

Table1. Percentage establishment of grafts with *P. vulgaris* as root stocks

Treatment	Root stock	Scion	Establishment (%)	Mean days to flowering in grafts
1	NY5-1	F_1 (NY5-1 / <i>P. ac 78</i>)	80	21
2	NY5-1	F_1 (<i>P. ac19</i> / ICA Pijao)	65	25
3	NY5-1	F_1 (<i>P. ac 78</i> / <i>P.a</i>)	65	24.5
4	NY5-1	F_1 (<i>P. ac 19</i> / <i>P.a</i>)	75	26.5
5	NY5-1	NY5-1	40	19
6	NY5-1	ICA Pijao	95	24.5
7	ICA Pijao	F1 (ICA Pijao/Pa)	85	24.5
8	ICA Pijao	F_1 (NY5-1 / <i>P. ac 78</i>)	70	23
9	ICA Pijao	F_1 (<i>P. ac19</i> / ICA Pijao)	90	26
10	ICA Pijao	F_1 (<i>P. ac 78</i> / <i>P.a</i>)	60	23.5
11	ICA Pijao	F_1 (<i>P. ac 19</i> / <i>P.a</i>)	70	25.5
12	ICA Pijao	NY5-1	50	19.5
		LSD (0.05)	24.94	2.85

Rootstocks had no influence on days to flowering in the established interspecies grafts. In a few grafts, it was observed that flowering was hindered for the interspecies hybrids grafted on to a different species (treatments 3 and 10) when the scions selected were devoid of any reproductive buds. A detailed study on the hormonal influence on flowering involving various species has been started.

These results are very encouraging for breeders multiplying rare breeding material across different species. This technique will enable bean breeders to enhance breeding strategies through mass multiplication of early segregating breeding material involving various distantly related species.

INTERSPECIFIC HYBRID DERIVED-LINES DEVELOPED BY HERBERT LAMPRECHT: A SOURCE OF DISEASE RESISTANCE FOR COMMON BEAN

J. ERRON HAGGARD AND JAMES R. MYERS

Department of Horticulture, Oregon State University, Corvallis, OR 97331

Herbert Lamprecht studied transfer of traits between common and runner bean, and in the process, created a number of interspecific inbred lines (Lamprecht, 1935, 1948). After he died, some genetic stocks were transferred to the USDA NPGS collection at Pullman, WA via Stig Blixt (Weibulsholm Plant Breeding Inst., Landskrona, Sweden), and Mike Dickson, (N. Y. Agric. Exp. Sta., Geneva, NY). One hundred seventy-eight accessions are maintained in this collection of which 46 are interspecific-derived lines with the prefix “M0”. These may have been evaluated by Mike Dickson for white mold resistance, but if so, we have not discovered published data. We previously documented a high level of greenhouse and field resistance to white mold in M0162 (and L192, another Lamprecht line), so decided to test the remainder of the M0 collection. Forty-six lines were evaluated for white mold response using the straw test, modified from Petzholdt and Dickson (1996). Twenty-five lines were tested in the greenhouse in April 2005 and the other 21 in June 2005. An augmented analysis (Federer et al., 2001) was used to combine data from the two dates. Five plants per line in a single one gallon pot constituted a replicate with two replicates per line. Two replicates of G 122 (resistant check), and five replicates of OR 91G (susceptible check) were included in each test

While none of the lines we tested exceeded the resistance of G 122 ($p > 0.05$), thirty were not significantly different (Table 1). Being derived from interspecific crosses, these lines may harbor genes from runner bean that would be useful in breeding common bean cultivars for white mold resistance. Because *P. coccineus* is a source of resistance to several pathogens, these lines should be tested for reaction to other diseases of interest.

Table 1: Straw Test LSMeans and Comparison to Standard Checks of M0 lines^z

PI No.	Line	Straw Test LSMean	Standard Error	OR 91G Pr > t	G 122 Pr > t
PI 527832	M0061	3.45	0.89	<.0001	0.19
PI 527829	M0048	3.73	0.84	<.0001	0.35
PI 527834	M0070	3.78	0.85	<.0001	0.43
PI 527858	M0169	3.82	0.83	<.0001	0.42
PI 527838	M0107A	4.14	0.87	<.0001	0.94
PI 527851	M0157	4.18	0.83	<.0001	1.00
PI 527864	M0186	4.22	0.83	<.0001	0.94
PI 527873	M0207A	4.22	0.83	<.0001	0.94
PI 527830	M0056	4.28	0.83	<.0001	0.83
PI 527839	M0107B	4.28	0.89	0.00	0.86
PI 527835	M0082	4.48	0.91	0.00	0.62
PI 527850	M0156	4.51	0.84	0.00	0.51
PI 527853	M0159	4.52	0.83	0.00	0.47
PI 527849	M0155	4.58	0.83	0.00	0.40
PI 527857	M0163	4.62	0.83	0.00	0.34
PI 527868	M0196	4.62	0.83	0.00	0.34

PI No.	Line	Straw Test	Standard	OR91G	G122
		LSMean	Error	Pr > t	Pr > t
PI 527869	M0197	4.62	0.83	0.00	0.34
PI 527840	M0113	4.73	0.84	0.00	0.27
PI 527831	M0059	4.78	0.83	0.00	0.21
PI 527837	M0098	4.78	0.83	0.00	0.21
PI 527847	M0146	4.78	0.85	0.00	0.24
PI 527855	M0161	4.82	0.83	0.00	0.17
PI 527865	M0192	4.82	0.83	0.00	0.17
PI 527862	M0179	4.83	0.84	0.00	0.18
PI 527860	M0175	4.92	0.83	0.00	0.11
PI 527854	M0160	4.94	0.84	0.01	0.11
PI 527867	M0194	5.02	0.83	0.01	0.07
PI 527872	M0204	5.02	0.83	0.01	0.07
PI 527852	M0158	5.06	0.84	0.02	0.07
PI 527841	M0118	5.16	0.85	0.04	0.06
PI 527863	M0184	5.22	0.83	0.03	0.02
PI 527843	M0122	5.28	0.83	0.05	0.02
PI 527844	M0133	5.28	0.89	0.11	0.05
PI 527861	M0178	5.32	0.83	0.05	0.01
PI 527856	M0162	5.38	0.84	0.09	0.01
PI 527836	M0088	5.48	0.83	0.14	0.01
PI 527842	M0120	5.48	0.83	0.14	0.01
PI 527846	M0140	5.48	0.83	0.14	0.01
PI 527866	M0193	5.49	0.84	0.15	0.01
PI 527828	M0010	5.68	0.83	0.30	0.00
PI 527845	M0137	5.78	0.83	0.42	0.00
PI 527871	M0203	5.92	0.83	0.60	0.00
PI 527833	M0069	6.28	0.83	0.75	<.0001
PI 527848	M0150	6.78	0.83	0.15	<.0001
PI 527870	M0198	7.12	0.83	0.02	<.0001
PI 527859	M0173	8.62	0.83	<.0001	<.0001
	OR91G	6.14	0.75		<.0001
	G122	4.18	0.77	<.0001	

²Reaction to white mold was rated at 8 days after inoculation on a 1-9 scale, (1= no symptoms, 3= lesion progress down the stem to the first node, 5= lesion progress to the middle of 2nd internode, 7= lesion progress to second node, and 9= total plant collapse.

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TOLERANCE TO SUB-ZERO TEMPERATURES IN PHASEOLUS ACUTIFOLIUS AND DEVELOPMENT OF INTERSPECIES HYBRIDS WITH P. VULGARIS

J. Martinez-Rojo, V. Gurusamy, A. Vandenberg and K. E. Bett.

Dept. of Plant Sciences, University of Saskatchewan

51 Campus Dr. Saskatoon, SK S7N 5A8 Canada

INTRODUCTION. Cultivation of dry bean in the Canadian prairies is limited by its sensitivity to chilling temperatures. The climate results in a short growing season because of potential late frosts in May and early frosts in late August and early September. This climate has obliged breeders to develop varieties of various crops that are able to grow under these climatic conditions. Dry bean is a crop that does not have any appreciable tolerance to sub-zero temperatures and it is killed at ice formation temperatures (Balasubramanian *et al.*, 2004). However, because of its economic potential it has been introduced to this area.

Adaptation of dry bean to this region has been improved through plant breeding; however sensitivity to chilling and sub-zero temperatures are still a problem. A wild relative of *P. vulgaris*, *P. angustissimus*, has been identified as being more tolerant to sub-zero temperatures (Balasubramanian *et al.*, 2004). The ability to survive cold temperatures has led us to focus research on finding an effective way to transfer this tolerance into cultivated dry bean. Experiments have been conducted to generate hybrids between these two species, but the hybrids could not produce viable embryos even with extensive backcrossing and tissue culture manipulations. As a result, new approaches to find and transfer cold tolerance related genes into dry bean are necessary. Preliminary experiments by Balasubramanian (2004) demonstrated that some *P. acutifolius* accessions are more tolerant to sub-zero temperatures than *P. vulgaris*, although they were less tolerant than *P. angustissimus*. As it is more closely related to *P. vulgaris* than is *P. angustissimus*, a further exploration of this species as a source of tolerance to sub-zero temperatures was deemed valuable.

MATERIALS AND METHODS. The experiments were done in controlled growth chambers in the phytotron at the University of Saskatchewan. For these studies seven different genotypes were initially used: *P. vulgaris* cv. ICA Pijao, which is known to be susceptible to cold; *P. vulgaris* line NY5-161, which has been reported to be more tolerant of cold (Holubowicz and Dickson, 1989); *P. angustissimus* PI 535272 which is chilling tolerant (Balasubramanian *et al.*, 2004); *P. acutifolius* var. *acutifolius* (PI319445 & W615578) and *P. acutifolius* var. *tenuifolius* (PI430219 & W620127). These four *P. acutifolius* accessions have not been evaluated under sub-zero temperatures. The percentage of survival of these different genotypes was evaluated following exposure to decreasing levels of temperature. Plants were transferred to a chilling cabinet and ice nucleated with a fine spray of water at -1°C . The temperature was then lowered to -2.5°C and held for one hour. After this period one set of four plants was taken per genotype and placed at 5°C . The temperature for the rest was then dropped a further 0.5°C , held for 1 h and another set of genotypes was removed and placed at 5°C . This procedure was repeated until the temperature reached -4°C . After 24 hrs at 5°C the percentage of survival was evaluated. The experimental design use was an RCBD with 3 repetitions.

RESULTS AND DISCUSSION. ICA Pijao had the lowest level of tolerance with only 30% survival after 1 hour of exposure to -3.0°C and 0% survival at -3.5°C and -4.0°C (Fig. 1). *P. angustissimus* PI 535272 showed better levels of chilling tolerance with 50% survival at -3.0°C and -3.5°C , decreasing at 33% survival at -4.0°C . The four *P. acutifolius* accessions evaluated showed differing levels of tolerance to chilling. Only two of the *P. acutifolius* accessions (W6 15578 and PI 319445) had a significant level of tolerance to sub-zero temperatures in these initial experiments. *P. acutifolius* W6 15578 showed the greatest level of tolerance to sub-zero temperatures with 58% survival at -4.0°C . *P. acutifolius* PI 319445 also had reasonable tolerance with 42% survival at -4.0°C .

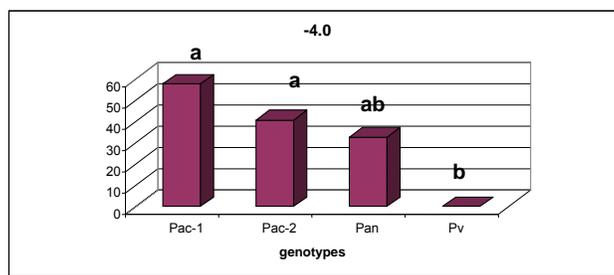


Fig 1: Percentage survival of four different *Phaseolus* genotypes following exposure to -4 °C for 1 hour. Pac-1: *P. acutifolius* W6 15578, Pac-2: *P. acutifolius* PI 319445, P.an: *P. angustissimus* PI 535272, Pv: *P. vulgaris* cv. ICA Pijao.

The development of F1 hybrids between *P. acutifolius* and *P. vulgaris* was not difficult and BC1 and BC2 individuals are currently being developed and grown. These will be evaluated for tolerance to sub-zero temperatures in the near future.

Cross combination	# embryo rescued	# of seeds obtained	# of hybrids established
W6 15578 x ICA Pijao	-	35	2
ICA Pijao x W6 15578	23	-	2
NY5-161 x W6 15578	108	-	3
(NY5-161 x W6 1557)x NY5-161	62	-	3
((NY5-161 x W6 1557)x NY5-161)x W6 1557	17	-	10 plantlets in culture so far

CONCLUSION. *P. acutifolius* var. *acutifolius* offers equal or better tolerance to sub-zero temperatures than *P. angustissimus* and hybridization with *P. vulgaris* is possible. Thus, *P. acutifolius* represents an important genetic source of variability for this trait in dry bean breeding.

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COMPARATIVE PHOTOSYNTHETIC RESPONSES OF WILD AND CULTIVATED *P. ACUTIFOLIUS* AND *P. VULGARIS* TO SALINITY

Jeannette S. Bayuelo-Jiménez^{1*} and Noé Jasso Plata¹

¹ Instituto de Investigaciones Agropecuarias y Forestales, Universidad Michoacana de San Nicolás de Hidalgo, Mexico. Km. 9.5 Carr. Morelia-Zinapécuaro Tarímbaro, Michoacán, 58880 México jsbayuelo@hotmail.com *Corresponding author.

Physiological processes as photosynthesis are highly sensitive to salinity and are, therefore, dominant in determining the plant's response to stress. Limitations on photosynthesis by salinity may be attributed to two major factors: (1) limited CO₂ diffusion to the intercellular spaces of the leaf as a consequence of reduced stomatal conductance and (2) impaired metabolism by direct inhibition of biochemical processes caused by ionic, osmotic or other conditions caused by loss of cellular water (Brugnoli and Lauteri, 1991). Therefore, the sensitivity of photosynthesis to salinity in different genotypes is an important factor to take into account (Bayuelo et al. 2003); given that photosynthesis is a major factor in the determination of plant growth. We examined the effects of salinity on leaf photosynthesis of two wild and two cultivated *Phaseolus* species.

MATERIALS AND METHODS

The experiment was conducted with accessions of different salt tolerance: two wild accessions representing two species (*P. vulgaris* PI325687, tolerant and *P. acutifolius* G40169, sensitive) and two cultivated accessions (*P. vulgaris* G04017, sensitive and *P. acutifolius* G40142 tolerant) were used. Plants were grown in nutrient solution under greenhouse conditions at Universidad Michoacana de San Nicolás de Hidalgo, Mexico between May and August 2006. Seedlings were allowed to grow with no salinity stress until the emergence of the first trifoliolate 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. Measurements of net CO₂ assimilation (A) and leaf diffusive conductance (g_s) were taken at 9, 14 and 19 days after the initiation of salt treatments using the second, third, and fifth trifoliolate leaf, which were the youngest fully expanded leaves, respectively. Measurements were made with a LI-COR 6400 portable photosynthetic system (LI-COR, Lincoln, NE). Net photosynthesis was measured at 34 MPa external CO₂ partial pressure (340 μmol CO₂ mol⁻¹ air) and a VPD of 1.8 KPa. The photon flux density (PPFD) was 1200 μmol m⁻² s⁻¹. All measurements were conducted between 9 00 h and 14 30 h, only on bright days. Data were analyzed using the GLM procedure (SAS Institute, Cary, NC, 1985). 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 ≤ 0.05.

RESULTS AND DISCUSSION

Salinity and the duration of salt stress significantly affected A and g_s. The CO₂ assimilation decreased gradually with salinity, showing significant reductions only at 60 mM NaCl, thereafter, a steady values was attained (Fig. 1a). Differences among cultivated and wild accessions were significant at any salt concentration. Overall, the salt-tolerant *P. acutifolius* G40142 had the highest A at 30 and 60 mM NaCl. A of the salt-sensitive accessions at high salinity decreased by an average of 11 and 27 % at Day 15 and 13 and 16 % at Day 20 compared with the control. By contrast, A of the salt-tolerant accessions at high salinity decreased by 12

and 24 % at Day 15 and by 10 and 19 % at Day 20. The reduction in g_s for both groups was greater than for A. At both measurements time (Day 14 and 19), g_s at 90 mM NaCl was reduced by an average of 33 and 65 % for the salt-tolerant and 33 and 71 % for the salt-sensitive accessions, as compared with the control. The salt-tolerant *P. acutifolius* G40142 had the highest g_s and the highest A at 90 mM NaCl. About 74 % of A at high salinity could be attributable to reduced g_s , with the consequent restriction of CO₂ availability for carboxylation, or to the acceleration of leaf senescence (Brugnoli and Lauteri, 1991).

ACKNOWLEDGMENTS

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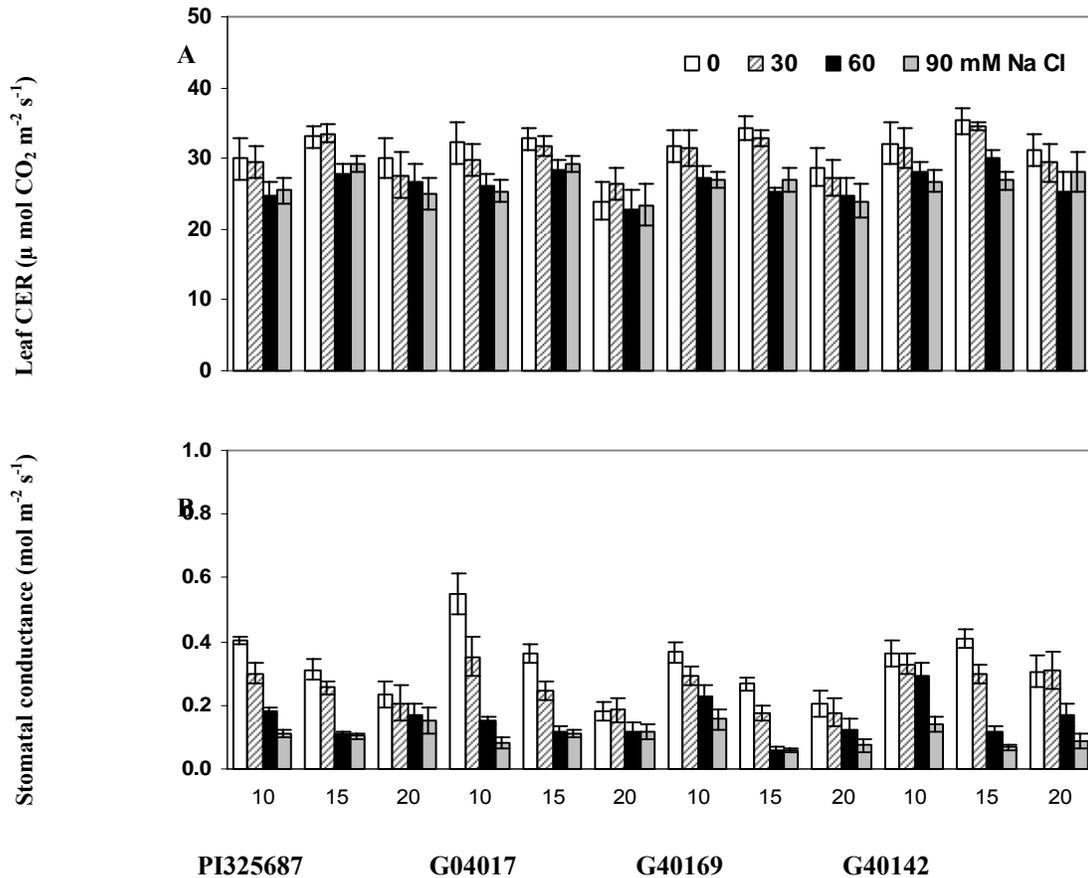


Fig. 1 Effects of increasing NaCl levels on Leaf CO₂ exchange rate (A) and stomatal conductance (B) at 9, 14 and 19 days. Data correspond to the average of four measurements on different individual plants. Standard error is shown as vertical bars.

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RELATIVE GROWTH OF WILD AND CULTIVATED PHASEOLUS ACUTIFOLIUS AND P. VULGARIS UNDER SALT STRESS

Jeannette S. Bayuelo-Jiménez^{1*} and Noé Jasso Plata¹

¹ Instituto de Investigaciones Agropecuarias y Forestales, Universidad Michoacana de San Nicolás de Hidalgo, Mexico. Km. 9.5 Carr. Morelia-Zinapécuaro Tarímbaro, Michoacán, 58880 México jsbayuelo@hotmail.com *Corresponding author.

In glycophytes, plant growth and development are generally limited by salinity. Most of the world's crop species are glycophytes, hence they do not grow under high soil salinity. However, with increasing amounts of arable land undergoing salinization (Gorham, 1992) and increasing food demand from the growing human population (Ghassemi et al. 1995), the need to develop salt-tolerant crop varieties is unavoidable. To develop salt-tolerant crops, it is necessary to identify the degree of salinity tolerance within crops and their wild-type relatives. Achieving this goal by breeding requires a better understanding of the role of the growth rate parameters in the salt tolerance of contrasting genotypes so that the traits leading to salt tolerance can be introduced in the new genotypes. We examined the effects of salinity on relative growth and components of two wild and two cultivated (*P. acutifolius* and *P. vulgaris*) species.

MATERIALS AND METHODS

The experiment was conducted with accessions of different salt tolerance: two wild accessions representing two species (*P. vulgaris* PI325687, tolerant and *P. acutifolius* G40169, sensitive) and two cultivated accessions (*P. vulgaris* G04017, sensitive and *P. acutifolius* G40142 tolerant) were used. Plants were grown in nutrient solution under greenhouse conditions at Universidad Michoacana de San Nicolás de Hidalgo, Mexico between May and August 2006. 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. Plants were harvested at 10, 15 and 20 days after transplanting and separated into roots, stem and leaves. Plant material was dried at 65 °C for 96 hours to determine dry weight. The relative growth rate, RGR ($\text{g g}^{-1} \text{d}^{-1}$), the unit leaf rate on a leaf area basis, ULR ($\text{g m}^{-2} \text{d}^{-1}$), and the leaf area ratio, LAR ($\text{m}^{-2} \text{g}^{-1}$), were calculated according to Hunt (1990). Data were analyzed using the GLM procedure (SAS Institute, Cary, NC, 1985). 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

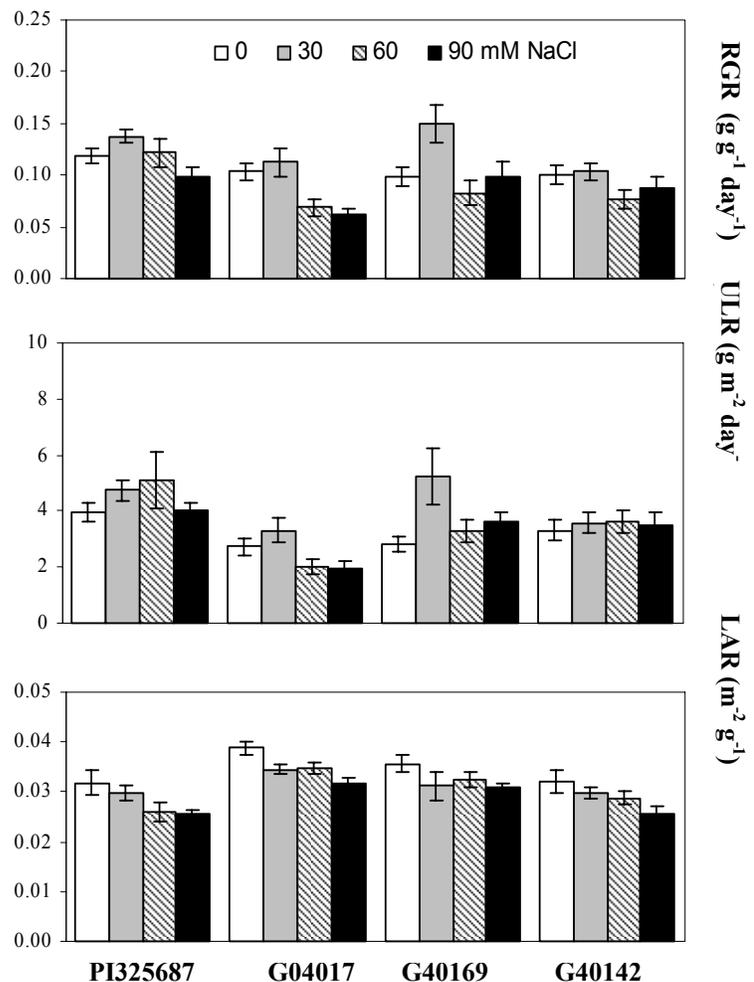
Salinity stress decreased relative growth rate (RGR) (Fig. 1a). Differences in RGR between unsalinized plants and plants treated with 30 and 60 mM NaCl were evident after 15 days (data not shown). Among genotypes, RGR for the control plants ranged from 0.10 to 0.12 $\text{g g}^{-1} \text{day}^{-1}$, whereas RGR of salinized plants varied between 0.06 to 0.15 $\text{g g}^{-1} \text{day}^{-1}$ (Fig. 1a). RGR decreased with high levels of salinity and with the period of exposure. Unit leaf rate (ULR) declined over time in all genotypes, particularly in high salinized plants (Fig. 1b). The decline showed a similar trend to that of RGR (Fig. 1b), except for salt-tolerant PI325687 genotype for which ULR was maintained between 10 and 15 days for 60 mM NaCl. Leaf area ratio (LAR)

maintained relatively steady values with increasing salinity and with the period of exposure in all genotypes except for wild salt-tolerant PI325687 whose LAR were significantly decreased by 60 mM NaCl between 15 and 20 days (Fig. 1c). Unit leaf rate was highly correlated with RGR in all genotypes. The coefficients of determination, R^2 , ranged from 0.43 to 0.79 for the cultivated and between 0.59 and 0.88 for the wild genotypes. The growth of the salt-sensitive G40169 and G4017 and salt-tolerant G40142 genotypes was reduced by salinity primarily due to a decline in the specific activity of the leaves (ULR) rather than a reduction in leaf area. In contrary, 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-tolerant PI325687.

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Fig. 1. **A** Relative growth rate (RGR), **B** Unit leaf rate (ULR), and **C** Leaf area ratio. 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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EXPRESSION OF SOME MODEL PLANT EMBRYOGENESIS GENES IN PHASEOLUS OVULES

Souleymane Silué¹, Jean-Marie Jacquemin² and Jean-Pierre Baudoin¹

¹ Faculté Universitaire des Sciences Agronomiques de Gembloux, Unité de Phytotechnie Tropicale et d'Horticulture. 2, Passage des Déportés, BE-5030 Gembloux, Belgique.

² Centre Wallon de Recherches Agronomiques, Département de Biotechnologies Chaussée de Charleroi, 234 BE-5030 Gembloux Belgique.

Introduction. Embryogenesis studies in plants reveal the importance of many genes during the embryo development. Seed development in plants requires a coordinated differentiation of the embryo proper, suspensor, endosperm tissues, and seed coat. Many genes must be expressed as the zygote divides in a regulated manner, completes morphogenesis, and differentiates into a mature embryo capable of surviving desiccation and producing a viable plant (1). These genes show temporal as well as spatial patterns of gene expression during seed development. The stage-specific cDNAs have been used as markers of cell differentiation and to follow the development of the embryos (2). Embryo development studies explored in part the analysis of embryo-defective mutants. A large number of embryo-lethal mutants was identified and analyzed in *Arabidopsis*. The number of genes that can be easily mutated to give an embryo lethal phenotype was estimated to vary between 250 to 750 (1, 3, 4). Some of these genes are heat shock proteins (HSPs), BEL, TITAN (TTN), PASTICCINO (PAS), Leafy Cotyledon (LEC) and lipid transfer proteins (LTP). The HSPs are molecular chaperonins that regulate protein homeostasis and membrane fluidity and ultimately prevent or delay cell death during heat stress (5). BEL transcription factors are essential for inflorescence and fruit development (6). The TTN genes encode chromosome scaffold proteins of the condensing and cohesion classes required for chromosome function at mitosis (7). The PAS genes, which are involved in the control of cell division, proliferation and differentiation, are required for normal organization of the apical region in the embryo (8). LEC genes are central regulators of embryogenesis that play key roles in processes that occur during both the morphogenesis and maturation phases (9). LTP genes are involved in the polar transfer of the lipids towards the peripheral layers of the cells (10). Expression of the above-mentioned genes was analyzed during *Phaseolus* seed development.

Material and methods. The genotype BAT93, a cultivated form of *P. vulgaris*, was used as plant material. Plants were grown in growth chambers under the following conditions: 27°C/23°C (day/night), 75% relative humidity and 12 hrs photoperiod. Total RNAs were extracted from 100 mg of ovules. mRNAs were purified using the “mRNA Purification Kit” from Amersham and used for RT-PCR with the “Titan One Tube RT-PCR kit” from Roche. The 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, Ann. Temp. for 45 sec, 68°C for 45 sec), 68°C for 5 min with Elongation Factor 1 α (EF-1 α , F, CTCAGGATGTBTACAAGATTG & R, GCAGCCTTGGTVACCTTGCWCC) used as internal control.

Results and discussion. mRNAs extracted from ovules harvested 12 days after anthesis were used for RT-PCR. Primers were designed after alignment of species mRNA coding sequences from each gene family. Annealing temperature and MgCl₂ concentrations used are given in table

1. RT-PCR products loaded in 1.5% agarose gel showed one band for PAS, LEC, and LTP genes, two bands for BEL gene, several bands for HSP and TTN genes (Figure 1). Expected size for each gene was as follows: 270 bp for BEL gene, 350 bp for LTP genes, 440 bp for LEC gene, 500 bp for HSP gene, 550 bp for PAS gene and 590 bp for TTN gene. The presence of several bands for some gene families could be explained by unspecific amplifications, due to some primers degeneracy. High intensity band for LEC and LTP genes was obtained after 35 cycles of PCR. Band intensities are intermediate for HSP and PAS genes, and low for BEL and TTN genes. These results mean that transcripts of LEC and LTP genes accumulate more than the others; these genes are strongly expressed in the *Phaseolus* ovules. Amplified fragments will now be sequenced. Sequence homology analyses in gene banks will be performed by using the National Center for Biotechnology Information (NCBI) BLAST network service. The gene sequences with high homologies in genes banks and unknown in *Phaseolus* data base (BEL, TTN, PAS, etc.) will be submitted to gene banks such as NCBI.

Table 1. Oligonucleotides, annealing temperatures and MgCl₂ concentrations used for RT-PCR

Gene families	Annealing temperature & MgCl ₂ concentrations
BEL	45°C, 1.5 mM
HSP	45°C, 2.5 mM
LTP	56°C, 1.5 mM
PAS	50°C, 1.5 mM
LEC	50°C, 1.5 mM
TTN	45°C, 2.5 mM

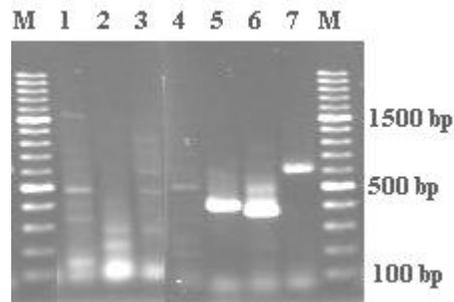


Figure 1. RT-PCR products separated on agarose gel, with one band for PAS (4), LEC (5), and LTP (6) genes, two bands for BEL (2) gene, several bands for HSP (1) and TTN (3) genes. EF-1 α (7) used as internal control.

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MICROSATELLITE DIVERSITY OF MESOAMERICAN COMMON BEANS (PHASEOLUS VULGARIS L.)

Lucy M. Díaz and Matthew W. Blair

CIAT – International Center for Tropical Agriculture - A. A. 6713, Cali, Colombia,

Introduction:

Cultivated common beans (*Phaseolus vulgaris* L.) originated in two centers of diversity giving rise to two gene pools: Mesoamerican from Central America and Mexico and Andean from the Andes mountains of South America (Singh et al., 1991a, b). Some authors also refer to the Mesoamerican gene pool as the Middle American gene pool but both terms refer to the same set of characteristics. The differences between Mesoamerican and Andean gene pools of common bean include seed size, phaseolin patterns, plant morphology, isozymes and RFLP, RAPD or AFLP markers. Cultivated bean gene pools have further been divided into races according to morphological criteria and agroecological adaptation where the term ‘race’ is used to denote a group of related landraces (Singh et al., 1991c). Members of each race have distinctive and specific physiological, agronomic, biochemical and molecular characteristics and differ from other races in the allelic frequencies at specific loci. Race structure has been analyzed by RAPD markers (Beebe et al., 2000) but less molecular evidence has been accumulated for within gene pool differences as compared to between gene pool differences. The objective of this study was to describe the race structure of the Mesoamerican gene pool using microsatellite markers.

Materials and Methods:

A total of 60 genotypes were used of which 35 were from Mexico, 8 from Guatemala, 7 from Brazil, 3 from El Salvador, 2 from Colombia and 1 each from Costa Rica, Ecuador and the United States, all of these representing the Mesoamerican genepool; with 2 additional genotypes, ‘Calima’ from Colombia and ‘G19833’ from Peru, used as an Andean outgroup. Genotypes were selected based on previous race designations (principally Beebe et al., 2000 and Singh et al., 1991a, b, c) and the phaseolin pattern of each genotype was known to be typical of the Mesoamerican gene pool (S, Sb, Sd and M). The Mesoamerican genotypes ‘ICA Pijao’ and ‘DOR364’ from Colombia and El Salvador/CIAT, respectively, were considered control genotypes for the genepool since they had been evaluated previously (Blair et al., 2006). DNA extraction involved germinating 10 seeds selected at random from each accession, which were scarified to ensure uniform germination in the laboratory and pre-germinated in darkness on germination paper. DNA was diluted to 10 ng/ml for further experiments. The DNA was used to amplify a total of 52 microsatellites of which 22 were cDNA based and 30 were genomic. Microsatellites were selected based on their high polymorphism information content from Blair et al. (2006) and their even distribution around the genome. PCR conditions were as per this earlier article. Gels were stained with silver nitrate and allele sizes were evaluated relative to a 10 bp molecular weight size standard (Invitrogen, Carlsbad, CA). The allele information coded for band presence or absence was used to determine population structure and other common parameters of genetic diversity (percentage polymorphic loci, allele frequencies, observed heterozygosity (H_o), indices of genetic differentiation (G_{st}), and gene flow (N_m) for each of the races.

Results and Discussion:

A total of 267 bands were generated with an average of 5.1 alleles per marker and 0.297 heterozygosity across all microsatellites. Correspondence analysis identified two major groups equivalent to the Mesoamerica race and a group containing both Durango and Jalisco race genotypes. Two outlying individuals were classified as potentially of the Guatemala race although this race does not have a defined structure and previously classified members of this race were classified with other races. Population structure analysis with $K=1$ to 4 agreed with this classification (Figure 1). The genetic diversity based on Nei's index for the entire set of genotypes was 0.468 (Table 1) while this was highest for the Durango-Jalisco group (0.414), intermediate for race Mesoamerica (0.340) and low for race Guatemala (0.262). Genetic differentiation (G_{ST}) between the Mesoamerican races was 0.27 while genetic distance and identity showed race Durango and Jalisco individuals to be closely related with high gene flow (N_m) both between these two races (1.67) and between races Durango and Mesoamerica (1.58). Observed heterozygosity was low in all the races as would be expected for an inbreeding species. The analysis with microsatellite markers identified subgroups, which agreed well with commercial class divisions, and seed size was the main distinguishing factor between the two major groups identified. These results have implications in terms of multiple domestications within the Mesoamerican gene pool and introgression between wild and cultivated genotypes.

Table 1. Genetic diversity parameters for Mesoamerican and Andean genotypes.

Groups	N	na	ne	Obs Het	Nei's	P	%
Race Durango (D)	16	2.769	1.720	0.046	0.339	45	86.54
Race Jalisco (J)	14	3.288	2.221	0.045	0.425	48	92.31
Total D-J	30	3.808	2.081	0.046	0.414	48	92.31
Race Guatemala (G)	2	1.577	1.526	0.067	0.262	28	53.85
Race Mesoamerica (M)	26	3.212	1.939	0.028	0.340	42	80.77
Total Mesoamerican	58	4.789	2.352	0.039	0.444	49	94.23
Andean checks	2	1.346	1.339	0.010	0.171	18	34.62
Grand Total	60	5.077	2.447	0.038	0.468	52	100.0

Abbreviations: Number of genotypes (N), observed number of alleles (na), effective number of alleles (ne), observed heterozygosity (Obs_Het.), genetic diversity according to Nei (1973), number of polymorphic loci (P), percentage polymorphic loci (%).

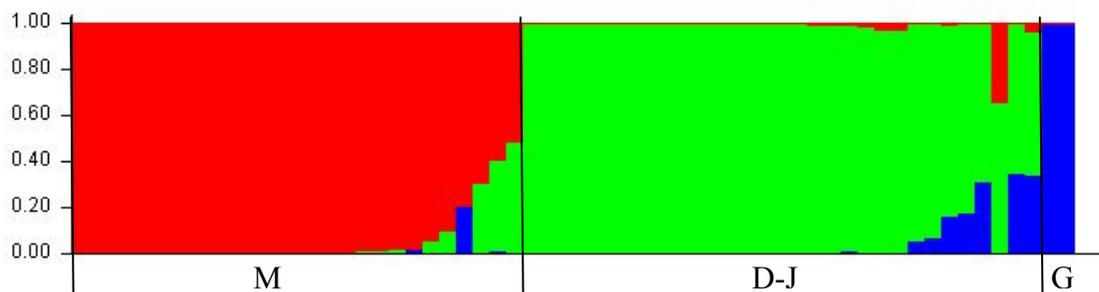


Figure 1. Graph of the population structure ($K=3$) for 58 Mesoamerican genotypes (x-axis) sorted by membership coefficients (y-axis) within clusters defined as in Table 1.

MORPHO-AGRONOMICAL CHARACTERIZATION OF DOMESTICATED COMMON BEAN CORE COLLECTION FROM MÉXICO

M. L. Patricia Vargas-Vázquez¹, José S. Muruaga-Martínez¹, Rigoberto Rosales-Serna², Patricia Pérez-Herrera¹, Homar R. Gill-Langarica³, Miguel Á. Martínez-Damián⁴ and Netzahualcoyotl Mayek-Pérez³

INTRODUCTION. Characterization deficiencies are the major challenge for systematic use of common bean (*Phaseolus vulgaris* L.) diversity in genetic breeding programs. Due to complications observed for detailed crop evaluations in huge number of accessions it is necessary to create representative and manageable core collections. Core collections are small, but thoroughly representative samples which facilitated diversity characterization in several crops. There are 7,846 accessions at INIFAP's (Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias; National Institute for Forestry, Agriculture and Livestock Research) common bean germplasm bank. A subset consisting in 200 accessions were selected to represent the gene bank's entire common bean holdings. Selection was made considering seed color and germplasm origin, representing regions where bean domestication could have been carried out such as the borders of states of Guanajuato, Jalisco and Michoacan (Payró-de la Cruz *et al.*, 2005). Other regions where common bean was traditionally planted or recently introduced were also considered. Evaluating the core collection for a wide range of traits such as morphological, agronomic and disease resistance could be used to identify parents which can be incorporated into breeding programs. Objectives were to characterize using morpho-agronomical traits and to evaluate the representative degree included in a subset of 200 accessions selected as a core collection in INIFAP's common bean germplasm bank.

MATERIALS AND METHODS. Two hundred accessions from INIFAP's common bean core collection were characterized in 2003. Accessions were planted in May 19th at Santa Lucía de Prías, México in one 3 m row, 0.8 m apart and three replications. An additional empty row was leaved between accessions to facilitate the growth of Type IV vining germplasm. Determinations were made for 45 morpho-agronomical traits grouped in: passport data, phenology, leaf characteristics, plant architecture, yield components, seed quality and disease reaction scored according to 1-9 scale (CIAT, 1987). Field readings were made for Anthracnose, Rust, Common Bacterial Blight, Halo Blight, Angular Leaf Spot and White Mold. Principal component analysis (PCA) was performed using all recorded data. Analysis was performed using Systat Ver. 5.02 and results were plotted in Sigma Plot Ver. 8.

RESULTS AND DISCUSSION. Seed commercial classes with higher accession number was cream type known as "Bayo" (28) and black (24), while pink cream stripped "Flor de Junio" (2) and cream "Canario" type (4) showed the lowest accession number. Superiority observed for Bayo and black seeded cultivars are due to those commercial classes have been planted in larger areas of the Mexican Highlands and in the tropics. Natural diversity present in each commercial class influenced the number of cultivars included in core collection. PCA showed low values for PC1 (12.7 %) and PC2 (12.5 %) due to a high variation detected in the cultivar subset selected in the common bean germplasm bank. Three main groups were observed (Figure 1): I. Nueva Granada race with determinate bush (Type I) cultivars; II. Mesoamerican black seeded cultivars and III. Highlands diversity complex with cultivars included in Jalisco and Durango races. Group I. included cream types known as "Canarios" and Cranberry (Cacahuat) cultivars with different color patterns such as red and black. Group II included mainly black (opaque and shiny) seeded cultivars with sub-groups for Type II (Mesoamérica) and Type III (Jalisco) growth habits. Group II also included recombinant cultivars with different grain color such as "Jaspeado" or "Rebosero" (cream

¹ Bean Program. INIFAP-Campo Experimental Valle de México. km. 18.5 Carretera Los Reyes-Lechería, C. P. 56230. Chapingo, México. Fax: 01 (595)954-65-28. e-mail: vargas.patricia@inifap.gob.mx.

² Bean Program. INIFAP-Campo Experimental Valle del Guadiana. km 4.5 Carretera Durango-Mezquital, C. P. 34170. Durango, Dgo. México.

³ Centro de Biotecnología Genómica, IPN. Apdo. Postal 152, C. P. 88730. Reynosa, Tamaulipas, México.

⁴ ISEI-Colegio de Postgraduados. km. 36.5 Carretera México-Texcoco, C. P. 56230. Montecillo, México.

or gray background and gray, red or black mottled) and black striped cultivars similar to Ojo de Cabra type. Some Bayo cultivars were included in group II, which showed some traits similar to black seeded germplasm such as purple flowers. Diversity complex was observed in group III which included several commercial classes sown in Mexican Highlands. Highest diversity by commercial class was observed in Bayo (Cream), Morado de Agua (Purple), Garbancillo (Yellow) and Pinto (Cream with brown and black spots). Some Cranberry Type IV cultivars were also included in group III. Recombinant cultivars with intermediate traits were observed between groups. Similar results were observed using bred germplasm since many bred cultivars were obtained using massal selection in landraces. In spite of the apparent over-representation observed in some commercial classes, due to similarities in grain color, differences in other morpho-agronomical traits were detected. Diversity observed in determinate Type I cultivars was low due to its introduction in México (Nueva Granada race), while huge accession number and diversity were observed in commercial classes related to Mesoamérica gene pool (Mesoamérica, Durango and Jalisco races). Results helped to ensure that actual common bean core collection are truly representative of the many diverse environments in which beans evolved and are grown. Results need to be corroborated using molecular markers to ensure representativeness of common bean genetic diversity included in the core collection. Morpho-agronomical and molecular characterization will be used in broadening the knowledge and exploitation of common bean genetic diversity to obtain increments in disease resistance and seed yield.

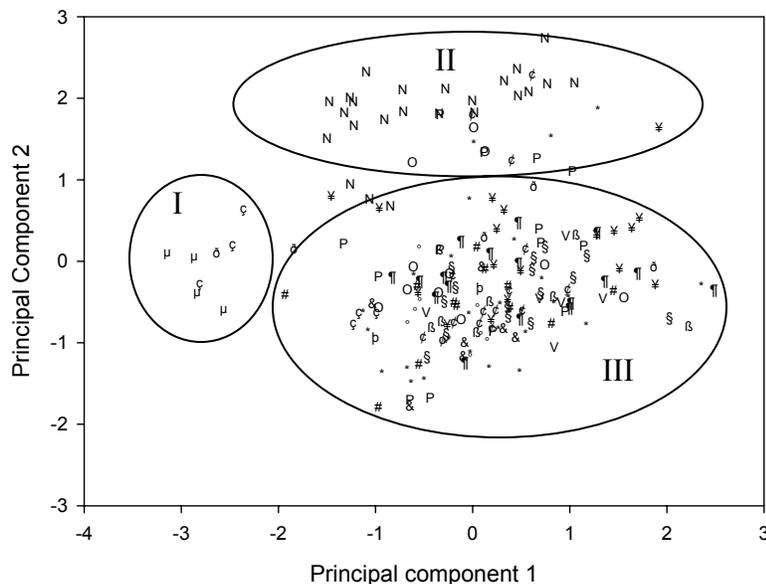


Figure 1. Principal component analysis for Mexican common bean core collection. I. Nueva Granada race, II. Black seeded and recombinant cultivars and III. Highlands diversity complex (Jalisco and Durango races). &= White; #= Yellow; *= Bayo; °= Cream-Gray; ϕ= Cranberry; §= Brown; μ= Canario; β= Flor de Junio; β= Flor de Mayo; ¶= Garbancillo; ϕ= Jaspeado; ¥= Purple; N= Black; O= Ojo de Cabra; P= Pinto; δ= Red and V= Vaquita.

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GENETIC ANALYSIS OF CULTIVATED PHASEOLUS VULGARIS L. CORE COLLECTION OF INIFAP-MEXICO

Homar R. Gill-Langarica¹, M. L. Patricia Vargas-Vázquez², José S. Muruaga-Martínez², Rigoberto Rosales-Serna² & Netzahualcoyotl Mayek-Perez^{1*}

¹ Centro de Biotecnología Genómica-Instituto Politécnico Nacional, Reynosa, México, 88710; ² Campo Experimental Valle de México-Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (CEVAMEX-INIFAP), Chapingo, México, 56230; E-mail: nmayek@ipn.mx

Common bean (*Phaseolus vulgaris* L.) is a basic food for Mexican people and is cultivated up two million hectares annually. Despite the importance of common beans, the broad genetic diversity has been lost due to man and environment impacts (3). The genetic diversity analysis can verify the bean genetic changes to improve conservation strategies (6, 7, 8). Classical criteria to quantify genetic variability are based on either expected heterozygosity or genetic diversity index calculations (11). Molecular marker methodologies have been applied to analyze genetic variability because they are neutral and can be used to analyze evolution and conservation of new morphotypes in crops (7, 8). Molecular markers can also be used to determine historical and geographical relationships among plant species and/or populations (1).

Four AFLP (Amplified Fragment Length Polymorphisms) oligonucleotide combinations were used to characterize genetic relationships and diversity in 200 accessions from the Core Collection of cultivated *Phaseolus* form of INIFAP, which includes samples from all geographical regions of Mexico where common bean is cultivated. As outgroups, ten cultivars (Azufrado Pimono 78, Bayo Zacatecas, Blanco Tlaxcala, Canario 101, Flor de Junio Marcela, Flor de Mayo, Garbancillo Supremo, Negro Jamapa, Pinto Villa, Negro Michigan) released by INIFAP and 30 landraces from Oaxaca, Veracruz and Chiapas, México were included. The DNA was isolated using the protocol of Dellaporta *et al.* (4) and the AFLP method was carried out as described by Vos *et al.* (12). Amplified products were separated by electrophoresis in acrylamide gels using a semi-automated sequencing system (model IR2, Li-Cor, Lincoln, NE). The numbers of amplified and polymorphic AFLP products, genetic diversity index (DI) and cluster analysis among accessions based on geographical origin (state and altitude) were determined using Info-Gen (2) and Phylip 3.6 (5) programs.

The AFLP analysis detected 530 amplified products, 469 were polymorphic (89.3%). The greatest DI was found in accessions from Jalisco (35%) and Aguascalientes (33%) and the lowest in accessions from Guerrero (24%) and Veracruz (23%). Landraces from Oaxaca and cultivars showed the lowest DI values (20 and 22%, respectively). Cluster analysis based on states of origin showed two major groups, where group A was divided on two subgroups: A1 included accessions from Central Mexico and A2 accessions from Michoacan, landraces from Oaxaca, Veracruz and Chiapas and cultivars. The group B included accessions from Central and Northern Mexico (subgroup B1) and Central and Southern Mexico (subgroup B2) (Fig. 1A). Cluster analysis based on altitude of origin showed clear separation among accessions from core collection, landraces and cultivars. Accessions from 0-500 and 501-1000 masl (C0 and C1) showed the highest genetic similarity. As altitude origin was increasing, the genetic distance among accession was increased too (Fig 1B). The low DI values and close relationships among

bean accessions indicated a few number of major genes affect genetic variability in common beans but a high number of genes can be affected by artificial selection during domestication of the genus (13). Major genes have been associated to seed dispersion mode, dormancy, growth habit, sensitivity to photoperiod and harvest index (10). Our results emphasize the intensive gene flow in the cultivated form of *P. vulgaris* (9) due accessions from contrasting climate and geographical regions were genetically similar. In addition, clear differences between core collection, landraces and cultivars were found based on geographical origins (state and altitude) indicating the clear impact of bean breeding in *Phaseolus* genetic resources from Mexico.

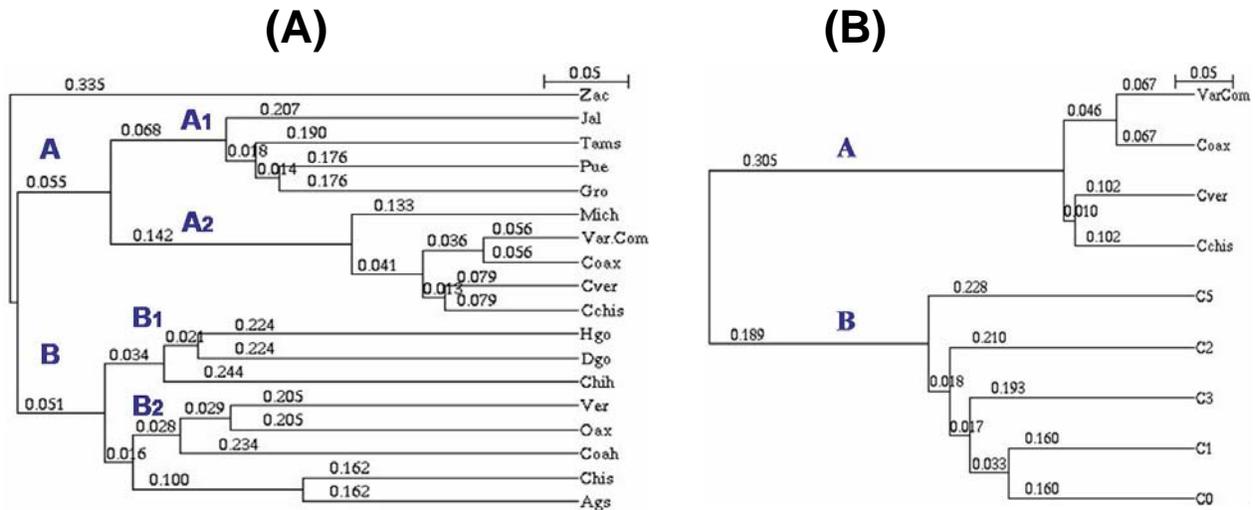


Fig. 1. Dendrograms of genetic dissimilarities among *P. vulgaris* accessions from INIFAP core collection based on state (A) and altitude of origin (B).

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SEED YIELD OF BLACK SEEDED LINES INTROGRESSED WITH WILD PHASEOLUS VULGARIS

Jorge A. Acosta-Gallegos¹, Bismarck Aguilar Garzón¹, Raúl Rodríguez-Guerra¹, Manuel Mendoza Hernández¹, Horacio Guzman-Maldonado¹ and James D. Kelly².

¹ Bean Program of INIFAP, Campo Experimental Bajío, Km 6.5 carretera Celaya-San Miguel de Allende, CP 34110, Celaya, Guanajuato, México. jamk@prodigy.net.mx

² Bean Program at Michigan State University, 207 PSSB, E. Lansing, MI 48824

The common bean cultigen was domesticated from wild *P. vulgaris*, a viny indeterminate plant from the mid-altitude Neotropics and subtropics that has a wide distribution range from northern Mexico to northwestern Argentina (Gepts and Debouck, 1991). Contemporary bean breeding programs are limited by the under-utilization of the available genetic diversity in the wild species because of the necessity to pre-breed exotic material. As such, wild *P. vulgaris* is an untapped resource for traits such as resistance to insects and diseases, higher N, iron and calcium content in seeds, which will ultimately contribute to improvements in nutritional quality and yield.

Scientists at CIAT have used the inbred backcross method to introgress wild *P. vulgaris* from one gene pool into the domesticated germplasm of the other gene pool. One such example is the development of an inbred backcross population between an elite black bean cultivar Negro Tacaná (Lopez-Salinas *et al.*, 1997) from Mexico and a wild bean accession G24423 from Colombia. The original cross and two backcrosses were made at CIAT and the inbred progenies were evaluated through the Bean/Cowpea CRSP network in Mexico and the U.S. One BC₂F_{4:7} line (115M) produced the highest recorded bean yield in a Michigan State University yield trial (5790 kg ha⁻¹), and outperformed the recurrent parent, Negro Tacaná by 27% (Kelly, 2004). The line has continued to outperform commercial checks in National Cooperative Dry Bean Nurseries from 2002-05. The most striking observation in these inbred backcross populations derived from wild *P. vulgaris* was the similarity of the progeny to the domesticated recurrent parent after only two backcrosses, despite the obvious undesirable characteristics of the wild parent.

Here we report the yield of five inbred backcross lines (IBLs) derived from the above-mentioned population that were tested in uniform yield trials in Guanajuato, Mexico and compared to two bred cultivars and two lines derived from crosses between elite parents. Thirteen trials were conducted from 2003 to 2006 at either irrigated or rainfed conditions. A RCBD with four replicates was utilized and data were recorded on several traits, but only seed yield is reported here. The recurrent parent, Negro Tacana, was not included in the trial, however in previous rainfed trials at Texcoco, State of Mexico the average yield of the five IBLs reported here was 11 % higher than the recurrent parent Negro Tacana (1861 vs 1656 kg ha⁻¹) and UG-21141-102(F₁)-1-1-M outyielded the recurrent parent by 17%. In our trials there was significant genotype x trial interaction for seed yield with different genotypes at the top in each trial. In general, higher yields were observed under rainfed (R) conditions than under irrigation (I), particularly in 2005 and 2006 at the Bajío Experimental Station (CEB; Table 1). The irrigated trials were conducted earlier in the season when temperatures were higher during the seed filling stage which resulted in smaller seed size and slightly lower yields than under rainfed conditions (data not shown). One IBL was the top yielder in six out of 13 trials, while in the rest of the trials either a line or a released cultivar was the top-yielder. Averaged as a group, the five IBL lines yielded (1645 kg ha⁻¹) similar to the two elite lines (1646 kg) and the two improved cultivars (1682 kg) across all trials. Individually, Negro 8025 was the top yielder followed by UG-21141-71(F₁)-1-1 that was significantly superior to Negro Altiplano and the two elite lines.

Table 1. Seed yield (kg ha⁻¹) of five black seeded IBLs introgressed with wild *P. vulgaris* (UG -21141- code), two elite x elite lines (NG code) and two bred cultivars grown in four locations in Guanajuato Mexico under either irrigated (I) or rainfed (R) conditions from 2003 to 2006.

Locations/ Genotypes	CA 03 R	SLP 03 I	CEB 03 I	CEB 03 R	LA 04 R	OCA 04 R	CEB 04 I	CEB 05 I(1)	CEB 05 I(2)	CEB 05 R	CEB 06 I	CEB 06 R	LA 06 R	Ave.
UG-21141- 87(F ₁)-1-1	1871	1644	2030	1158	928	1710	3263	933	1257	2827	2085	2012	148	1682
UG-21141- 70(F ₁)-1-1	1934	1404	1833	1224	873	1994	1202	2200	906	2982	2212	3245	627	1741
UG-21141- 71(F ₁)-1-1	1851	1578	1645	1128	773	2032	2450	2207	1636	2659	2483	2416	1267	1856
UG-21141- 102(F ₁)-1-1	1937	1547	2190	1202	358	1741	2888	2067	1250	2556	2046	3048	531	1797
UG-21141- 10(F ₁)-1-1	2142	1689	1861	1194	861	1063	1734	2126	1283	2838	2253	3105	611	1751
NG99038	1763	3485	-	-	964	1941	1622	1766	1030	2746	2087	2965	569	1611
NG99279	2403	2289	-	-	856	2397	1322	2720	1103	2687	2191	3467	420	1681
Negro 8025	2853	1249	1658	1139	925	2055	2967	2100	1347	3215	1997	2935	496	1918
Negro Altiplano	1917	1823	-	-	992	1518	1238	1707	953	2792	2079	2925	857	1446
Trial Ave.	2075	1856	1869	1174	837	1828	2076	1981	1196	2811	2159	2902	614	

The seed harvested in 2005 from all genotypes in the trial was taken to the food quality lab to determine protein content and other traits related to quality. The introgressed IBLs were similar to the standard cultivars in the trial, thus the phenotypic selection practiced for yield and seed size among the 100 IBLs, did not affect the recovery of critical quality traits from the recurrent parent. In addition, the genotypes in the trail were also inoculated in the greenhouse with three anthracnose races (1472, 448 and 328) widely distributed in the Mexican Highlands and three of the five IBL lines (UG-21141-70(F₁)-1-1, UG-21141-71(F₁)-1-1 and UG-21141-10(F₁)-1-1) along with the two elite lines (NG99038 and NG99279), were resistant to all three races. These results suggest that the use of wild *P. vulgaris* as parental stock in bean breeding will not hamper but should enhance the breeding process.

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Acknowledgments: The bean program in CIAT for making available the germplasm of introgressed lines used in this research. The financial support from Fundación Guanajuato Produce, Project 317/04, is highly appreciated.

INHERITANCE OF PLANT HEIGHT, INTERNODE LENGTH AND BRANCH NUMBER IN CLIMBING COMMON BEAN POPULATIONS (*PHASEOLUS VULGARIS* L.)

Oscar E. Checa¹, Hernán Ceballos², Matthew W. Blair³

¹Faculty of Agricultural Sciences, Universidad de Nariño, Pasto, Colombia ² Universidad Nacional de Colombia, Palmira Campus; ³Centro Internacional de Agricultura Tropical, A.A. 6713, Cali, Colombia

Introduction:

Climbing common bean (*Phaseolus vulgaris* L.) genotypes have among the highest yield potential of all accessions found in the species and are distinct from bush bean varieties of common beans because they have tall growth, long internodes and twining ability. They are an important component of traditional agriculture in several parts of Latin America, especially Mexico, Guatemala, Colombia, Ecuador, and Peru and have spread to the Great Lakes region of Africa. Climbing beans are often grown in association with maize, either in relay or simultaneous plantings, and maize provides the support required for the climbing beans to grow upwards. In monoculture, climbing beans are planted with the support of wood or bamboo stakes or trellis systems. Trellising, a widespread system in the Andean region, is an alternative that reduces the need for stakes, but requires an investment in wires, string and labor for tying up bean vines. Trellising of climbing beans is economically justified because yields in monoculture may surpass 4500 kg ha⁻¹. Therefore, climbing beans are particularly useful for small landholdings in situations where labor is not limiting and where demand for beans is high. Genetic improvement of climbing beans would benefit from an understanding of the inheritance of climbing capacity (made up of plant height and internode length traits). Therefore, the objective of this study was to determine the inheritance of climbing capacity traits in three crosses made within and between gene pools using generation means analysis.

Materials and Methods:

Three populations were developed, both within and between gene pools i.e. Andean × Andean (BRB32 × MAC47), Mesoamerican × Mesoamerican (Tío Canela × G2333), and Mesoamerican × Andean (G2333 × G19839). Each cross combination contrasted for growth habit with either a type II (BRB32, Tío Canela) or type III (G19839) parent crossed with a type IV (MAC47 or G2333) parent. A total of 50 pollinations were made per cross to generate F₁s and 40 pollinations per backcross of the F₁ hybrids with their respective parents to create the BC₁P₁ and BC₁P₂ generations. For each population, the six generations (P₁, P₂, F₁, F₂, BC₁P₁ and BC₁P₂) were planted in a randomized complete block design experiment with three replications in Darién, Colombia. The experiments were planted as bean monocultures using trellis systems in which each individual plant was tied with a string made of polypropylene to a heavy weight wire that was suspended horizontally above the row at a height of 2 m above the soil level on bamboo posts. Plant density was every 10 cm with 1.0 m between rows. Each plant was evaluated for plant height (PH) in m, internode length (IL) in cm, and number of branches (BN). PH, IL and GS were evaluated twice during the growing season on an individual plant basis from plants within the row (not considering border plants), first at 40 and then at 70 days after planting (DAP).

Results and Discussion:

Analysis of variance for each of the three populations was conducted separately and significant differences were observed between treatments for every variable except BN at 40 dap. Table 1 shows the separation of means carried out with Tukey's multiple comparisons ($P \leq 0.05$) for the six treatments of the three populations. For the G2333 \times G19839 population, dominance was important in controlling both PH and IL and this trend was more evident early in the season at 40 DAP rather than late in the season at 70 DAP. Means of the backcross generations were observed to be similar to the means of their respective recurrent parents which themselves were contrasting for both PH and IL. In the other two populations, the F_1 and F_2 treatments had means intermediate between the parents. Those variables that showed significant differences by orthogonal contrasts between parents P_1 and P_2 , were submitted to generation means analysis using the methodology proposed by Mather and Jinks (1971) which showed the importance of additive compared to the dominant-additive portion of the genetic model for all three population. Broad sense heritabilities for the traits varied from 62.3 to 85.6% for plant height and from 66.5 to 83.7% for internode length. Narrow-sense heritabilities calculated from additive and environmental variances were similar to broad sense heritabilities, with the highest values for G2333 \times G19839 and BRB32 \times MAC47 (66.9 and 65.4%, respectively) and the lowest for Tio Canela \times G2333 (52.5%). The generation means analysis and estimates of heritability suggested that the inheritance of plant height and internode length in climbing beans is relatively simple and mostly additive although a dominant-additive model was also significant in the inter gene pool cross.

Table 1. Tukey's multiple means comparison for plant height and internode length evaluated at 40 and 70 days after planting (DAP) in three populations.

Treatment	Plant Height – 40 DAP	Plant Height – 70 DAP	Internode length – 40 DAP	Internode length – 70 DAP
G2333 X G19839				
P1 (G2333)	1.893 A	2.626 A	21.38 A	21.81 A
BC ₁ P ₁	1.630 A	2.806 A	18.74 A	19.89 A
F ₁	1.710 A	2.626 A	18.42 A	20.98 AB
F ₂	1.173 B	2.136 B	14.25 B	16.60 BC
BC ₁ P ₂	0.74 C	1.523 C	9.75 C	13.45 C
P2 (G19839)	0.396 D	0.803 D	6.20 C	8.07 D
Tio Canela x G2333				
P2 (G2333)	1.743 A	2.573 A	19.95 A	18.16 A
BC ₁ P ₂	1.443 B	2.503 A	17.73 B	17.93 A
F ₁	1.150 C	2.176 B	16.50 B	15.35 B
F ₂	0.860 D	1.693 C	12.05 C	13.52 B
BC ₁ P ₁	0.506 E	1.016 D	7.68 D	9.76 C
P1 (Tio Canela)	0.173 F	0.396 E	3.03 E	4.44 C
BRB32 x MAC47				
P2 (MAC47)	1.650 A	2.636 A	22.01 A	23.10 A
BC ₁ P ₂	1.536 A	2.360 AB	19.20 AB	20.01 A
F ₁	1.233 B	2.170 BC	15.00 BC	16.10 B
F ₂	1.183 B	1.936 C	13.95 C	14.72 BC
BC ₁ P ₁	0.90 C	1.576 D	11.53 C	12.77 C
P1 (BRB32)	0.476 D	0.83 E	7.04 D	7.49 D

^a Means followed by the same letter within each column not significantly different at $P = 0.05$.

CHARACTERIZATION OF COMMON BEAN (*PHASEOLUS VULGARIS* L.) VARIETIES FROM SERBIA

Zorica T. Nikolić¹, Mirjana A. Vasić², Mirjana B. Milošević¹,
Milka Lj. Vujaković¹, Jelica M. Gvozdanović-Varga²

¹National Laboratory for Seed Testing, Novi Sad, Serbia

²Research Institute of Field and Vegetable Crops, Novi Sad, Serbia

E-mail: nikolicz@ifvcns.ns.ac.yu

Introduction

The variability at the protein level has been well documented for common bean (*Phaseolus vulgaris* L). Isozyme analysis (Koenig and Gepts, 1989) and the analysis of phaseolin seed storage protein pointed out to two different groups of *P. vulgaris*. It was found out that there was a relationship between geographic distribution and phaseolin type in wild and cultivated bean varieties. Samples from Central America had primarily S phaseolin type, with a few exceptions having M type. Samples from Andes had primarily T phaseolin type, and some had C, H, A, J, or I type. There are bean varieties with S and C/T phaseolin type, revealing that multiple events of gene recombination happened during domestication process (Brown et al., 1982, Gepts et al., 1986).

The aim of this work was to evaluate 15 bean varieties, using phaseolin seed protein and isozymes analysis, the genetic variability as well as to relate their origin to the Mesoamerican and Andean gene pools. The results may contribute to improvement of germplasm bank management and may improve the efficiency of the breeding process.

Material and methods

Fifteen bean genotypes of different origin i.e. selections were studied in this paper. Eight varieties of Department of vegetables, Research Institute of Field and Vegetable Crops, Novi Sad, two domestic populations, two Bulgarian varieties, one variety from Institute in Smederevska Palanka, one American variety, and one variety from Croatia.

Isozyme systems: malate dehydrogenase (MDH), malic enzyme (ME), phosphohexose isomerase (PHI), phosphogluconate dehydrogenase (PGD), phosphoglucomutase (PGM), shikimate dehydrogenase (SKDH), isocitrate dehydrogenase (IDH), alcohol dehydrogenase (ADH) were analyzed according to Stuber et al. (1988). Preparation of samples and 1D-SDS PAGE electrophoresis of phaseolin were done according to Rodino et al. (2001). Four individual seeds were tested from each samples.

Results and Discussion

The origin of Serbian bean germplasm is unclear. The biochemical marker phaseolin and isozymes were used in this work to display the variation of common bean germplasm. It was confirmed by experiment that significant polymorphism of enzymatic system was not expected since commercial bean varieties were studied.

From 8 analyzed enzymic systems, enzymes MDH, SKDH, ME and IDH were polymorphic, while there were no differences in zymograms for enzymes PGM, PHI, PGD, ADH (Figure 1). Three different allelic variants were found for enzyme SKDH and two for IDH.

Analysis of phaseolin revealed two types: S and T (Figure 2). S type of phaseolin was found in most of analyzed genotypes (9 from 15), which revealed that in the process of

development of new varieties under climatic conditions of our country and the region, germplasm from Central America was used. According to Genčev et al. (2002) Bulgarian bean varieties with dominating S phaseolin were better adapted, to climatic conditions of high temperature, and irregular rain falls, in comparison to others. Phaseolin type T is found in three varieties of Novi Sad selection, domestic populations, Croatian variety. Those varieties were developed from domestic populations from north-west region of Balkan, Slavonia, and Vojvodina.

Results obtained in this work suggested that both gene pools were used in process of introduction and breeding of common bean in Serbia. Data on isozymic variability in combination with data on phaseolin type give a fine picture on genetic diversity of bean varieties (Santalla et al., 2002). Analysis of specific region of genes for phaseolin, identification of variation in exon and introne offer more precise data on genetic diversity (Kami et al., 1995).

Analysis and characterization of varieties of Department of vegetables, Research Institute of Field and Vegetable Crops, Novi Sad at the level of protein was done for the first time. Obtained results present a solid starting base for further investigation of gene bank and application of molecular markers.

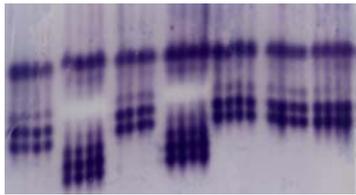


Figure 1. Zymogram pattern of MDH in common bean genotypes

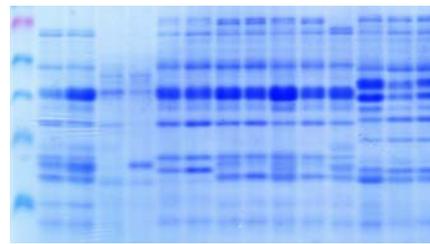


Fig.2. Different types of phaseolin in common bean genotypes

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GENETIC PROGRESS AFTER FIVE CYCLES OF PHENOTYPIC RECURRENT SELECTION FOR EARLY FLOWERING OF CARIOCA COMMON BEAN

Flávia Barbosa Silva¹; Magno Antonio Patto Ramalho¹;
Ângela de Fátima Barbosa Abreu²

¹Universidade Federal de Lavras, Lavras, MG, P. O. Box 3037, e-mail:
flaviabs28@hotmail.com; magnoapr@ufla.br

²Embrapa Arroz e Feijão/Universidade Federal de Lavras, Lavras, MG, P. O. Box 3037; e-mail:
afbabreu@ufla.br

Introduction

In the environmental conditions of Brazil, common bean has one of the shortest cycles among cultivated species (normally between 85 and 90 days). This is the main reason for the trend to cultivation under irrigation and in rotation with other species. Moreover, owing to the short cycle, three growing seasons per year are possible. Nevertheless, the search for even earlier cultivars is still going on, mainly to reduce water consumption, energy for irrigation as well as the production costs, and to attain a greater flexibility of crop rotation.

In this setting, this study was conducted to verify the efficacy of phenotypic recurrent selection in common bean for the number of days to flowering and evaluate the response correlated to selection in other important traits.

Material and Methods

The recurrent selection program for early flowering in common bean was conducted on an experimental field of the Universidade Federal de Lavras, in Lavras, Minas Gerais, Brazil. Firstly, the base population was developed by means of a partial diallel mating system from five early lines and ten of normal cycle. From this diallel, 49 F₁ hybrids were derived. These were evaluated for early flowering and the 11 hybrid populations with the lowest number of days to flowering selected. Then 300 carioca seeds were collected from each population. The seeds were all mixed to form the base population S₀ (cycle 0) of the recurrent selection program.

The plants of the S₀ population that presented the first flower buds were crossed on the field. This intercrossing was repeated for five successive days. The hybrid seeds were blended to obtain cycle I of the recurrent selection program. The procedure was repeated to obtain cycles II, III, IV, and V.

To estimate the genetic progress 53 S_{1:2} progenies of each cycle were used. The S_{1:2} progenies were evaluated in February 2006. The experiment was arranged in a 18 x 18 simple lattice design, with 318 S_{1:2} progenies and six controls. Plots consisted of a 2m row, with a sowing density of 15 seeds per meter. The following traits were evaluated: number of days from emergence to flowering; severity of angular leaf spot; number of days from plant emergence to physiological maturity; grain yield in grams per plot and grain type.

Results and Discussion

Significant differences were observed in the mean performance of the progenies between the selection cycles for the trait number of days to flowering, indicating the existence of

variability, an essential condition for the selection process. A tendency towards a reduction in the number of days for the beginning of flowering was observed along the selection cycles. It was further observed that the average number of days to flowering of the progenies was 15.3% smaller than of the controls. It is further noteworthy that the mean number until flowering of cultivar Pérola, one of the most planted in Brazil, was about 34 days. The progenies were therefore in the mean eight days earlier.

The genetic progress estimated for the number of days to flowering (earliness) of -0.73% can basically be considered as a small magnitude (Table 1). Some points must however be taken into consideration. Firstly, of the 49 hybrid populations developed in the beginning, involving the crossing of lines of early and normal cycle, only the 11 earliest were selected to form a base population of the recurrent selection program. In the literature, there are reports that earliness is controlled by few major genes (Bassett, 2004), although the existence of modifier genes is evident (Singh, 1991). It is therefore probable that the population selection targeted the major genes. The action of recurrent selection was towards the minor genes, letting one expect a smaller response to selection, since the base population was already quite early.

Another point is that the phenotypic selection is applied in all growing seasons, that is, one cycle of recurrent selection per growing season. In the south of Minas Gerais, three growing seasons per year are possible, which allows three selection cycles per year, resulting in a progress of -2.2% per year.

No association was stated either of the beginning of flowering with grain yield ($r_G=0.07$) and severity of angular leaf spot ($r_G=0.00$), indicating the possibility of selecting early-flowering progenies associated to good yield and resistance to angular leaf spot.

TABLE 1. Estimates of the coefficients of linear regression between number of cycles (x) and the trait mean (y): number of days to flowering (NDF), number of days to maturation (NDM), grain yield (GY), severity grades of angular leaf spot (ALS) and grain type grades (GTG) obtained in the evaluation of S_{1,2} progenies of the recurrent selection cycles.

Traits	b ₀	b ₁	P	R ² (%)	Progress(%)
NDF	26.92	-0.197	0.040	65.45	-0.73
NDM	72.81	0.053	0.921	0.27	-
GY	312.4	-0.717	0.872	0.71	-
ALS	5.14	-0.031	0.650	5.48	-
GTG	2.79	-0.018	0.541	9.67	-

b₀ – intercept; b₁ – linear regression coefficient; P – probability of significance by t test; R² – determination coefficient; progress (%) computed by the equation b₁/b₀ x 100.

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EFFECT OF SOAKING AND COOKING ON THE OLIGOSACCHARIDES AND LECTINS IN RED KIDNEY BEANS (*PHASEOLUS VULGARIS* L.)

G. Nyombaire¹, M. Siddiq¹, K. Dolan^{1,2}

¹Department of Food Science and Human Nutrition, ²Department of Biosystems and Agricultural Engineering, Michigan State University, East Lansing, MI, 48824

INTRODUCTION

Common beans (*Phaseolus vulgaris*), owing to their nutrient-dense attributes, offer a potential to be developed as multiple-use products. However, in general, the consumption of beans has rarely gone beyond traditionally processed bean products and their uses. The reason for this stagnancy in the growth of processed bean consumption may be linked to 2 antinutritional factors found in common beans: 1) mainly due to the presence of flatulence-causing oligosaccharides, namely raffinose and stachyose (Silva-Queiroz et al., 2002), and 2) to a lesser extent, the presence of toxic lectins (Sharon and Lis 2002) that have been associated with food poisoning. Thus, any newly developed bean product would find acceptability among consumers only if these negative characteristics of common beans were minimized or eliminated. Pre-soaking beans at suitable conditions for oligosaccharides reduction and then cooking them at temperatures above 80 °C for elimination of lectins are necessary to produce a safe bean product. The objective of the present study was to see the effect of soaking beans at an elevated temperature and cooking for short time on hydration, oligosaccharides and lectin content.

MATERIALS AND METHODS

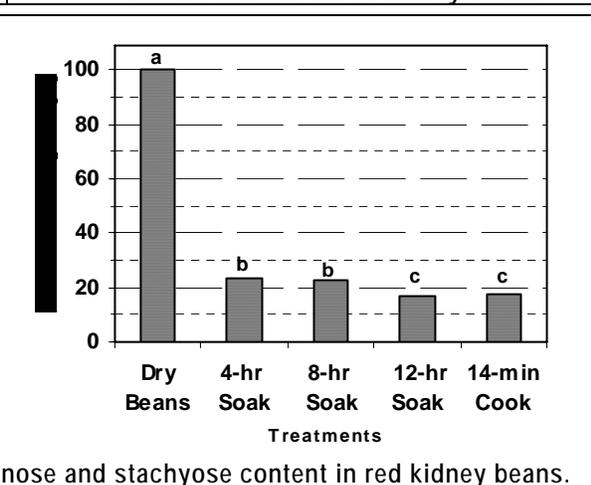
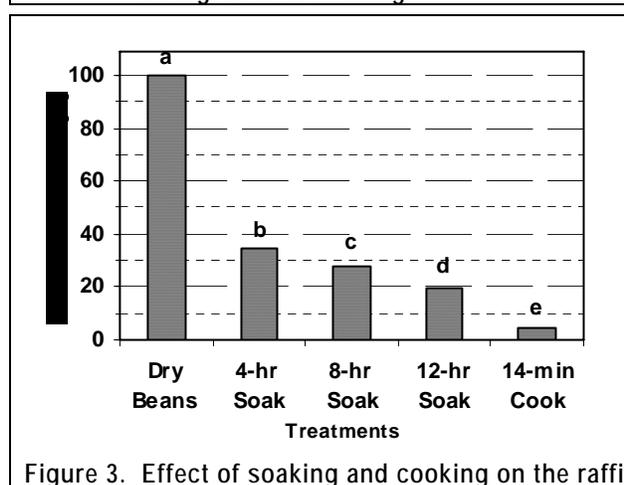
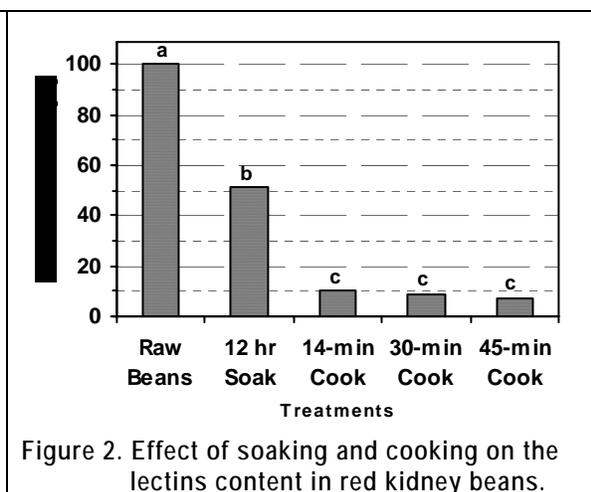
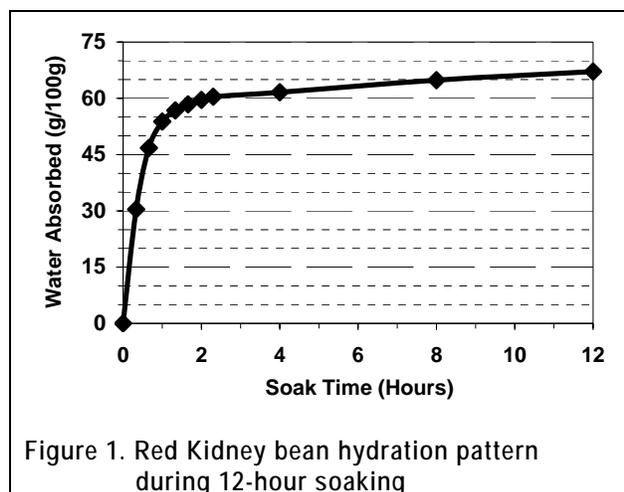
Whole dry red kidney beans were purchased from a local source. Beans were soaked for 12 hour in distilled water containing sodium bicarbonate and sodium polyphosphate (Dolan et al., 2006). The initial soak temperature was 77 °C and samples were then let to equilibrate to room temperature, 24 ± 1 °C. The initial soak temperature of 77 °C was chosen because bean processors in Michigan use this for commercial production of canned beans. Soaked beans were cooked in boiling water (~99.3 °C) for 14 minutes. Bean hydration, oligosaccharides (raffinose and stachyose), and lectins were determined by the methods of Matella et al. (2005) and Siddiq et al. (2006).

RESULTS

The total weight gain at the end of 12-hour soak was 67.1 g/100 g dry beans. About 80% of the weight gain was attained during the first hour of soaking (Fig. 1).

There was a significant reduction in lectin activity at the end of 12-hour soak, as evidenced by a 48.88% drop. Following 12-hour soak, cooking beans in boiling water for 45 min reduced lectins by a total of over 93%, with very little or no differences between 14, 30, or 45 min cook times (Fig. 2).

Soaking for 12 hours resulted in 80.83% reduction in raffinose, however, as was the case with weight gain, a significant portion of the total loss (65.28%) occurred during the initial 4 hours of soaking (Fig. 3). Total reduction in raffinose content, including cooking, was about 96%. As compared to raffinose, the rate of stachyose reduction was higher during soaking cycle, accounting for 83.44% loss. Cooking beans at 99.3 °C for 14 min further reduced raffinose content significantly ($p < 0.05$) but had no such effect on stachyose reduction (Fig. 3).



CONCLUSION

In conclusion, it was demonstrated that the soaking and cooking conditions used in our study were effective in significantly reducing antinutrient factors in red kidney beans, namely flatulence-causing oligosaccharides (raffinose and stachyose) and toxic lectins.

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A NEW MINIMALLY PROCESSED RED KIDNEY BEAN (*PHASEOLUS VULGARIS* L.) SUGAR-COATED SNACK

G. Nyomba¹, M. Siddiq¹, K. Dolan^{1,2}, J.B. Harte¹

¹Department of Food Science and Human Nutrition, ²Department of Biosystems and Agricultural Engineering, Michigan State University, East Lansing, MI, 48824

INTRODUCTION

Beans (*Phaseolus vulgaris*) are consumed all over the world and are a staple food in many countries. Beans are a good source of protein, energy (approx. 380 kcal/100 g of seeds), vitamins (thiamine, riboflavin, niacin, vitamin B-6, folic acid), dietary fiber (especially soluble fraction), and minerals (calcium, iron, zinc, phosphorus, potassium, magnesium) (Reyes-Moreno and Paredes-Lopez 1993; Wu et al. 2004), and antioxidant (dark colored varieties) and are low in fat. Pre-soaking beans at suitable conditions may help to reduce flatulence-causing oligosaccharides such as raffinose and stachyose (Silva-Queiroz et al. 2002) and then cooking them at temperatures above 80 °C for elimination of lectins (Coffey et al. 1993) are necessary to produce a safe bean product. The objective of the present study was to develop a red kidney bean snack product that was safe to consume, having no or very low oligosaccharides and lectins, and possessing acceptable sensory attributes of color, texture, flavor, and overall acceptability.

MATERIALS AND METHODS

Whole dry red kidney beans in this study were purchased from Bayside Best Beans, LLC (Sebewaing, Mich., U.S.A.). Beans were processed according to the method of Dolan et al. (2006). Briefly, beans were soaked for 12 h in 5-times volume of distilled water containing sodium bicarbonate and sodium polyphosphate. The initial soak temperature was 77 °C and samples were then let to equilibrate to room temperature, 24 ± 1 °C. The initial soak temperature of 77 °C was chosen because bean processors in Michigan use this for commercial production of canned beans. Beans were then cooked in boiling water at 99.3 °C for 14 min in steam-jacketed kettles, in 3-times volume of distilled water containing sodium bicarbonate and sodium polyphosphate. Finally, beans were sugar-coated by dipping in 20%, 35% or 50% sugar-in-water solution (w/w) at 70 °C for 45 min; the ratio of beans to syrup was 1:3 (w/w) and excess syrup was drained completely at the end of process. Samples were presented in random order and panelists were asked to rate their likeness for color, texture, sweetness, flavor and overall acceptability on a 1-9 hedonic scale (1=dislike extremely, 2=dislike very much, 3=dislike moderately, 4=dislike slightly, 5=neither like nor dislike, 6=like slightly, 7=like moderately, 8=like very much, 9=like extremely). Panelist entered their responses on computer using a direct data entry system. A score of 5 was considered the lower limit of acceptability for all sensory attributes tested.

RESULTS

Mean sensory ratings by the consumer panel are shown in Table 1. A score of 5, that corresponded to “neither like nor dislike,” or below was designated as the limit of acceptability for sensory quality of beans. All bean samples evaluated scored higher than 5 for all the sensory attributes tested. Since this product is not currently marketed in the U.S., sample rating of

greater than 5 was considered promising. For all three levels of sugar syrups used for coating beans, there were no significant differences in subjective color or texture. For sensory attributes of flavor, sweetness, and overall acceptability, beans coated with 20% sugar syrup differed significantly ($p < 0.01$) than those coated with 50% syrup, which were consistently rated the highest for these three attributes. Beans coated with 20% syrup were not different than the other two samples, the only exception being significant difference in the sweetness as compared to beans coated with 50% syrup. Beans coated with 50% syrup were the only sample that was rated higher than a score of 6 for all 5 sensory attributes tested.

Table 1. Consumer panel¹ sensory scores of cooked red kidney beans coated with different sugar syrups.

Sensory Attribute	Sensory Scores ² of Sugar-Coated Beans		
	20% Syrup	35% Syrup	50% Syrup
Color	6.15a ³	6.00a	6.01a
Texture	5.78a	5.73a	6.10a
Flavor	5.59b	5.95ab	6.30a
Sweetness	5.48b	6.03a	6.27a
Overall Acceptance	5.46b	5.85ab	6.19a

¹N=105; 33 males, 72 females

² Scoring scale of 1-9: 1-dislike extremely, 9-like extremely

³ Means sharing the same letter in rows are not significantly different from each other (Tukey's HSD test, $p < 0.05$)

CONCLUSION

Based on the consumer panel sensory results, it is concluded that red kidney beans coated with either 35 to 50% sugar syrup can find acceptability among consumers and offer potential for commercial production and marketing of such products.

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THE USE OF A LOW-COST EXTRUDER TO INACTIVATE PHYTOHEMAGGLUTININS IN LIGHT RED KIDNEY BEANS (*PHASEOLUS VULGARIS* L.)

G. Nyomba¹, K. Dolan^{1,2}, M. Bennink¹

¹Department of Food Science and Human Nutrition, ²Department of Biosystems and Agricultural Engineering, Michigan State University, East Lansing, MI, 48824

INTRODUCTION

Dry beans (*Phaseolus vulgaris* L.) are an important leguminous crop. Dry beans are generally recognized as a good source of food protein and dietary fiber. Dry beans (*Phaseolus vulgaris* L.) are an extremely beneficial component of all diets because they are high in complex carbohydrates, protein and dietary fiber, and low in fat, calories and sodium, and are cholesterol free. In the developed world where cholesterol is a major health problem, dry beans are considered as the major source of dietary fiber. The inactivation of antinutritional factors such as phytohemagglutinins (lectins) is very important in bean cooking. Lectins in undercooked kidney beans have been linked to food poisoning in the United Kingdom. Extrusion processing using a combination of moisture, pressure, temperature and mechanical shear results in physical and chemical changes, such as inactivation of antinutritional factors and starch gelatinization. The purpose of this study was to determine how effective the low-cost extruder was on the inactivation of phytohemagglutinins in light red kidney beans.

MATERIALS AND METHODS

Dry red kidney beans (*Phaseolus vulgaris* L.) used in this study were purchased from Bayside Best Beans, LLC (Sebewiang, MI). The damaged beans were segregated from the main population, and then raw bean material was ground using a hammer mill (Model D Comminuting Machine, The W.J. Fitzpatrick Company, Chicago, USA). Extrusion runs of raw ground red kidney beans were carried out using a low cost laboratory co-rotating twin-screw extruder model JS30A manufactured in China by Qigong Chemical Industry Equipment Co, Ltd. The screws are 30 mm in diameter and the barrel has a L/D of 14. Red kidney bean flour was extruded at 25 % and 36 % moisture content wet basis; screw speed was 118, 194 and 255 r.p.m; feed rate was 85 and 120g/min and extrusion barrel temperature was 105 and 125°C (die end). Extruded beans were dried overnight at 60°C and then ground to pass a 0.5mm sieve. Phytohemagglutinin activity in the extruded samples was determined in triplicate by Enzyme-Linked Immunosorbent Assay (ELISA). Data were analyzed using JMP IN 5.1 software (SAS Inst., Cary, NC).

RESULTS

Lectin activity in terms of phytohemagglutinating activity (PHA) in light red kidney beans was effectively reduced by all extrusion processing conditions used in this study (see table below). There was a significant difference between control (raw dry beans) and extruded samples. Extrusion conditions reduced phytohemagglutinin activity in all extruded samples by over 90%. However, there was no clear trend of phytohemagglutinin activity in regard to extrusion variables used. The explanation for this phenomenon is that lectins in extruded samples were inactivated at a temperature lower than the product temperature at the die. Our result confirm with Coffey and others (1992) who reported complete inactivation of lectins at 93°C. Myer et al. (1981) and Myer and Froseth (1983) reported that 90 % reduction of lectins in red kidney bean and soybean mixes extruded at barrel temperatures of 140°C.

Table: Percent reduction of lectins in light red kidney extruded at different conditions

Run	Barrel Temperature (°C): Zone 1 (nearest die)/Zone 2	Product temperature at die (°C)	Flour Feed Rate (g/min)	Screw Speed (rpm)	Moisture content (%), wet basis	Percent reduction of Lectins (PHA activity)
Raw beans	N/A		N/A	N/A	N/A	0
1	120/105	123.0	80	118	25	92.7±0.017
2	120/105	127.3	80	184	25	90.3±0.003
3	120/105	130.0	80	253	25	90.6±0.005
4	120/105	124.0	120	118	25	90.0±0.006
5	120/105	129.3	120	184	25	92.4±0.003
6	120/105	128.3	120	253	25	90.8±0.004
7	120/105	119.3	80	118	36	92.1±0.004
8	120/105	118.0	80	184	36	90.8±0.004
9	120/105	117.3	80	253	36	91.5±0.004
10	120/105	126.0	120	118	36	92.6±0.003
11	120/105	129.4	120	184	36	92.4±0.001
12	120/105	124.0	120	253	36	90.8±0.004
13	130/115	126.0	80	118	25	90.7±0.002
14	130/115	123.2	80	184	25	92.1±0.003
15	130/115	135.1	80	253	25	91.6±0.004
16	130/115	137.2	120	118	25	90.3±0.003
17	130/115	135.5	120	184	25	91.1±0.003
18	130/115	127.2	120	253	25	90.5±0.014
19	130/115	123.3	80	118	36	90.6±0.003
20	130/115	124.8	80	184	36	93.4±0.006
21	130/115	126.6	80	253	36	94.6±0.009
22	130/115	127.8	120	118	36	93.3±0.013
23	130/115	127.0	120	184	36	93.2±0.006
24	130/115	126.1	120	253	36	91.5±0.005

Percent reduction is a mean of triplicate values. Standard deviation is given in parenthesis.

DISCUSSION AND CONCLUSION

1. Extrusion processing reduced lectins by over 90 %.
2. Low-cost extruder can be used to produce cheap and safe instant bean flours.

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FUNCTIONAL CHARACTERISTICS OF EXTRUDED PINTO AND NAVY BEAN FLOUR (*PHASEOLUS VULGARIS* L.)

G. Nyomba¹, E. Birmingham¹, J.B. Harte¹, S.Venkatesh¹, M. Siddiq¹, K.D. Dolan^{1,2}

¹Department of Food Science and Human Nutrition, ²Department of Biosystems and Agricultural Engineering, Michigan State University, East Lansing, MI 48824

INTRODUCTION

Dry beans are an important food crop consumed in many countries around the world as a major source of protein. Beans are rich in dietary fiber, vitamins, and essential minerals such as iron, zinc, and calcium. Extruded bean flour has potential to be used for making bakery products, such as cookies and muffins for celiac patients. Celiac disease is a digestive disease that damages the small intestine and interferes with absorption of other nutrients from food. People who have celiac disease cannot tolerate gluten, a protein found in wheat, rye, and barley. The objective of this study was to evaluate a new method of producing navy and pinto bean flours for applications in gluten free bakery products.

MATERIALS AND METHODS

The pinto and navy beans (*Phaseolus vulgaris* L.) used in the study were obtained from Bayside Best Beans, LLC (Sebewiang, MI). Any damaged beans were removed. Raw beans were washed and soaked for 4 hours to reduce flatulence-causing oligosaccharides by leaching into the water. After soaking, the beans were dried in an oven at approximately 65 °C for 12 hours. Pinto and navy beans were ground using a hammer mill model (Model D comminuting Machine, W.J. Fitzpatrick Company, Chicago, USA). Extrusion runs of raw pinto and navy beans were carried out using a laboratory co-rotating twin-screw extruder model JS30A (China by Qitong Chemical Industry Equipment Co, Ltd). The extruder screws are 30 mm in diameter and the barrel has an L/D of 14. Pinto and navy beans were extruded at 85, 100, and 120°C (die end); moisture content was 36 % wet basis; and feed rate was 120 g/min. The bean extrudates were dried overnight (70°C) and then ground to pass a 250 micron screen. The extruded bean flours were compared with the commercial control navy bean and pinto bean flours that were steam cooked at 82°C for overall quality of baked products. Line-spread tests of all the flour/water slurries (sol) were done at different temperatures to find the thickening properties of the flour (Figure 1). Each unit on the line spread scale is equal to 4 mm. Two types of baked products were chosen to compare the functionality of flours: Gluten-Free Cinnamon Sugar Cookies and Gluten-Free Spiced Muffins. These baked products were prepared using recipes for gluten free products and baked at 350 °F (Heartland Finest, Hillman, MI). The total baking time was 14 min and 25 min for the cookies and muffins, respectively. The diameters of the cookies were measured to evaluate the cookie spread. Seed displacement was used to compare baked muffin volumes. Observations of the cookie and muffin appearance, color, and flavor were also used as a basis of comparison.

RESULTS AND DISCUSSION

The experimental pinto and navy flours extruded at 85°C yielded the best results in terms of flour functionality and were most similar to that of the control. The samples had a uniform color distribution in the heat formed sol. At slurry temperatures above 75°C the control navy and pinto bean flours and both of the experimental flours had nearly identical spreads and did not exhibit much thickening. The flours' spread ranged between 8.5 and 10.75 (34mm and 43 mm) on the line spread scale. The control pinto bean flour exhibited more thickening as the temperature approached 100°C and ranged from 5.75 to 10.13 (23mm to 40.5mm) on the same

line spread scale. The control pinto bean flour exhibited the most thickening as the temperature of the sol approached 100°C (Figure 1). The experimental flours that were extruded at 120 and 100°C were inferior to the controls in terms of thickening ability. The 85°C flours also yielded nearly identical products when used in baking of cookies and muffins. Average diameters and heights of the cookies made using the pinto bean flours were identical (2.44 and 0.84 in., respectively). The average diameter and height of the cookies made using the extruded navy bean flour and control navy bean flour (2.24 and 0.9in. vs. 2.5 and 0.73in. [diameter, height], respectively) were very similar. The experimental flours extruded at 120 and 100°C exhibited poor baking performance in cookies and muffins. Muffins made with the experimental flours had an average seed displacement of 115 ml whereas the muffins made with the control flours had an average displacement of 123ml.

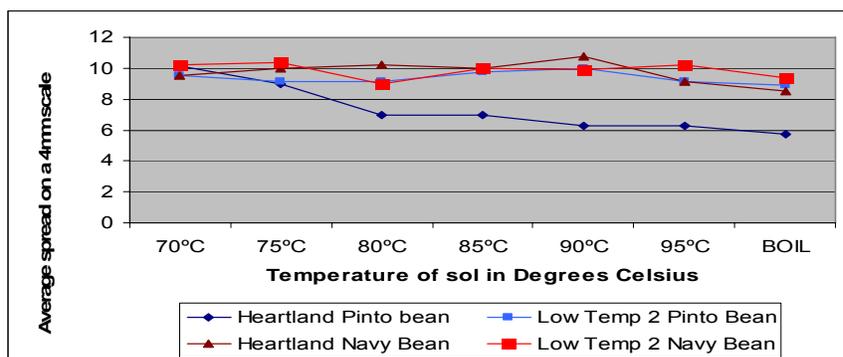


Fig 1: Average Spread of Control and Extruded Navy and Pinto Bean Flour Sols (slurry) during Line-spread test.

CONCLUSION

The experimental flours extruded at 85°C are comparable to the control flours in terms of functional characteristics. The baked cookies and muffins made using the experimental flours were nearly identical in appearance and flavor profile. Batter viscosity was fairly smooth and consistent and comparable to the respective control flours. All had similar baked volume.

ACKNOWLEDGEMENT

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ABSORBANCE OF THE SOAK WATER TO PREDICT CANNING QUALITY

Shawna Bushey¹ and George Hosfield²

¹Pybas Vegetable Seed Co, Inc. Santa Maria, CA 93455, ²USDA-ARS, Retired

Breeding dry bean (*Phaseolus vulgaris* L.) for improved canning quality characteristics is a long process. It can take up to six generations from the time the first cross is made until there is enough seed of a breeding line to evaluate canning quality characteristics. In black bean, excessive leaching (color loss during thermal processing) can cause beans to appear gray and washed-out. Typically, this would result in the breeding line being discarded from the program due to unacceptable canning quality, specifically poor visual appeal. Determining the propensity of a breeding line to leach in earlier (F₃ or F₄) generations could be a valuable tool much like marker assisted selection allowing the breeder to discard those lines that have a propensity toward leaching earlier in the breeding process.

Fifty randomly selected F_{2:8} breeding lines derived from crosses between ‘Shiny Crow’ and ‘Black Magic’ or between ‘Black Magic’ and ‘Raven’ were used in the soak water color test (1) to quantify the amount of particulate matter that had leached into the brine as well as to determine the correlations between the absorbance of the brine, the soak water color score, the amount of water imbibed by the seed, and the shininess or opaqueness of the seed coat. Ten beans from each randomly selected breeding line were blanched and soaked according to the protocol for the soak water color test (1). The color of the soak water was scored on the 1 to 5 scale, the percent water imbibed was determined, and the shininess or opaqueness of the beans was documented. The absorbance was measured using a spectrophotometer to assign a quantitative value to the visual color score of the soak water. The spectrophotometer used for measuring the soak water was calibrated using the same brine solution used for the soak water color test at a wavelength of 600nm. The soak water from each vial of the soak water color test was then transferred to a cuvet to have the absorbance measured. Anthocyanins rapidly oxidize in the presence of light so to prevent any degradation from occurring that could influence the absorbance readings; the absorbance was measured immediately following the completion of the experiment.

There were significant positive correlations ($P < 0.001$) between absorbance and shininess or opaqueness of the seed coat, percent water uptake and color of the soak water (Figure 1). The Pearson’s correlation coefficients were 0.54, 0.60, and 0.58 respectively. The lower the absorption, the less water the sample imbibed, the lower the color score of the soak water and the less likely it was to have an opaque seed coat. The significant correlation between the absorbance and the color score of the soak water indicated that the soak water color could be used as a predictive tool for the relative amount of leaching that will occur.

Based on scanning electron microscopy measurements (data not shown) taken on 20 black bean samples from the near isogenic populations used for this study, there was some variation in the size of the vacuoles in the seed coat. Variation in vacuole size has also been noted in other cultivars of black bean (2). Anthocyanins are localized in the vacuoles of the seed coat palisade cells; therefore, larger vacuoles may initially contain more anthocyanins than their smaller counterparts. The amount of leaching observed for ‘Raven’ and ‘Black Magic’ was quantified (3) and showed that ‘Raven’ exhibited a higher percentage of leaching in relation to the total anthocyanin content of the seed coat. Therefore, the size of the vacuoles, the initial

anthocyanin content of the vacuoles and the rate at which a cultivar will leach will all contribute to the appearance of the bean seed coat following thermal processing. This test predicts the propensity of a breeding line to leach but may not predict if the breeding lines will have an acceptable canning quality appearance following thermal processing.

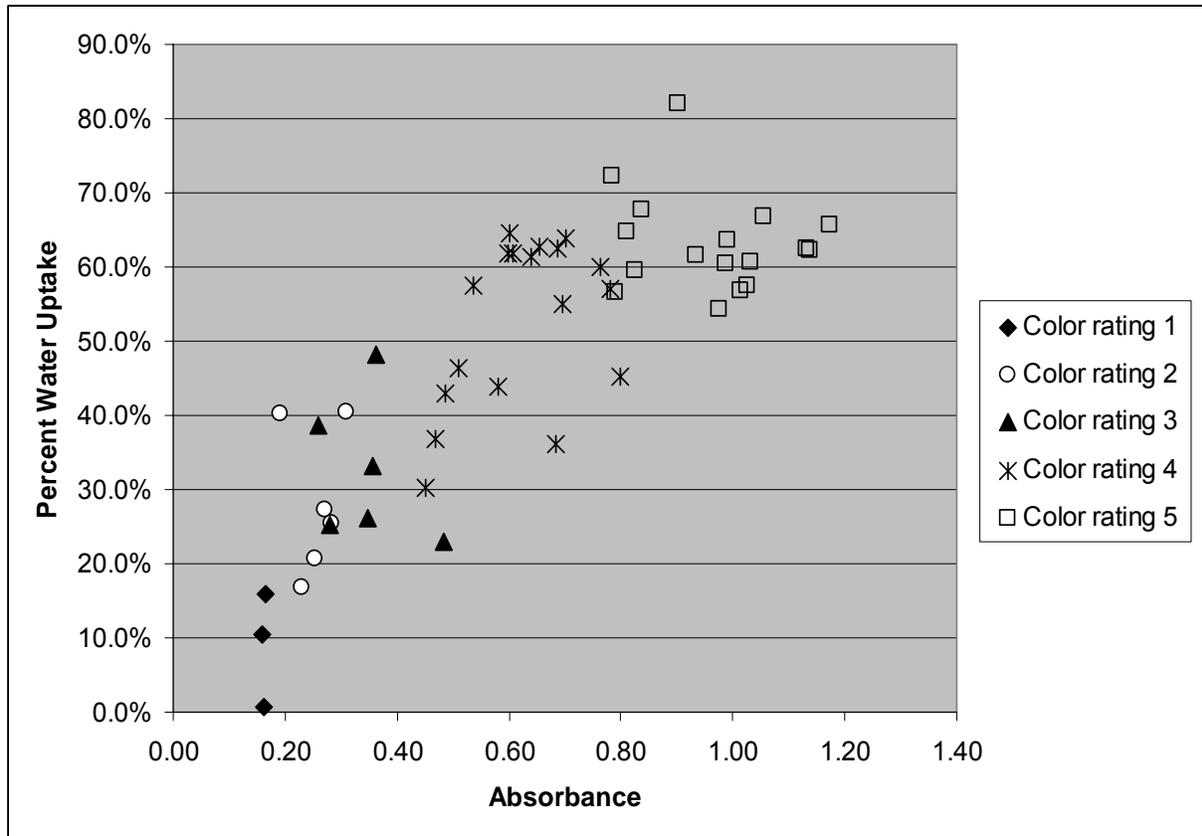


Figure 1. Graph showing correlations between percent water uptake, color of the soak water and absorbance.

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A TEST TO PREDICT COLOR LOSS IN BLACK BEAN DURING THERMAL PROCESSING

Shawna M Bushey¹ and George L Hosfield²

¹Pybas Vegetable Seed Co, Inc. Santa Maria, CA 93455, ²USDA-ARS, Retired

A major problem associated with black bean (*Phaseolus vulgaris* L.) is the excessive loss of color (leaching) during soaking and thermal processing (Anonymous). Leaching can cause black beans to appear gray or reddish-colored and have a washed-out seed appearance making them visually unappealing to the consumer (Bushey et al., 2000). Only a few of the advanced breeding lines that make it to the canning stage of evaluations are ever released as cultivars due in part to unacceptable canning quality. It can take six generations from the time the first cross is made until there is enough seed of a potential new cultivar to evaluate canning quality characteristics. Early generational testing of the propensity of a particular breeding line to leach can assist in selecting and eliminating those lines that exhibit profuse color leaching earlier in the breeding process.

A population consisting of 98 near isogenic lines variable for seed coat luster and the two parents, 'Shiny Crow' and 'Black Magic' was developed to study the relationship between leaching, water imbibition, and shininess or opaqueness of the seed coat. 'Black Magic' is an opaque cultivar that leaches moderately and 'Shiny Crow' is a shiny cultivar that exhibits little to no leaching. 'Raven', an opaque cultivar that leaches profusely was used as a check variety. The seed of this population was grown near Christchurch, New Zealand in the winter of 2002 and at the Saginaw Valley Bean and Sugar Beet Research Station near Saginaw, Michigan in the summer of 2002.

This experimental protocol, the soak water color test, is a technique for predicting the amount of leaching that will occur during thermal processing. The test involves taking 10 fresh beans with intact seed coats, weighing them and placing them into a vial with 20 ml of distilled water (23°C) adjusted to 100 mg· ml Ca⁺² (brine). The vials are sealed (caps put on lightly) and then placed in an 83° C water bath (blanched) for 10 minutes. The calcium concentration of the brine and temperature of the water bath were chosen to simulate conditions used in commercial canning operations. Once the vials have been blanched for 10 minutes, the vials are removed and placed in the dark for 110-minutes at room temperature (23° C). Upon completion of the 110-minute soak the vial is gently agitated to incorporate any anthocyanins that may have leached out and settled to the bottom of the vial into the soak water. The ten beans are then removed from the vial, blotted dry, and weighed taking care to retain as much of the soak water as possible. The soak water is then scored from 1 to 5 with one being clear and 5 being very dark. The amount of water absorbed by the ten seeds through imbibition is determined and expressed as the percent increase in weight. 'Raven' with a soak water color of five and 'Shiny Crow' with a soak water color of one can be used as checks to develop a scale for the soak water color test.

There is a strong positive correlation among the percent water uptake, color of the soak water, and opaqueness of the seed coat (Table 1). The color of the soak water was indicative of the degree of leaching that occurred during the 10 minute blanch. Although less precise, shininess of the seed coat is also a good predictor of the amount of leaching that will occur. In general, shiny beans imbibe less water and exhibit a lesser degree of leaching than their opaque counterparts. Because this test requires such a small seed sample, breeders can use this test on

early generational (F₃ or F₄) material to predict which lines will have a propensity toward leaching. This test is predictive of the amount of leaching that can occur however, the test has not been correlated with overall canning quality yet. Additional work has shown that there are location and year effects that influence the amount of leaching in addition to variation in the amount of anthocyanins initially contained within the seed coat. Black beans that contain a greater amount of anthocyanins in the seed coat prior to soaking and blanching can potentially leach more anthocyanins into the soak water before the color differences become apparent to the naked eye.

This test was used on a second near isogenic population of 108 breeding lines developed from a cross between ‘Raven’ and ‘Black Magic’ with similar results (data not shown). However, a recent study comparing ‘Raven’ grown in two different locations in the same year showed that there was a great degree of variability in the amount of leaching observed from one location to the next. Additional work needs to be done to determine if this test can be adapted to account for the variability in different populations and environments.

	Shininess	Color of soak water	Percent water uptake
Shininess	1.00	-0.82	-0.81
Color of soak water	-0.82	1.00	0.93
Percent water uptake	-0.81	0.93	1.00

¹ p<0.001.

The authors would like to thank Evan Wright and J.D. Kelly at Michigan State University for their work on the additional testing of this protocol.

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SEED IRON AND ZINC LEVELS IN A COLLECTION OF COLOMBIAN RELEASED VARIETIES GROWN AT TWO LEVELS OF PHOSPHORUS FERTILIZATION

M.W. Blair, C. Astudillo, A. Hoyos, Y. Viera, O. Mosquera

Centro Internacional de Agricultura Tropical, A.A. 6713, Cali, Colombia

Introduction:

Bean breeding in Colombia has produced a wide range of large-seeded Andean varieties for many different agroecological zones. The country is home to a wide range of traditional farmer varieties and landraces. To obtain a baseline of information on nutritional quality in Colombian germplasm we assembled a nursery for the major bush bean varieties released in the country. The specific objectives of this research were to test a representative number of improved and traditional Colombian varieties for seed iron and zinc levels. We were also interested in the interaction of seed mineral accumulation with fertilization level especially as it relates to the major nutrient limitation for beans in Colombia, which has low phosphorus soils. The genotypes represented all the bush bean varieties released by ICA and CORPOICA over the past thirty years along with standard check varieties and a few standard landraces. The majority of the varieties evaluated were red mottled 'Calima' or 'Nima' classes followed by large red 'Radicales' or 'Duva' types.

Materials and Methods:

A total of 40 varieties were evaluated including 24 large-seeded red mottled (Calima) or red (Radical) varieties released by ICA or CORPOICA (namely DIACOL Calima, Catio, Nima, Nutibara and ICA Bachue, Cafetero, Caucaya, Cerinza, Citara, Cuna, Duva, Frijolica P-1.1, Guali, Guanenta, L-24, L-59, L-66, Palmar, Quimbaya, Radical Cerinza, Radical Froylan, Tone and Tundama), 9 landraces from Berruecos, Darien, Sevilla and Tenerife (Valle), Pasto (Nariño) and Ocaña and Zaragosa (Norte Santander) and seven CIAT lines as controls (A36, AFR188, AFR612, AFR619, AFR735, AND279 and CAL96). Landraces included popular varieties such as Andino, Blanquillo, Cargabello, Chocho, Mina, Palisero, Rosado (1 each) and Sangretoro (2 entries). The genotypes were grown in a split plot replicated yield trial in Darien in semester 2004A under two fertilization treatments: low phosphorus, 50kg/ha (7.5 kg P205) and high phosphorus, 350 kg/ha (45kg P205). Native soil P is 2 to 10 ppm. Experimental design consisted in three repetitions in randomized complete blocks with plots separated by DOR390 as check rows. Darien was a favorable site for all the genotypes given its location at 1500 masl and average temperature of 19°C and annual rainfall of 1.200 mm (500 during the season). Data was collected on days to flowering, days to maturity and yield (in g/pl and kg/ha). Seed mineral content was evaluated by grinding 3 g of grain into a fine powder using a modified Retsch mill with teflon chamber and zirconium grinding balls, transferring the resulting powder to 25 ml plastic tubes and analyzing it for both iron and zinc concentration measured in parts per million (ppm) with a wet digestion method and Atomic Absorption spectrophotometry. An analysis of variance was carried out with the software program Statistix v. 8.0 which was also used to

calculate Pearson's correlation coefficients.

Results and Discussion:

The analysis of variance for the seed iron and zinc showed significant differences for both genotype and phosphorus level. The genotype x phosphorus fertilization interaction was significant for iron ($P=0.02$), while it was not significant for zinc (Table 1). Among all varieties, iron levels were higher on average under the high phosphorus treatment (56.7 ppm) than under the low phosphorus treatment (52.5 ppm). In contrast zinc levels were higher under low phosphorus (26.3 ppm) than under high phosphorus (23.1 ppm). The range in iron concentration was from 35.1 to 77.0 ppm in the low phosphorus treatment and from 32 to 79 ppm in the high phosphorus treatment. The range in zinc concentration was from 20.1 to 30.7 ppm in low phosphorus and 16.9 to 30.4 ppm in high phosphorus (Figure 1). Coefficients of variation for seed iron and zinc ranged from 9.9 to 18.0 % and were lower for zinc estimates than for iron estimates. Varieties with the highest seed iron levels were ICA Tundama (79.44 ppm), AFR188 (76 ppm), AFR735 (75.14 ppm) and ARS-59 (75.12 ppm) in high phosphorus and Radical de Restrepo (69.98 ppm), AFR612 (77.04 ppm) and AFR188 (67.36 ppm) in low phosphorus. AFR188 and AFR612 were also high in seed zinc concentration, along with Cargabello, especially under low phosphorus. The correlations between iron and zinc concentrations in low and high phosphorus treatments were high ($r=0.79$ and $r=0.57$, respectively ($P=0.000$)). The correlations of seed mineral content between phosphorus treatments for both iron and zinc was also high ($r=0.55$ and $r=0.56$, respectively ($P=0.000$)). On the other hand, correlations were generally not significant between mineral content and the agronomic characteristics evaluated for the experiment (including days to flowering, days to maturity and yield, both in g/plant and kg/ha). In terms of yield, the more recently-developed breeding lines tended to do better than the released varieties. Two notable breeding lines in this respect were AFR188 and AND279. Overall, the average yield of the breeding lines (2129.5 kg/ha) exceeded that of the landraces (1776.0 kg/ha) and the improved varieties (1446.3 kg/ha) under high P treatment. The same pattern was seen for breeding lines (1114.1 kg/ha), landraces (989.5 kg/ha) and released varieties (712.7 kg/ha) under low P treatment.

Table 1. Analysis of variance for seed iron and zinc in 40 Colombian varieties grown under high and low phosphorus levels in Darien.

a) Iron

Source	DF	SS	MS	F	P
P level	1	3982.4	3982.42	90.41	0.0000
Genotype	38	12966.8	341.23	7.75	0.0000
P level x Genotype	38	2711.6	71.36	1.62	0.0211
Error	162	7136.2	44.05		
Total	239				

b) Zinc

P level	1	766.40	766.403	156.14	0.0000
Genotype	38	1352.72	35.598	7.25	0.0000
P level x Genotype	38	179.50	4.724	0.96	0.5385
Error	162	795.17	4.908		
Total	239				

iron CV 11.55 % // zinc CV 8.82 %

GENE EXPRESSION ANALYSIS OF GENES INVOLVED IN THE PHYTIC ACID PATHWAY OF COMMON BEAN (*P. VULGARIS* L.) USING REAL-TIME PCR

M. Fileppi*, I. Galasso**, B. Campion*, R. Bollini**, F. Sparvoli**

* Istituto Sperimentale per l'Orticoltura, Montanaso Lombardo, Lodi, Italy

** Istituto di Biologia e Biotecnologia Agraria, CNR, Milano, Italy

Myo-inositol phosphates are phosphate esters of a cyclic alcohol derived from D-glucose-6P. The best-known component of this family is phytic acid, its features are 12 replaceable protons in its structure and it can be found in many different forms, called phytates. The negatively charged phytic acid chelates positively charged divalent cations, determining a poor absorption of the bound metals in small intestine of humans and monogastric animals.

Common bean, as other legume seeds, contains high levels of phytic acid. In humans it has been demonstrated that the phytate determines a dose dependent inhibition on absorption of Fe, Zn and Ca (Sandberg, 2002). This reduced availability of such microelements results in alimentary deficiencies, especially in developing countries where food is mainly seed-based. In developing countries with high mortality, about one-sixth of the entire disease is attributed mainly to underweight, however, a substantial proportion is also attributed to micronutrient deficiencies (World Health Report, www.who.int/whr/2002/en/). Also in developed countries the increased number of vegetarians among young people might lead to iron deficiencies, for its bioavailability may be crucial in a vegetarian diet.

Therefore, there is a strong interest to develop seeds with a reduced content in phytates. However, the manipulation of phytic acid amount in the seed requires knowledge on the key enzymes involved in its biosynthetic pathway.

The first step in the phytic acid biosynthesis is the conversion of D-glucose-6P to D-myo-inositol-1P (InsP₁) by the isomerase myo-inositol phosphate synthase (MIPS). After this well characterized step, several kinases, many of which yet to be identified, catalyse the sequential addition of phosphate units to the InsP₁, to produce InsP₂, InsP₃, InsP₄, InsP₅ and InsP₆. In *Arabidopsis thaliana* several kinases involved in the phytic acid pathway have been isolated and characterized: Inositol-(1,3,4,5,6)-pentakisphosphate 2-kinase (*AtIpk1*), Inositol-(1,4,5) trisphosphate 3/6-kinase (*AtIpk 2α/β*) and Inositol (1,3,4) trisphosphate 5/6-kinase (*Ipk3*) (Stevenson-Paulik et al., 2005).

To identify the orthologous of the above *Ipk*s in *Phaseolus vulgaris*, the *AtIpk1*, *AtIpk2α/β* and *Ipk3* genes from *Arabidopsis* were used as query against plant genome (EMBL, TIGR) and EST databases. Full-length cDNAs and genomic DNAs with high similarity (66–82%) to the *Arabidopsis AtIpk1* sequence were found in rice (*OsIpk1*), maize (*ZmIpk1*), and wheat (*TaIpk1*). When *AtIpk 2α/β* and *Ipk3* were used as queries only very few highly homologous ESTs of soybean and *P. coccineus*, were identified. Multiple sequence alignment of the above identified *Ipk*s sequences revealed several motifs that define conserved regions present in all *Ipk* members. Several pairs of PCR primers were designed along these conserved regions. All tested primers produced abundant and specific PCR products of the expected size. The PCR-products were gel purified and sequenced. BLAST analysis of the obtained nucleotide sequences evidenced that the three genes isolated and called *PvIpk1*, *PvIpk2* and *PvIpk3* have a high degree of sequence

identity with the corresponding *Ipk* genes present in the EMBL database.

To monitor in developing seeds the expression of the genes coding for these three kinases and MIPS we used a real time quantitative RT-PCR approach. The most commonly used method is the relative quantification whereby gene expression level is normalized to that of an internal reference gene. In this study, we chose the 18S gene coding for ribosomal RNA and we analysed the results with the ΔC_t method (Livak and Schmitteng, 2001).

The analysis of the expression of these genes in common bean, carried out on different stages of seed development 6-12-16-20 DAF (days after fertilization), revealed that the highest expression of MIPS gene is at very early stages of seed development (6 DAF), then its expression rapidly decrease (up to 500 fold lesser). This result on MIPS expression is in agreement with our previous data carried out with northern blot analysis (Fileppi et al., 2004).

As regards *PvIpk1*, *PvIpk2* and *PvIpk3*, transcripts were detected at constant low levels during all stages of seed development here analysed. It has been reported that phytic acid accumulation peaks at 22 DAF and then there is no more increase (Coelho et al., 2005). Since these enzymes act on later stages of phytic acid biosynthesis that take place in more advanced seed developmental stages, we are planning to extend the expression analyses to verify whether the expression remains constant during the development or is subject to changes according to phytic acid accumulation.

The knowledge provided with this study, together with the previous one obtained on *MIPS* gene (Fileppi et al., 2004), will be basic for the identification of low phytic acid mutants in EMS mutagenised population of *P. vulgaris*, that we have recently obtained, using the TILLING reverse genetic approach

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BREEDING FOR HIGH SEED-ZN AS A VALUE ADDED TRAIT IN NAVY BEAN

J.R. Gelin*, K. Grafton, G.A. Rojas-Cifuentes, and A.J. Vander Wal

Department of Plant Sciences, North Dakota State University

* Email: robert.gelin@syngenta.com

Introduction

Navy bean grown in North Dakota is sensitive to low available Zn in the soils, and symptoms can range from discoloration in the leaf tissues to low seed yield. Earlier reports indicate that seed-Zn efficiency in dry bean is controlled by a single major gene (Singh and Westermann, 2002; Cichy et al., 2005), which makes it possible to develop cultivars with high seed-Zn content. Conventional and molecular breeding strategies have offered new opportunities to breeders to develop varieties with novel or value-added traits (Korban, 2005). The objective of this research was to field evaluate seven elite lines of navy bean developed for high seed-Zn content.

Materials and Methods

Seven advanced navy lines developed from a cross between ‘Albion’ (Asgrow Seed Company, 1987) and ‘Voyager’ (Rogers Seed Company, 1995) were field evaluated in 2006 at Hatton, and Johnstown, ND. These lines were transgressive segregants for high seed-Zn content, which was confirmed in greenhouse experiments (Moraghan, J., personal communication, 2004). Navy bean Norstar (Grafton et al., 1993) was used as a check along with the two parents to give 10 entries. A randomized complete block design (RCBD) was used with 3 replicates at each location. Plant height, maturity, seed weight, and seed yield were measured as previously described (Gelin et al., 2004), and statistical analysis was performed with SAS (SAS Institute, 1988).

Results and Discussion

Significant differences ($P < .05$) between the two locations were found for all four traits, but genotype means were significantly different only in Johnstown (Table 1). Seed yield was also lower at Johnstown due to standing water after rainfall for a long period of time. Differences among genotypes were found also for the across location means for plant height, maturity, and seed weight. Although no difference was observed for seed yield, two genotypes (AV60 and AV63) had a mean equal to or greater than the mean of either parent or the mean of Norstar. Our results suggest that combining high seed-Zinc with good agronomic traits in navy bean is achievable through breeding techniques.

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Table 1. Agronomic performance of high seed-Zn navy bean genotypes grown in 2006 at Hatton, and Johnstown, ND.

Genotype (mg kg ⁻¹ Zn)*	Plant Height (cm)			Maturity (days)			Seed Weight (g of 100 seeds)			Yield (kg ha ⁻¹)		
	----- Location -----											
	Hatton	Johnstown	Mean	Hatton	Johnstown	Mean	Hatton	Johnstown	Mean	Hatton	Johnstown	Mean
Albion (21.2)	53.3	44.7	49.0	100.7	92.7	96.7	22.4	17.2	19.8	2278.9	1212.4	1740.0
Voyager (29.5)	57.0	43.7	50.3	99.3	88.3	93.8	20.6	14.0	17.3	1841.1	1302.2	1560.4
Norstar (25.1)	56.3	47.3	51.8	100.7	89.7	95.2	21.5	15.7	18.5	2346.2	1571.6	1964.6
AV60 (30.8)	49.0	48.0	48.5	101.3	70.0	85.7	21.1	15.7	18.4	2739.1	1762.5	2245.2
AV63 (34.5)	53.0	45.0	49.0	101.3	94.3	97.8	21.7	17.5	19.6	2615.7	1302.2	1964.6
AV18 (31.9)	51.7	39.7	45.7	102.7	92.7	97.7	20.3	16.2	18.3	2323.8	1392.0	1852.3
AV72 (31.3)	51.0	46.7	48.8	102.3	84.7	93.5	20.4	13.7	17.0	2301.3	1392.0	1841.1
AV14 (35.8)	56.3	50.0	53.2	100.7	91.7	96.2	21.3	20.9	21.1	1582.9	1717.6	1650.2
AV64 (31.4)	48.7	46.0	47.3	105.3	85.0	95.2	19.5	14.7	17.1	1706.4	1481.8	1594.1
AV42 (30.4)	57.0	55.0	56.0	102.7	86.0	94.3	19.7	12.7	16.2	2099.3	1055.2	1571.6
Mean	53.3	46.6	49.9	101.7	87.5	94.6	20.8	15.8	18.3	2177.8	1414.5	1796.2
LSD (.05)	ns**	9.0	8.6	ns	12.9	7.2	ns	4.3	2.0	ns	628.7	ns

* Source : Forster, S.M. (2002) ; ** not significant at .05 level

ALPHA-GALACTOSIDASE ACTIVITY IN COMMON BEANS

Carmen Jacinto-Hernández¹; Ramón Garza García¹; Irma Bernal-Lugo²

¹Laboratorio Calidad de Frijol y Programa de Frijol INIFAP, CEVAMEX, Correo-e: carmenh8@yahoo.com.mx. ²Laboratorio de Bioquímica, Facultad de Química, UNAM. Ciudad Universitaria, D. F. 104510 México.

INTRODUCTION

Dry beans (*Phaseolus vulgaris* L.) are basic grains for Mexican nutrition, however a limiting factor is the PRESENCE OF raffinose-family oligosaccharides (RFO's), which are implicated in causing flatulence. On the other hand there is evidence that α -oligosaccharides play an important role in the acquisition of seed desiccation tolerance and also as a substrate for embryo growth during germination. Those means the it would be desirable to have varieties with enough amount of RFO's to favore agronomic performance and make it possible to partially reduce the amount of RFO's once grain is ready for consumption to reduce the flatulence potencial. There exist differences in soaking effectiveness for RFO removal from different cultivars (Jacinto *et al* 2006), this may be due to variable levels of α -galactosidase activity which selectively cleaves galactose from raffinose, stachyose and verbascose leaving behind sucrose. The objective of this work was to determine α -galactosidase activity in 13 common bean genotypes previously characterized according to its oligosaccharide content.

MATERIAL AND METHODS

Thirteen genotypes from four market classes representing a significant genetic diversity for market classes were used. Water absorption capacity was measured and expressed as % of weight increased after water bean soaking by 15 h. The oligosaccharides content in dry as well as in water soaked (18 h at 23 °C) grains was analyzed. Cotyledons were ground and then extracted with aqueous ethanol (75%, 30 ml) with an internal standard (melezitose) for 2 h at 50-60 °C. The mixture was centrifuged at 12000 x g and supernatants recovered and passed through nylon filters (0.4 μ m). Samples (20 μ l) of sugars were analyzed with a Waters HPLC (Milford MA), USING a Waters 2414 refractive index detector. The oligosaccharides were eluted from the column (WAT044355) with acetonitrile:water (75:25 v/v). α -galactosidase activity was determined trough method of Lima *et al* 2004). All analyses were made with three replicates. A one-way analysis of variance (ANOVA) and Tukey (α 0.05) test were performed.

RESULTS AND DISCUSSION

Considerable variability existed among common bean genotypes in oligosaccharide content, as well as in sucrose content. Stachyose was the main α -galactoside with values between 9.4 and 36.7 mg g⁻¹ while raffinose was between 1.63 and 7.04 mg g⁻¹.

The amount of RFOs removed through soaking was since 7 % of raffinose+stachyose in Flor de mayo M-38 up to 59-60 % in Bayo Zacatecas and Bayo Victoria (figure). α galactosidase content varied from 430 up to 729 p-nitrofenil nmols $\text{mg}^{-1} \text{min}^{-1}$. There were no significant differences among varieties. In most of the cultivars there was a tendency to increase α galactosidase activity after 18 hours soaking, however in some others, activity of the enzyme decreased after the soaking period. There was no statistic association between galactosidase activity and diminishing of oligosaccharides (raffinose+stachyose) after soaking period. Even though, the six cultivars showing the larger oligosaccharide diminution had the higher enzymatic activity (0.73*). Whereas, the four cultivars with the smaller oligosaccharide reduction during soaking did not showed any correlation with α galactosidase activity but, exhibited an association of water absorbance capacity during soaking and oligosaccharide diminish (0.70*). The above results suggested that in some cultivars oligosaccharide could be lost mainly by lixiviation during grain soaking while in others hydrolysis by α galactosidase could be the mechanism to decrease oligosaccharide content.

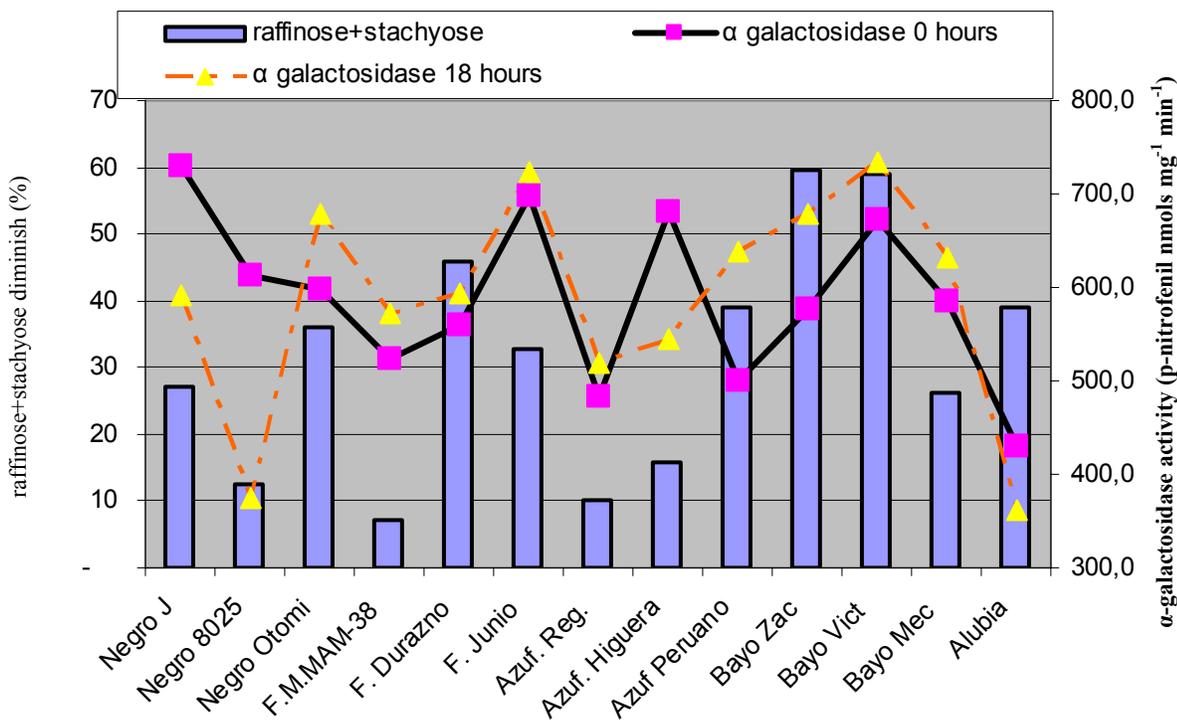


Figure 1. Raffinose+stachyose reduction by water soaking of common bean in relation to its α galactosidase activity before and after grain soaking

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POLYPHENOL OXIDASE IN BEAN CULTIVARS WITH DIFFERENT PRONENESS TO AGE

Carmen Jacinto-Hernández,¹ Ramón Garza-García¹ Albino Campos Escudero¹ Irma Bernal-Lugo²

¹Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias. CEVAMEX. A. Postal 10. Chapingo, edo. México C.P. 56230. (México). jacinto.carmen@inifap.gob.mx ²Facultad de Química, UNAM D.F. 04510, México

INTRODUCTION

Ageing of common beans is a challenge for farmers and bean traders. Beans subjected to long periods of storage undergo gradual loss of quality, such as changes of the seed coat color, soaking characteristics and cooking time (Liu, 1995, Jacinto *et al.*, 2004). In many fruit and vegetables, post-harvest enzymatic browning is catalyzed by the enzyme polyphenol oxidase. The objective of the study was to detect polyphenol oxidase (PPO) activity in various cultivars of common bean.

MATERIAL AND METHODS

Seven common beans (*Phaseolus vulgaris* L.) genotypes with different proneness to age were evaluated.

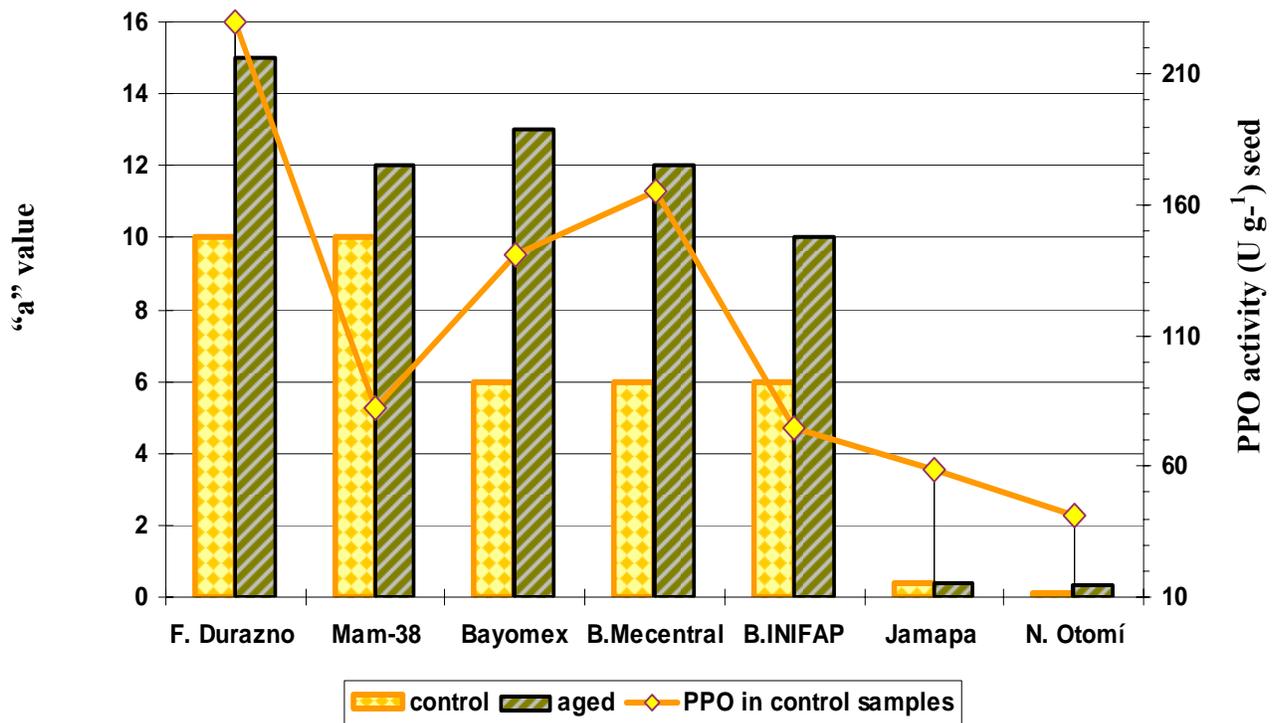
Grain moisture content was adjusted to 12%, separated into two lots, one was stored at 40 °C, 75% Relative Humidity, (in a saturated NaCl solution) during 28 days. The other lot of each variety was kept at 5 °C until analyzed. Coat color was measured with a Hunter Lab MinSanXEPlus L 50. Seed coats were obtained from dry grains using a scalpel. PPO activity was determined at room temperature according to the method described by (Anderson and Morrison). One unit of PPO was defined as the amount of protein which produces a change of 0.001 in absorption at 540 nm. The weight and volume of one hundred seeds wt. and volume were measured, and cooking time was evaluated using a sensorial method (Guzmán *et al.*, 1995). Data were processed through an analysis of variance.

RESULTS AND DISCUSSION

In all the cultivars storage increased cooking time, decreased water absorption, and darkened seed color. One hundred seed weight and volume exhibited no differences. Not all color variables changed, L value (luminosity) did not showed statistically significant difference. However “a” value increased, that means red tones were augmented also “b” which means more yellow tones in seed coat. There were significant differences among varieties in polyphenol oxidase activity. Activity of this enzyme in control samples was associated to darkening of seed coat in aged samples in terms of “a” value ($r=0.69^{**}$). The extent of the change at storage was cultivar dependent and the same was for the polyphenol oxidase activity. Even among the same

commercial class, there were different responses to seed coat color change, for instance, Flor de Mayo M-38 was less prone to darkening than Flor de Durazno, both of them belonging to Flor de mayo class. Among the Bayos, Bayo INIFAP was less affected in terms of seed coat darkening than Bayo Mecentral and Bayomex. Black seed varieties exhibited low PPO activity in comparison to the clear seed coat ones.

Results suggest the possibility of using PPO activity to predict proneness to ageing of cultivars in terms of seed coat darkening which could be used to develop bean varieties with greater stability for the ageing parameters.



Color change ("a" variable) in aged beans and polyphenol oxidase activity in control samples of common bean cultivars.

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POLYPHENOLIC PROFILES OF THREE BEANS VARIETIES

Devanand L. Luthria^{1*}, Long-Ze Lin¹, James M. Harnly¹, and Marcial A. Pastor-Corrales²
¹Food Composition Laboratory, Beltsville Human Nutrition Research Center, BARC (East),
²Vegetable Laboratory, Plant Sciences Institute,
10300 Baltimore Avenue, ARS, USDA, Beltsville, MD 20705-3000

Introduction

Phenolic compounds are important phytonutrients that are ubiquitously distributed throughout the plant kingdom. These compounds are associated with the sensory and nutritional quality of fresh and processed plant foods. Several varieties of dry beans (*Phaseolus vulgaris* L.) varying in seed shape, size, and color are consumed throughout the world primarily as an important source of plant proteins (1). However, in recent years dry bean polyphenols have received considerable attention mainly due to their health promoting properties (1,2). Preliminary results suggest that phenolic compounds found in beans and other plant products provide protection against certain types of cancers, cardiovascular, and other chronic diseases due to their antioxidant activity and ability to chelate metal catalyst (1-4).

In our previous communication, we reported the determination of phenolic acid content in the dry bean cultivars of phenolic acids commonly consumed in the United States (5). In continuation of our research, we have determined the polyphenolic content in three dry bean (Black, Navy and Pinto) varieties.

Materials and Methods

All bean samples (Pinto, Navy, and Black) were provided by Dr. M.A. Pastor-Corrales of the vegetable Laboratory, USDA (Beltsville, Maryland). Standards of phenolic acids (caffeic, ferulic, para-coumaric and sinapic) were purchased from Sigma (St. Louis, MO, USA). (myricetin, quercetin dihydrate, kaempferol, and rutin trihydrate were purchased from Sigma Chemical Co. (Saint Louis, MO). HPLC grade quercetin 3-*O*-glucoside and kaempferol 3-*O*-glucoside were purchased from Extrasynthese (Genay, Cedex, France). Cyanidin chloride, pelargonidin chloride, petunidin chloride, delphinidin chloride, malvidin chloride, pelargonidin 3-*O*-glucoside chloride, pelargonidin 3,5-*O,O*-diglucoside chloride, cyanidin 3-*O*-glucoside chloride and malvidin -3-*O*-glucoside chloride were purchased from Indofine Chemical Co. (Somerville, NJ).

Extraction and analysis of polyphenols

All bean samples were ground in a coffee grinder and sieved through a size 20 standard sieve to obtain a uniform particle size fraction (particle size < 0.825 mm). The ground material was stored at -60°C in an inert nitrogen atmosphere until analyzed. The identification of phenolic compounds was carried out using an LC-DAD-ESI/MS instrument (6). Briefly, it consisted of an Agilent 1100 HPLC (Agilent, Palo Alto, CA) coupled with a diode array (DAD) and mass spectrometer (MSD, SL mode) detectors. A Waters (Waters Corp., Milford, MA, USA) Symmetry column (C18, 5 µm, 250 x 4.6 mm) with a sentry guard column (C18, 5 µm, 3.9 x 20 mm) was used at flow rate of 1.0 ml/min. The column oven temperature was set at 25 °C. The mobile phase consisted of a combination of A (0.1 % formic acid in water) and B (0.1% formic acid in acetonitrile). The gradient was varied linearly from 10-26 % B (v/v) in 40 min, to 65 % B at 70 min, and finally to 100 % B at 71 min and held at 100% B to 75 min. The DAD was set at 270, 310, 350, and 520 nm for real-time read-out and UV/VIS spectra, from 190-650 nm, were continuously collected. Mass spectra were simultaneously acquired using electrospray ionization in the positive and negative ionization (PI and NI) modes at low and high

fragmentation voltages (100 V and 250 V, respectively) for the mass range of 100-2000 *amu*. A drying gas flow of 13 L/min, a drying gas temperature of 350 °C, a nebulizer pressure of 50 psi, and capillary voltages of 4000 V for PI and 3500 V for NI were used. The LC system was directly coupled to the MSD without stream splitting. The hydrolyzed bean extracts were used to detect the flavonoid aglycones and some hydroxycinnamic acids. PI/NI selective ion monitoring (SIM) detection was used to confirm the existence of trace amount of some aglycones in the hydrolyzed extracts.

The polyphenols were extracted by sonicating mixture of powdered dried beans (250 mg) with methanol-water (5.0 ml, 60:40, v/v) using an ultrasonic bath (FS30 Ultrasonic sonicator, 40 KHz, 100 W, Fisher Scientific, Pittsburg) for 60 min at ambient temperature. The polyphenol enriched extract was separated from the solid residue by centrifuging the mixture at 420 g for 15 min. The supernatant solvent was removed and filtered through a 0.45 µm 13 mm PVDF syringe filter (VWR Scientific, Seattle, WA) and 25 µl of the filtered extract was assayed by LC-DAD-ESI/MS analysis.

Results and Discussion

Identification of polyphenols in bean samples was carried out by UV and mass spectral analyses of the hydrolyzed aglycon and the conjugated forms. All dry beans contained similar phenolic acid profiles, but the flavonoid components of the three beans showed distinct differences. Black beans contained primarily the 3-*O*-glucosides of delphinidin, petunidin and malvidin, while pinto beans contained kaempferol and its 3-*O*-glycosides. However, insignificant quantities of flavonoids were detected in navy beans.

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NICHE MARKET SHELL BEAN VARIETY TRIAL

Carol Miles, Liz Nelson, Lydia Garth, and Erin Klingler
Washington State University, Vancouver Research & Extension Unit, Email: milesc@wsu.edu

Introduction

Niche market varieties of dry beans (*Phaseolus vulgaris*) are gaining in popularity among small-scale growers because they are relatively easy to produce, harvest and store. A single dry bean crop can also be harvested at several growth stages to produce three distinctly different crops: 1) green beans, 2) fresh shell beans, and 3) dry beans. Harvesting green beans or shell beans from a dry bean crop is a way for a farmer to diversify crop production while maintaining a single crop. Fresh shell beans are also high-value (\$6 per pound in Portland). While not many bean varieties are suitable for green bean and dry bean production, many varieties are suitable for both fresh shell and dry bean production. Preferred characteristics for fresh shell beans include large bean size, large pod size, and a large number of beans per pod. Currently, white bean color tends to be preferred however a color pattern or pink may be desirable.

Materials and Method

We evaluated 34 dry bean entries for fresh shell bean production at Washington State University Vancouver Research Extension Unit in 2006. Entries included common varieties and breeding lines from Phil Miklas, USDA-ARS Washington, and Jim Kelly, Michigan State University. Dry beans were planted May 15 in a randomized complete block design with 4 replications. Plots were 2 rows wide and 10 feet long, spacing between rows was 2 feet, and spacing in the row was 2 inches. The field was managed using organic practices. Ten plants were harvested from the center of each plot when pods were fully formed and starting to turn yellow.

Results

Entries differed significantly for all measured yield parameters (Table 1). Cannellini, Vermont Cranberry, White Marrow and French Flageolet Flaro were the highest yielding while Supremo, Jacob's Cattle and Black Calypso were the lowest. French Flageolet Flaro and Cannellini had the greatest number of pods per plant while all entries in the Cranberry market class, except for Vermont Cranberry, had the lowest. Supremo had the greatest pod length while Black Calypso had the lowest. Belneb-RR-1 and White Marrow had the largest number of beans per pod while entries in the White Kidney market class tended to have the lowest. PS01-207-2-B3, Tongue of Fire and PS01-203-2-B3 (all Cranberry types) had the largest 100-bean weight while both Flageolet entries had the smallest.

Discussion and Conclusions

Cannellini and Flageolet are currently preferred bean types for fresh shell bean production however they tend to be sensitive to cold soils. The Cranberry market class is also commonly used and in this study had the largest 100-bean weight. However, except for Vermont Cranberry, yields of all Cranberry entries tended to be low. Supremo produced the greatest pod length and the largest beans, both desirable characteristics for fresh shell beans, but yield was very low due to an extremely low number of pods per plant. White Marrow had the largest number of beans per pod and a large bean size but emergence was poor in this study. These results indicate that

many varieties have several of the desired characteristics for fresh shell bean production but no variety in our study had them all.

Table 1. Days after planting (DAP) to 50% emergence, 50% flowering and harvest, plant height mid season (cm), and plant stand at harvest of beans grown for fresh shell bean production at WSU Vancouver REU in 2006.

Entry	DAP to 50% Emergence	DAP to 50% Flowering	DAP to Harvest	Plant Ht (cm)	Plant Stand
Great Northern					
ABL 6	21	57	94	37	40
Beryl	21	57	97	32	40
Belneb-RR-1	26	59	100	32	30
Great Northern	22	56	100	37	46
Matterhorn	22	60	93	37	44
Orion	22	60	95	38	42
PS01-145-4-2-B2	20	56	97	42	48
USGN-5	20	62	98	40	42
White kidney					
Beluga	20	53	98	38	39
Cannellini	No 50%	52	101	31	22
USWK-CBB-16	20	53	99	39	39
Cranberry					
95-8186C	19	52	95	36	45
Capri (old Coral)	19	49	94	32	48
Cardinal	21	49	94	30	37
PS01-203-3-B3	18	47	94	33	48
PS01-207-2-B3	19	47	93	30	44
Taylor Hort	19	56	95	31	50
Tongue of Fire	20	50	95	36	46
UI-686	20	61	96	33	50
USCR-14	19	47	94	30	45
USCR-15	19	50	96	41	44
Vermont Cranberry	26	51	98	31	30
Supremo	19	48	94	33	48
Soldier					
Red Coat Soldier	21	51	98	34	41
Soldier	21	51	95	38	34
Dark Red Kidney					
Montcalm	19	51	94	39	41
Red Hawk	19	50	93	50	47
Flageolet					
French Flageolet- Flagrano	28	52	102	28	33
French Flageolet-Flaro	27	55	102	31	29
Others					
Black Calypso	22	49	93	22	41
Jacob's Cattle	19	47	93	30	37
Marrow Fat	21	56	96	45	43
Roma II	20	50	94	36	39
White Marrow	No 50%	62	99	33	23
Mean	21	53	96	35	41
p-value	0.0000	0.0000	0.0000	0.0169	0.0026

MODIFIED ATMOSPHERE PACKAGING OF GREEN-SHELLED COMMON BEANS

Nalini Pandya¹, James Beaver², Carol Harper³

¹Department of Food Science and Technology, ²Department of Agronomy and Soils,
³Department of Agricultural & Biosystems Engineering, University of Puerto Rico, Mayaguez,
Puerto Rico 00681

Many consumers in Puerto Rico prefer to eat green-shelled common beans (*Phaseolus vulgaris* L.) compared to canned, dried or frozen beans. Like fresh vegetables and fruits, green-shelled beans remain metabolically active postharvest. As a consequence, green-shelled beans have a short postharvest shelf-life of no longer than a few days. Modified atmosphere packaging (MAP) is a packaging system that uses a semi-permeable packaging material, composed of a gas atmosphere other than air, that slows the basic level of metabolism. For the last decade, MAP has been used to extend the shelf-life of respiring products. The shelf-life of green-shelled beans could be extended with MAP in conjunction with the appropriate storage temperature, thereby delaying physiological deterioration. MAP depends on 2 variables, the respiration rate of the product that is packed and the permeability of the packaging material (Church and Parsons, 1995). Therefore, the objective of this research was to experimentally determine the respiration rate of beans and to develop a modified atmosphere packaging system for green-shelled common beans, in order to increase shelf-life with minimal physiological changes.

Green-shelled seeds of the white bean cultivar 'Morales' were used for this research (Beaver and Miklas, 1999). The beans were harvested at the Isabela Substation of the University of Puerto Rico, Agricultural Experimental Station, and transported to a laboratory at 4 °C. The bean pods were shelled mechanically using a 'Little Sheller' manufactured by Taylor Manufacturing Co, Moultrie, GA. Green-shelled beans with no visible damage were used for the study. In the respiration rate study, the experimental unit was 250 g of green-shelled beans placed in a 950 ml glass jar. The jars were placed in controlled temperature environments of 5, 10, 15 and 24 °C. The four treatments were replicated three times. Everyday the jars were sealed for 4 hours. The respiration rate, percentage of CO₂, was measured each day 4 hours after the jars had been sealed. This procedure was performed on the same jars. Daily respiration rates were measured until physiological changes or microbiological deterioration began to appear. Gas samples were extracted from the jars using a 10 ml syringe and analyzed in a gas analyzer (Servomex Food Package Analyzer Series 1400, Norwood, MA). The respiration rate was calculated as ml CO₂/kg*hr on a fresh weight basis, using the following equation: CO₂ = Head space volume (ml) * Change in CO₂ % in the jar. Final rate of CO₂ (Rco₂) is expressed in mg CO₂/kg*hr. In the second study describing a MAP system, the green-shelled beans were harvested, handled and sorted, as described in the respiration study. Color, pH, titratable acidity, texture and water activity analyses were performed on the green-shelled beans before packaging them under different modified atmospheres. The experimental unit was 225 g of green-shelled beans packed in Cryovac PD-961EZ plastic bags. The four gas combinations used to pack the beans were: 1) 4% O₂, 10% CO₂, and 86% N₂ (MAP-I), 2) 2% O₂, 5% CO₂, and 93% N₂ (MAP-II) and 3) 21% O₂, 0.03% CO₂, and 78% N₂ control (AIR). All of the packages were stored at 5 °C for 26.5 days. In addition to the above mentioned quality evaluations, changes in CO₂ within the packages were analyzed from 3 replicates of each gas treatment at 4-day intervals. On each day of analysis, 6 packages were taken out of the 5 °C storage. Gas and quality analyses were performed on 3 replicates, while the other 3 replicates were stored at 20 °C for 2 days, for

temperature abuse simulation. The gas and quality analyses on the 3 replicates of temperature abused packages were performed after 2 days.

The mean respiration rates of green-shelled beans were 15.94, 48.88, 73.95, 123.92 mg CO₂/kg*hr at 5, 10, 15 and 24 °C, respectively (Fig. 1). The green-shelled common beans stored in AIR maintained their overall quality at 5 °C for 10 days ($p \leq 0.05$), while beans in MAP-I were able to maintain a fresh-like quality up to 18 days at 5 °C ($p \leq 0.05$). During their shelf-life, beans in both the AIR and MAP-I treatments maintained their color, texture, titratable acidity, pH and they remained free of off-odors, similar to freshly harvested green-shelled beans. The tenderness of the beans increased in both treatments, without any stickiness or off-odor, which could be beneficial in terms of reduced cooking time. Temperature abuse of any treatment, whether AIR or the gas mixtures of 4% O₂, 10% CO₂ and 86% N₂ (MAP I) and 2% O₂, 5% CO₂, and 93% N₂ (MAP II) was unfavorable, as it shifted the fermented off-odors within the packages to rotten odors and produced a softer texture that was sticky to the touch. Microbiological analysis along with the determination of ethanol and acetaldehyde, should be performed in order to determine if microorganisms contributed to the observed fermented and rotten off-odors. Additional sensory analyses would provide a more precise estimate of the actual shelf-life of the green-shelled common beans.

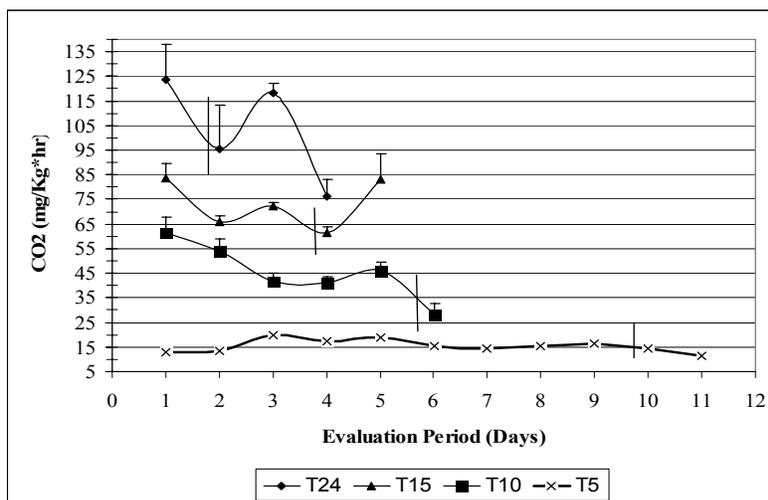


Figure 1. Respiration rate of green-shelled common beans in the form of mg CO₂/kg*hr at four different temperatures (5, 10, 15 and 24 °C). The vertical line signifies the end of shelf-life as determined by undesirable physiological changes or microbiological deterioration.

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NEW BULGARIA VARIETIES OF GARDEN BEAN (*PHASEOLUS VULGARIS* L.) WITH IMPROVED QUALITY

S. Sofkova and G. Pevicharova

Maritsa Vegetable Crops Research Institute, Plovdiv, Bulgaria

e-mail for contacts: svetlas_76@yahoo.com

Introduction: Garden bean production in Bulgaria has been increasing slowly within the last five years. Growers are interested in better varieties that will produce quality packed, ecologically pure and healthy products. Several varieties, recently released by “Maritsa” Vegetable Crop Research Institute in Plovdiv and Institute of Plant Genetic Resources “K. Malkov” in Sadovo, were developed to meet some of these requirements such as high yield, good taste and flavor, firm stringless pod flesh, straight flat pods suitable for canning, and early pod market maturity combined with multiple disease and pests resistance.

Materials and methods: Three garden bean varieties were bred and have been extensively analyzed for yield, disease and pest resistance potential, morphological, chemico-technological and sensory characters over five seasons (2002-2005). The contents of dry matter by Manuelyan (1966), total sugars by Shoorel-Regenbogen, starch, cellulose by Heneberg-Shtoman (Genadiev, 1968), protein nitrogen by Kjeldal and sensory traits were analyzed using mean sample of 0.500 kg green pods at optimal market maturity (seed beds in pods were not well formed and the pods possessed shallow and narrow suture).

Results and discussion: Plants of the new varieties have medium dense foliage, determinate growth habit (Type II) with tendency to open and spread when they are heavy with pods. The new varieties’ yield potential exceeds the standard and all of them demonstrate yield stability during the different environmental and soil condition through experimental years showing good adaptive capacity (Table 1). Pagane, Tangra and Plovdiv yellow reach market maturity from 10 to 13 days earlier than the standard, avoiding unfavorable high temperature and low humidity during the summer period (July-August). Garden bean pod quality is a complex characterization, defined by multiple morphological, chemico-technological and sensory traits which varied according to the producers’ objectives. Morphological pod characters are similar and low variable to all of the three varieties: pods are saturated green (Pagane and Tnahgara) and yellow (Plovdiv yellow), 12.1-15.3 cm long and smooth (Table 1). Pod curve indexes are higher than the standard, defining their straight and good shape. The pod round indexes of Tangra and Pagane are higher than the standard and Plovdiv yellow (with flat pod shape), classifying their pods between flat and round shape. Both characteristics are very important for processing of green bean pods and define pod ability for canning. The new varieties possess pods without seed and fiber development at market maturity. Furthermore under some conditions, the pods develop fiber more rapidly after reaching market maturity. Growers are therefore advised to check their crop closely and harvest prior to maturity in 2-3 times to insure a high quality production. The pod set is medium concentrated, heavy and well-scattered through-out the plant. The plant habit and pod set give a certain advantage for pod quality when mechanical harvesting is utilized.

Table 1. Morphological and yield characterization of garden bean varieties (*Ph.vulgaris* L.)

V a r i e t y	Pod length/cm/	Pod width/cm/	Pod curve index/i/	Pod round index(ii)	Plant high /cm/	Days to maturity	Yield potential kg/ bushel
BG standard	9.2	1.5	0.88	0.65	55	60	1.0
Plodv.yellow	15.3	1.4	0.84	0.55	57	47	1.3
Pagane	12.3	1.0	0.95	0.82	52	50	1.2
Tangra	12.1	1.2	0.94	0.87	49	49	1.4

Total sugars, starch, cellulose and protein nitrogen are a part of the chemical composition, which forms the biological value of garden bean. Sensory traits complete the quality of the green pod product (Martinez et al, 1995; Pevicharova and Poryazov, 2002). The contents of these characters in sterilized pods of the new Bulgarian varieties is established to be comparatively high, which determinate their high biological value (Table 2). They possess different level of multiple resistance to the most economically important for Bulgaria diseases and pests, such as: BCMV, CMV, CIYVV, Halo blight, Rust and Sclerotinia, (Sofkova, S, 2005) and bean weevil.

Table 2. Chemical components and multiple disease and pest resistance of garden bean varieties

Variety	Dry matter (per 100 g)	Total sugars (per 100 g)	Starch (per 100 g)	Cellulose (per 100 g)	Protein Nitrogen (per 100 g)	Multiple Disease and Pest Resistance
BG standard	12.4 n.s.	2.8 n.s.	1.5 n.s.	1.3 n.s.	2.2 bc	-
Plovdivski yellow	11.9 n.s.	2.4 n.s.	1.6 n.s.	1.4 n.s.	3.1 d	Sclerotinia; Rust
Pagane	11.6 n.s.	2.7 n.s.	1.4 n.s.	1.4 n.s.	1.7 a	BCMV; CMV; Halo Blight; Sclerotinia; Rust; been weevil
Tangra	11.2 n.s.	2.4 n.s.	1.4 n.s.	1.3 n.s.	1.9 bc	BCMV; CMV; Sclerotinia; been weevil

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

The new varieties obtain comparatively high sensory evaluation along with the standard (Table 3). The pods are stringless and meaty. The processed pods have a specific strong "beany" flavor which is highly desirable. The epidermal tissue of the pods does not slough off during the canning and cooking.

Table 3. Sensory characterization of garden bean varieties (*Phaseolus vulgaris* L.)

Cultivars	Appearance	Colour	Aroma	Skin integrity	Pod suture uniformity	Pod flatness	Crispness	Succulence	Tenderness	Stringless	Parchment layer free	Taste	Total sensory evaluation
BG standard	4.1 n.s.	4.4 n.s.	4.6 n.s.	4.9 n.s.	4.0 b	4.3 n.s.	3.8 b	4.3 n.s.	4.1 n.s.	3.6 b	3.9 b	3.9 b	3.9 b
Plovdiv yellow	4.6 n.s.	4.8 n.s.	4.5 n.s.	4.8 n.s.	4.5 a	4.6 n.s.	4.6 a	4.7 n.s.	4.6 n.s.	4.6 a	4.7 a	4.7 a	4.7 a
Tangra	4.1 n.s.	4.2 n.s.	4.5 n.s.	4.5 n.s.	4.3 ab	4.6 n.s.	4.3 ab	4.4 n.s.	4.4 n.s.	4.4 a	4.4 a	4.4 ab	4.3 ab
Pagane	3.9 n.s.	4.4 n.s.	4.5 n.s.	4.3 n.s.	4.5 a	4.5 n.s.	4.4 ab	4.4 n.s.	4.4 n.s.	4.4 a	4.6 a	4.3 ab	4.4 a

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

The above reported results show that the recombinogenesis during the hybridization of the new varieties had not only accumulated multiple diseases and pest resistance, but also had improved sensory characteristics of the pods and keep their high biological value. This fact proves the possibility for obtaining cultivars, combining disease and pest resistance with high sensory properties. Implementation in practice of such garden bean varieties gives producers the opportunity to obtain high quality and healthy saving products, using environmental-friendly and cost-saving farming avoiding utilization of pesticides.

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EVALUATION OF PLASTID PIGMENTS CONTENT IN GARDEN BEAN (PHASEOLUS VULGARIS L.) PODS

S. Sofkova and V. Petkova

Maritsa Vegetable Crops Research Institute, Plovdiv, Bulgaria

e-mail for contacts: svetlas_76@yahoo.com

Introduction

Chlorophyll -protein complexes become the building blocks of the photosynthetic apparatus. Chlorophyll is the dominant pigment in a mature **Gandul-Rojas B.**,. Although much of the biochemical pathways in the chloroplast do not involve chlorophyll directly, the pigment plays a key role in development of the organelle and through photosynthesis captures the energy in light to drive biosynthetic reactions. Determination of chlorophyll content is often accomplished to assess the impact of most environmental stresses, as the pigment content is linked to the visual symptoms and photosynthetic plant productivity (Gandul-Rojas et al, 2004). Pigments system formation in the chloroplasts is defined by their genome, which determined the necessity of evaluating plastid pigments contents in various garden bean accessions.

Materials and methods.

There were used 30 accessions from Maritsa Vegetable Crops Research Institute bean germplasm collection. Conventional equipment was used for planting bean accessions on the experimental plots of 3 m² of four replications at the Institute. Chlorophyll and carotenoid levels were estimate at stage of the commercial ripeness of bean pods by method of Wettstein (1967). Plastid pigments were extract in 96% ethyl alcohol and measured by spectrophotometer VSU-2P. Dry matter was defined by putting plant culture samples in dryer under 105°C for 30 min and drying under 75°C afterwards. All the tests were taken in four types of replication.

Results and discussion

In a plant cell chlorophyll is represent in two forms (chlorophyll *a* and *b*), which are responsible for the carbohydrate synthesis during photosynthesis. The most wide –spread pigment is chlorophyll *a*, which contain almost ½ of the hole pigment content. Chlorophyll *a* values at market maturity of the pods varies between 0.110 mg.g⁻¹FW (in acc. № 28) and 0,186 mg.g⁻¹FW (in acc. № 1). Accession № 3 is the exemption here (Table1). There were leveled three groups among the studied accessions according to their chlorophyll *a* content:

- Accessions with low chlorophyll *a* content (under 0.140 mg.g⁻¹FW)- № 3, 4, 22, 23, 24, 27, 28, 29 and 30;
- accession with high chlorophyll *a* content (over 0.160 mg.g⁻¹FW)- № 5, 9, 10, 12, 13, 25;
- The rest of the accessions are between.

It is determined that the development of the chloroplasts is relevant either with the chlorophyll or carotenoid synthesis (Goodwin, 1958). According to Vlasenok et al. (1972) the center for chlorophyll biosynthesis is identical with the carotenoid one. Carotenoid in photosynthetic eukaryotic plays protective role against abiotic stress (Griffiths et al., 1965). Our results showed that the carotenoid values vary restrictedly - from 0,024 to 0,068 mg.g⁻¹FW. Accessions № 8, 9, 14, 15, 16, 22 and 23 possessed the highest carotenoid content. Content of the chlorophyll *b* is relevant to that of chlorophyll *a* and carotenoid. Its values are between 0,035 mg.g⁻¹FW in accessions № 28; 30 and 0,059 mg.g⁻¹FW in accession № 16. Accession № 3 is the one exemption here as well (Table 1). According to total chlorophyll (*a+b*) and carotenoid content the evaluated accessions were distinguished as follow:

- Accessions with low content (under 0.240 mg.g⁻¹FW)- № 3, 18, 22, 24, 26, 28, 29, 30;
- Accessions with high content (over 0.270 mg.g⁻¹FW)- № 1, 5, 8, 9, 10, 12, 13, 25;
- The rest of the accessions are between.

Conclusion: There was determined gene-specific distinctiveness in plastid pigments content. One of the tested accessions (№ 3) was found to be rather distinguished from the rest according either to its much more lower plastid pigments content of the pods, and to the interaction between its chlorophyll and carotenoid values.

Table 1. Plastid pigments content in garden bean pods at market maturity

Accession №	mg.g ⁻¹ Fresh Weight /FW/				
	Chlorophyll <i>a</i>	Chlorophyll <i>b</i>	Chlorophyll (<i>a+b</i>)	Carotenoid	Total Chlorophyll (<i>a+b</i>) + carotenoid
1	0,157 d	0,052 c-f	0,209 e	0,062 bcd	0,271 a-f
2	0,146def	0,050 c-g	0,196 fgh	0,061bcde	0,292 a
3	0,020 l	0,010 l	0,030 q	0,024 k	0,054 j
4	0,136 hi	0,042 ij	0,178 m	0,062bcd	0,240 efg
5	0,163 c	0,054 a-d	0,217 cd	0,063abcd	0,280 a-e
6	0,156 d	0,052 c-f	0,208 e	0,061bcde	0,269 a-f
7	0,158 d	0,049 d-g	0,207 e	0,061bcde	0,268 a-f
8	0,155 d	0,052 c-f	0,207 e	0,064 abc	0,271 a-f
9	0,166 bc	0,055 abc	0,221 bc	0,064 abc	0,285 a-d
10	0,170 ab	0,058 ab	0,228 a	0,060bcde	0,288 abc
11	0,147 de	0,054 a-d	0,201 f	0,058defg	0,259 a-d
12	0,173 a	0,049 d-g	0,222 bc	0,055fghi	0,277 a-e
13	0,174 a	0,052 c-f	0,226 ab	0,064 abc	0,290 ab
14	0,145def	0,049 d-g	0,194 hi	0,065 ab	0,259 a-d
15	0,142 ef	0,053 b-e	0,195ghi	0,068 a	0,263 a-e
16	0,141 fg	0,059 a	0,200gh	0,063abcd	0,263 a-e
17	0,149 d	0,049 d-g	0,198 fgh	0,059c-g	0,257 a-d
18	0,143 ef	0,042 ij	0,185 jk	0,052hij	0,237 efg
19	0,149 d	0,046 ghi	0,195ghi	0,051hij	0,246 c-g
20	0,143 ef	0,047 f-i	0,190ij	0,054ghij	0,244 d-g
21	0,146def	0,053 b-e	0,199fgh	0,061bcde	0,260 a-g
22	0,131 i	0,045 g-j	0,176 nm	0,063abcd	0,239 efg
23	0,137 gh	0,047 f-i	0,184kl	0,064abc	0,248 b-g
24	0,132 hi	0,040 j	0,172 n	0,050ij	0,222 gh
25	0,167 bc	0,048 e-h	0,215 d	0,061bcde	0,276 a-e
26	0,116 j	0,048 e-h	0,164 o	0,055fghi	0,219 gh
27	0,131 i	0,043 hij	0,174 mn	0,056efgh	0,230 fgh
28	0,110 k	0,035 k	0,145 p	0,049 j	0,194 h
29	0,132 hi	0,047 f-i	0,179lm	0,060b-f	0,239 efg
30	0,120	0,037	0,157	0,047	0,204

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

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ASSOCIATION BETWEEN BIOCHEMICAL DESCRIPTORS AND THE POD FIBROUSNESS IN THE CHARACTERIZATION OF SNAPBEAN GERMPLASM FOR LATIN AMERICAN FRESH CONSUMPTION

A. Tofiño¹, C. Ocampo², M. Blair³: ¹Universidad Nacional de Colombia, Bogotá; ²CIAT, Genetic Resources Unit; ³CIAT Bean Breeding Program Apartado aéreo 6713, Cali, Colombia.

Introduction:

The fibrousness and the appearance of the pod; as size, color and shape of the seeds are very important in the consumption of snap beans, which is different between countries and the mode of consumption (fresh or processed). In the Latin American market of fresh snap beans, the Blue Lake – like type (rounded, low in fiber, uniform, with a few white and poorly developed seeds) is desirable ((Myers and Baggett, 1999).

The morphologic descriptors of *Phaseolus* are based mainly on the characteristics of the seeds; in common beans, a high correlation between the morphological characteristics, type of phaseolin and some iso-enzymatic markers was found (Silbernagel *et al.*, 1991; Singh *et al.*, 1991), but little is known about the association in the snap bean germplasm between the relevant morphologic markers for the Latin American fresh consumption and the phaseolin and iso-enzymes. The existence of a correlation between fibrousness and other desirable characteristics of the snap bean with biochemical markers, would provide an important tool for the fast selection with genotypes from the banks of germplasm, permitting to include them in breed programs, reducing the costs for morpho-agronomic evaluations of a big quantity of genotypes. In a previous genetic variability analysis of snap bean germplasm using morphologic descriptors, phaseolin and iso-enzymes, following a group analysis, it was found that iso-enzymes discriminate more accurately the quality contrasting genotypes for the fresh market. The group obtained from each of the three markers was compared with the classification of quality for fresh market, including a desirable morphology, and the fiber degree of the pod (Tofiño *et al.*, 2003; Tofiño *et al.*, 2004). The method of estimation of the fiber used in the analysis is qualitative and generally used in the determination of the quality of the pod in the field, but its level of accuracy is unknown. Therefore, a complementary experiment was considered to determine (using the chemical analyses of the fiber, chlorophyll and protein contents in the pod at different growing stages), the reliability of the qualitative measurement to confirm the previous findings.

Materials and methods:

Five contrasting voluble genotypes of snap bean with specific allelic iso-enzymatic composition and fibrousness (according to the breaking resistance and cuticle length after superficial mechanical detachment) were chosen (qualitative method). Three of the genotypes were accessions of the Bank of Germplasm of CIAT (G9069, G18722, G10165), and two were commercial varieties with desirable morphologic characteristics but contrasting in fibrousness (Blue Lake and Millenium). A systematic design of sowing in eight furrows, in which each corresponded to a period of weekly sampling, from the appearance of the first trifolium leaf was used. In each one of the furrows, five plants were chosen randomly for the growth analysis, and the foliar area was also measured using the extrapolation between the weight of a well-known foliar area and the weight of the total foliar area of the plant. Using the data from the foliar area and the dry weight; the curve of accumulation of dry matter in plants and pods, as well as the rate of net assimilation were determined. During the anthesis day, the flowers were tagged to establish the pod age at any moment t_0 , and the fiber content was estimated using the proposed

methodology of Vansoest *et al.*, 1991, as well as the neutral detergent fiber and the acid detergent fiber. Besides of measuring the fiber by means of the qualitative method, the contents of protein (Bradford, 1976) and chlorophyll (Wintermanns and Motts, 1965) were determined weekly, from the 13th day after the anthesis until the 63rd day (four samplings).

Results and discussion:

The selected genotypes, according to the contrasting of the allelic grouping of eight iso-enzymatic systems (PRX, ME, MDH, DIAP, ACP, SKDH, PGI, 6PGDH) (Tofiño *et al.*, 2004) and its fibrousness (measured with the qualitative method), also showed remarkable characteristics in the dynamics of biomass accumulation, percentage of the fiber components, and protein and chlorophyll contents in the pods. Only when the pod are three weeks old post anthesis, the correlation between the length of the detached cuticle, break resistance and neutral detergent fiber (cellular wall: cellulose, hemi-cellulose, lignin, polysaccharides associated to pectins) and total fiber, were highly significant ($R^2= 0.64$, $R^2= 0.62$, respectively). According to the former study, the qualitative method of fiber determination is efficient solely during a defined period of time, and since the estimations in the previous study were made in 20-days old pods (Tofiño *et al.*, 2003), it was found that the characterization using the eight proposed iso-enzymatic systems could be used as a marker of variability of the characteristics of quality of the pod for fresh consumption. These iso-enzymatic systems could be associated with the development of the fiber in the pod, and can be used as a finer system of discrimination than the morphologic description of pods, excluding the qualitative determination of fibrousness, since groups of similar morphologic characteristics of the pod but with contrasting fibrousness were made. The eight proposed iso-enzymatic systems could be used as markers of the early estimation for the selection of accessions of snap bean in the banks of germplasm.

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GROWTH AND YIELD OF SNAP BEAN (*PHASEOLUS VULGARIS* L.) WITH APPLICATION OF GROWTH REGULATORS

Jesús Rubén Torres-García¹, J. Alberto Escalante-Estrada¹, Ma. Teresa Rodríguez-González¹, David Martínez-Moreno² and Carlos Ramírez-Ayala¹.¹Colegio de Postgraduados, Montecillo, Méx.
²Benemérita Universidad Autónoma de Puebla, Puebla, México

INTRODUCTION

Growth regulators (auxins, gibberellins and cytokinins) can be an alternative to increase the snap bean production (Christiansen and Lewis, 1982). The growth regulators effect is in function of the concentration and phenological stage of application (Stuart and Cathey, 1961). Moreover, is well documented that exist a synergic effect between this three groups of growth regulators (Jankiewicz, 2003). The objective of this study was to determinate growth regulators effect (auxins, gibberellins and cytokinins) on the phenology, growth analysis index and snap bean yield.

MATERIAL AND METHODS

Greenhouse research was conducted during June 14 to September 20 of 2006 at the Colegio de Postgraduados Campus Montecillo. A commercial mixture that contains auxins, gibberellins and cytokinins to the 500, 500 and 200 ppm concentration respectively was utilized. The treatments were the foliar application of 5 and 10 mL L⁻¹ of the product in vegetative stage (Veg, 28 days after sowing, das) and flowering beginning (FB, 41 das). In addition the application of the product in both phenological stages was included. The design was completely randomized with five replicates. Plant samples were taken to 28, 41 and 98 das to evaluate the number and fresh weight of pods; dry weight of stems, leaves and pods and the total biomass by plant. Statistical analysis for each variable was realized.

RESULTS AND DISCUSSION

The phenology was not affected by the treatments. The emergency occurred to the five das, FB to the 40 and the physiological maturity to the 98 das. The pod harvest was to 62, 70, 75 and 98 das. Five mL L⁻¹ at FB was achieved the highest dry weight (g), pod yield and biomass, (g) (figure 1ab). With 10 mL L⁻¹ and Veg applications were not significant changes with respect to control. Table 1 show that the treatment with highest yield was 5 mL L⁻¹ FB and the lowest was 5 mL L⁻¹ Veg. With 10 mL L⁻¹ at FB had a similar yield to 5 mL L⁻¹ but with lower productivity. The rest of the treatments showed very similar yield to the control. The growth analysis indexes highly related with fresh weight pod were the relative growth rate (RGR), the net assimilation rate (NAR) and leaf area efficiency during the reproductive period (LAE).

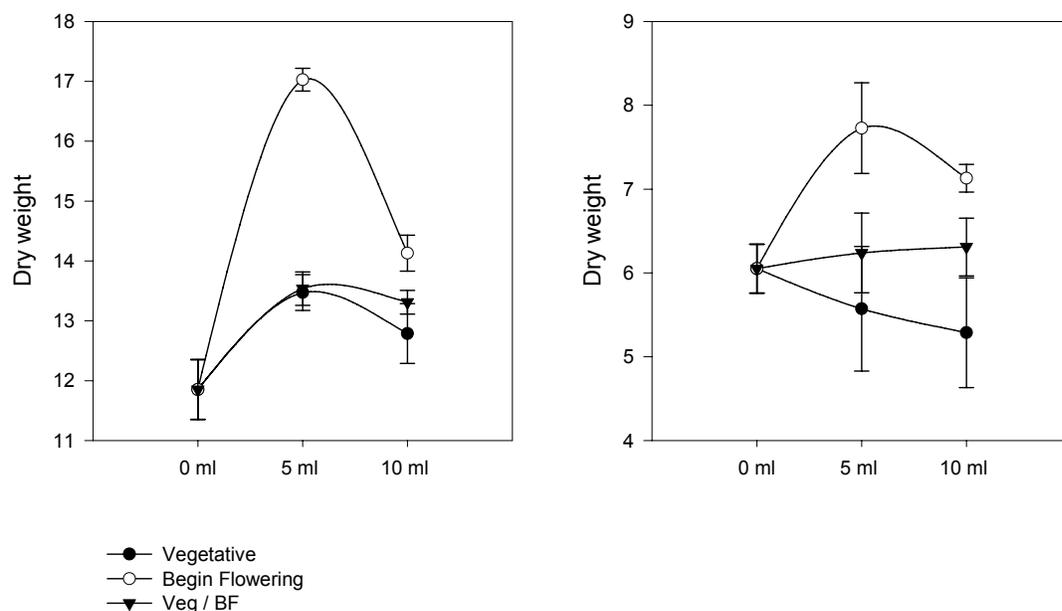


Figure 1. (A) Biomass and dry weight pod (g), in function of plant growth regulators. Bars denote standard error.

Table 1. Pod yield (fresh weight), growth analysis index in relation to foliar applications of growth regulators.

	Yield (g)	Pod number	RGR ($\text{g g}^{-1} \text{ week}^{-1}$)	NAR ($\text{g dm}^{-2} \text{ day}^{-1}$)	LAE ($\text{g dm}^{-2} \text{ day}^{-1}$)
Control	54	14	0,079	0,007	0,0013
5 mL Veg	53	13	0,076	0,005	0,0009
5 mL BF	64	19	0,101	0,008	0,0014
Prob. F	*	**	--	--	--

RGR= Relative growth rate, NAR= Net assimilation rate, LAE= Leaf area efficiency during reproductive stage. $P > F$ *,** 0.10 y 0.05, respectively.

CONCLUSIONS

The snap bean yield and biomass are increased with the foliar application of 5 mL L⁻¹ of the growth regulators mixture (auxins, gibberellins and cytokinins) at the beginning of flowering. The growth analysis indexes most related with the yield are relative growth rate (RGR), net assimilation rate (NAR) and Leaf area efficiency during reproductive stage (LAE)

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VARIABILITY STUDY OF 89 SNAP BEAN GENOTYPES USING THE AFLP MOLECULAR TECHNIQUE

A. P, Tofiño¹; J. F, Calderón¹; J. D, Palacio²; M, Blair³: ¹Universidad Nacional de Colombia, Bogotá; ² Instituto Humboldt; ³ Bean breeding Program CIAT, Cali.

Introduction:

Snap bean is a vegetable cultivated by small farmers in some mountainous and hillside regions of Colombian. Since the research of snap bean has not had the same importance as common beans in Colombia, little competitive varieties, compared to the quality of the Blue Lake variety have been released, with a culti-variety with low tolerance to the biotic constraints of the tropic (Silbernagel *et al.*, 1999). The lineages, coming from crossing this variety and other well adapted ones to the tropic genotypes of *Phaseolus vulgaris*, exhibit low commercial quality due to the rupture of the co-adapted genetic complex that determines the characteristics of the pod (Myers and Baggett, 1999). Using the AFLP technique, the genetic variability of 89 traditional varieties of snap bean (coming from primary and secondary domestication centers) was studied, to identify genotypes genetically closer to the Blue Lake type, combining desirable characteristics of the pod to be included in the set of basic materials associated to breed programs (Beattie *et al.*, 2003). This way, the genetic base of the culture would be bigger, without breaking the link group regarding the pod quality.

Materials and methods:

Eighty-nine traditional varieties of snap bean were evaluated; two commercial related varieties of snap bean, presenting contrasting fibrousness (Blue Lake and Millenium); two commercial varieties of common bean (ICA-Pijao, Diacol-Calima) and two wild beans, these four with different genetic pool (G 23441, Guatemalan and G 21117 Colombian) were used as controls. From a binary matrix of 51 polymorphic bands, using the software Tree NTSYS and the DICE coefficient, the similarity analysis was obtained, which was compared with a previous group according to the phaseolin type (Andean and Mesoamerican) (Tofiño *et al.*, 2004), with the purpose of knowing the process recombination between genetic pools and their relation with the increase of the desirable characteristics of the snap bean. The level of association and the degree of the variability between the analysis of molecular similarity and the morphologic grouping were also evaluated. Additionally, the total diversity, the population indexes, and the genetic flow using the software POPGENE, coming from the initial group of the genotypes by phaseolin type, were estimated.

Results and discussion:

The indexes of diversity and population structure, using the same technique, indicate a moderate genetic differentiation between genetic pools ($H_t=0.225$; $H_s=0.2064$; $G_{st}=0.0827$; $N_m= 5.534$; $I= 0.3413$), similar to the sample using iso-enzymes (Tofiño *et al.*, 2004); also, a total diversity comparable to that found in studies in wild Mesoamerican beans ($H_t = 0.22$), and superior in reported common Mesoamerican beans ($H_t= 0.12$; Papa and Gepts, 2003). However, a general homogeneity within the sample was observed, because the similarity dendrogram was split in two big groups at a level of 77% of similarity, different to other works made in common beans using the same technique, in which a minimum similarity of 24% was found (Nowosielski *et al.*, 2002). Each group presents a genetic pool predominance of Mesoamerican or Andean genetic pool with intermediate genotypes, that is Andean individuals grouped with Mesoamerican

genotypes and vice versa, similar to the observed by Tofiño *et al.*, 2004. In the same way, the genetic diversity was found bigger between individuals coming from Andean genetic pool (0.2225) than in individuals from Mesoamerican genetic pool (0.1902). This result suggests that the Andean genetic pool was enriched with the flow of genes coming from Mesoamerican genetic pool; since many individuals with Andean phaseolin are grouped with Mesoamerican individuals. The molecular analysis managed to discriminate the commercial controls appropriately because the dry Mesoamerican bean was grouped with genotypes of snap bean with Mesoamerican phaseolin, and the Andean control was grouped with genotypes of Andean-origin snap beans. On the other hand, the commercial controls of related snap bean were close grouped. These results strengthen the reach of the previously collected data, in spite of the low quantity of polymorphic bands. Additionally, the wild controls, although of Mesoamerican origin, were grouped together with genotypes of Andean-phaseolin snap bean. Also, the genotypes grouped according to their potential for the Latin American fresh market and their morphologic and fibrousness analysis, were close grouped under an index of similarity of 90%. Nevertheless, the genotypes G18722 and G10165, with contrasting fibrousness, were grouped together within the predominantly Andean group. Otherwise, the iso-enzymatic analysis allowed discrimination of genotypes with contrasting fibrousness in spite of the high morphologic similarity of the pod. According to the previous data (Tofiño *et al.*, 2004), the iso-enzymatic analysis allows a fine discrimination of the pod quality characteristics regarding the other evaluated markers. Additionally, the diversity found in the germoplasm of snap beans using AFLP, the morphologic and biochemical description are similar to the recorded germoplasm of common beans and something inferior to the registered one previously in snap bean using other molecular markers (0.388) (Skroch and Nienhuis, 1995).

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PHASEOLIN: VARIABILITY AND REFERENCE MATERIALS IN WILD AND CULTIVATED COMMON BEAN

O. Toro, C. H. Ocampo and D. G. Debouck
International Center for Tropical Agriculture CIAT, A.A. 6713, Cali Colombia

Introduction

Phaseolin has proven to be a particularly informative marker in studies of genetic diversity and evolutionary pathways in common bean, for both wild and cultivated forms (Gepts, 1993). The purpose of this note is to report on phaseolin types not published previously and to extend information on types already published (Gepts & Bliss, 1986; Gepts et al. 1986; Koenig et al. 1990; Toro et al. 1990; Debouck et al. 1993; Tohme et al. 1995; Beebe et al. 1997; Ocampo & Toro, 2005), with indication of source materials, available internationally as genetic stocks, and maintained by CIAT Genetic Resources Unit as inbred materials.

Materials and Methods

The accessions, which 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

Although this globulin has a narrow range of molecular weight (45-52 kD) and isoelectric point, a total of 62 banding patterns has been found so far, 30 being present in Mesoamerica, 21 in the Andean region, 8 in Colombia, and 3 in both Mesoamerica and Colombia. In relation to biological status, 38 patterns have been found in wild materials, 5 patterns in weedy forms and 19 patterns in cultivated materials (Table 1). Domestication that has been shown to happen in both major gene pools (Chacon et al. 2005), led to a reduction of phaseolin diversity or founder effect, perhaps stronger in Mesoamerica as compared to the Andean zone (our results, and Gepts, 1993). While the founder effect might be lesser than initially thought (Gepts, 1993; Sonnante et al. 1994), new phaseolin types continue to be found, namely in Colombia, suggesting more research in this area.

Table 1. Diversity of phaseolins and reference materials in wild, weedy and cultivated common beans (*Phaseolus vulgaris* L.).

No.	Phaseolin Types	Number G	Reference materials (Phs morphotypes)	Biological Status ¹	Genetic Pool ²	Country of origin ³
1	S	G12853	FI-2380	WILD	M	GTM
2	Sb	G12952	FI-5416	WILD	M	MEX
3	Sd	S33761	FI-2881	CULT	M	COL
4	M1	G23418	FI-5824	WILD	M	CRI
5	M2	G23652	FI-1930	WILD	M	MEX
6	M3	G12865	FI-1389	WILD	M	MEX
7	M4	G23678	FI-1697	WILD	M	MEX
8	M5	G12851	FI-4068	WILD	M	GTM
9	M6	G24365	FI-1712	WILD	M	MEX
10	M7	G12869	FI-1415	WILD	M	MEX
11	M8	G12879	FI-4414	WILD	M	MEX
12	M9	G12878	FI-1457	WILD	M	MEX
13	M10	G11034	FI-1363	WILD	M	MEX
14	M11	G50869	FI-3657	WDY	M	COL
15	M12	G10002	FI-1304	WILD	M	MEX
16	M13	G23439	FI-3144	WILD	M	GTM
17	M14	G12853	FI-1247	WILD	M	GTM
No.	Phaseolin Types	Number G	Reference materials (Phs morphotypes)	Biological Status ¹	Genetic Pool ²	Country of origin ³
18	M15	G24365	FI-1714	WILD	M	MEX

19	M16	G50726	FI-3976	WILD	M	HND
20	M17	G12882A	FI-1504	WILD	M	MEX
21	M18	G12855A	FI-2390	WILD	M	GTM
22	M19	G12854	FI-3349	WILD	M	GTM
23	M20	G24584	FI-5419	WILD	M	MEX
24	M21	G2721	FI-4045	CULT	M	PER
25	M22	G23511A	FI-1629	WILD	M	MEX
26	M23	G12890	FI-4089	WILD	M	MEX
27	M24	G12949	FI-1923	WILD	M	MEX
28	M25	G12851	FI-4070	WILD	M	GTM
29	M26	G23434A	FI-28	WDY	M	GTM
30	Dur	G11027A	MEXDU-01	WILD	M	MEX
31	T	G50015B	FI-2838	WDY	A	ARG
32	C	G21194	FI-4188	WILD	A	ARG
33	H1	G51049	FI-2753	CULT	A	COL
34	H2	G50401	FI-2514	CULT	A	COL
35	Ca	G12857	FI-1747	WILD	A	PER
36	Ca1	G50850	FI-3847	CULT	A	COL
37	To1	G24776	FI-4454	CULT	A	COL
38	To2	G23786B	FI-4456	CULT	A	PER
39	Ta	G23445	FI-1029	WILD	A	BOL
40	Nu	G12573	FI-13059	CULT	A	PER
41	K	G23422	FI-4105	CULT	A	PER
42	Ko	G23814	FI-4655	CULT	A	PER
43	J1	G19895	FI-998	WILD	A	ARG
44	J2	G23592	FI-934	WILD	A	ARG
45	J3	G19902	FI-976	WILD	A	ARG
46	J4	G21194	FI-4190	WILD	A	ARG
47	P1	G23423	FI-1805	WILD	A	PER
48	Pa	G23455	FI-1831	WILD	A	PER
49	I	G21244	FI-1	WILD	A	PER
50	A	G12857	FI-1748	WILD	A	PER
51	A1	G12078	FI-4842	CULT	A	PER
52	L	G24408	FI-2121	WILD	COL	COL
53	LI	G51019	FI-2493	CULT	COL	COL
54	CAR	G50843	FI-2432	CULT	COL	COL
55	HE	G51006	FI-2490	CULT	COL	COL
56	TI1	G51048	FI-2849	CULT	COL	COL
57	TI2	G51036	FI-2896	CULT	COL	COL
58	Mu	G50983	FI-4495	WDY	COL	COL
59	Qui	G24674	FI-4421	CULT	COL	COL
60	B	G24717	FI-2038 F2	CULT	M/COL	COL
61	CH	G50886	FI-3716 F ₂	WDY	M/COL	COL
62	Tel	G18970	FI-5791	CULT	M/COL	CRI

¹Biological Status: WILD (Wild), WDY (Weedy), CULT (Cultivated).; ²The common bean genetic pools (Tohme et al. 1996): M (Mesoamerican); A (Andean); COL (Colombia). ³Country of origin: MEX (Mexico), GTM (Guatemala), HND (Honduras), CRI (Costa Rica), COL (Colombia), PER (Peru), BOL (Bolivia), ARG (Argentina).

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MOLECULAR CHARACTERIZATION OF COMMON BEAN CULTIVARS BY PHASEOLIN AND RAPD MARKERS

M.C. Gonçalves-Vidigal¹, M.R. Costa², P.S. Vidigal Filho¹, A. Gonela¹ and A. L. Sansigolo

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

²Bioagro, Universidade Federal de Viçosa

Introduction

The landraces of common bean have been showing wide genetic variability for seed color, shape, brightness, size and have demonstrated adaptability to several environmental conditions, which can be observed through the resistance to diseases and elevated yield potential (Rodiño et al. 2003). The knowledge of patterns of genetic diversity among landraces and their relationship with new cultivars helps broaden the genetic base and maximizes use of available germplasm. The objective of this study was to characterize the phaseolin in common bean Jalo Listras Pretas and Jalo Vermelho using the SDS polyacrylamide gel electrophoresis (SDS-PAGE) and RAPD molecular markers.

Material and Methods

Eight genotypes were analyzed, one of them representative of the Andean (Jalo EEP558) and two of the Mesoamerican gene pools, were used as controls to analyze the phaseolin specificities. Seeds of each cultivar were characterized and proteins as well were extracted and applied to gradient gel of polyacrylamide (SDS-PAGE). The seed protein analysis and the designation of the different protein bands were carried out according to Vasconcelos (1994). Twenty-four landraces of common bean from Paraná State were evaluated in order to investigate the Andean and Mesoamerican polymorphism using RAPD analysis. DNA extraction method was used according to Edwards et al. (1991). Amplification reactions were performed similarly to that described by Young and Kelly (1996) using random primers (Operon Tech., Alameda, Calif.).

Results and Discussion

Figure 1 shows the polymorphism between Andean and Mesoamerican cultivars in SDS polyacrylamide gel. The pattern of protein distribution in the electrophoresis gel also corresponded to the amount of protein quantified by the spectrophotometer method. The bands identified on the gels with the black arrows are bands associated to phaseolins S and T.

Figure 2 shows an amplification standard obtained with the primer OPG19. The results among the identified polymorphic bands pointed out those with sizes of 1,790 pair of bases (in Mesoamerican cultivars) and marker with 1,400 pair of bases present in Andean landraces. Based on the electrophoresis analysis, the presence of a band that corresponded to the Phaseolin type T in Jalo Vermelho and Jalo Listras Pretas has demonstrated that these two cultivars belong to the Andean gene pool. In addition, RAPD analysis demonstrated that these cultivars possess the 'Diagnostic Band' present in Andean cultivars as well. The results confirmed that Jalo Vermelho and Jalo Listras Pretas are cultivars from the Andean pool.

Acknowledgements

We would like to thank CNPq, Capes and Fundação Araucária for the financial support. P. S. Vidigal Filho and M.C. Gonçalves-Vidigal were supported by grants from CNPq; G.F. Lacanallo and A. Gonela are supported by grants from Capes.

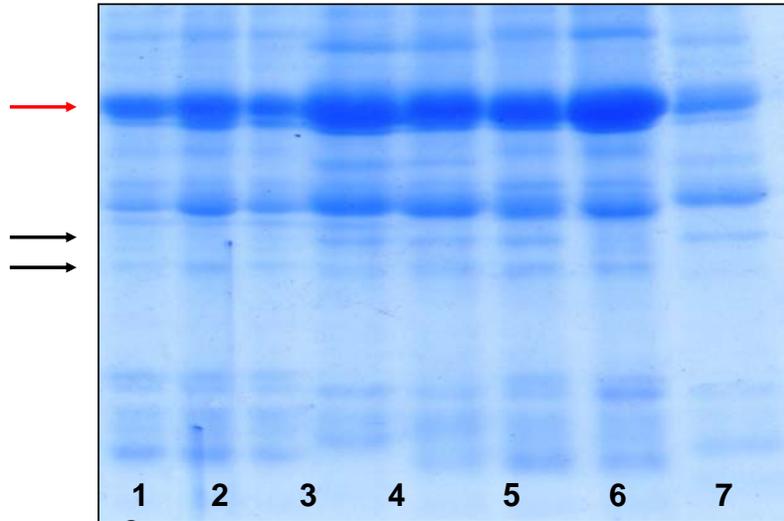


Figure 1 – Electrophoretic analysis of reserve proteins of eight genotypes, as follows: 1, Jalo Vermelho; 2, Jalo de Listras Vermelhas; 3, Jalo Listras Pretas; 4, Widusa I; 5, Widusa II; 6, Mesoamerican genotype; 7, Andean control (Jalo EEP 558), 8, Mesoamerican control (CSW).

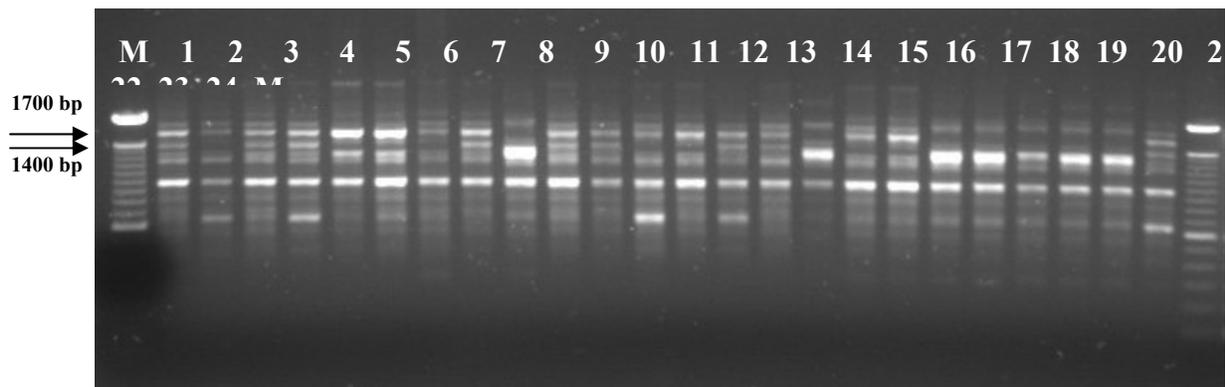


Figure 2 – Electrophoretic analysis of amplification products obtained with OPG19 RAPD marker. Lanes are as follows: M, molecular weight marker (100bp ladder); 1, Carioca 1; 2, Carioca 2; 3, Carioca 3; 4, Carioca 4; 5, Preto 1; 6, Preto; 7, Preto 3, 8, Carioca 5; 9, Jalo Pintado; 10, Carioca 6; 11, Carioca Pitoko; 12, Rosinha; 13, Preto 4; 14, Navy UEM; 15, Carioca Claro; 16, Jalo Pardo; 17, Iapar 31; 18, Carioca Pintado 1; 19, Jalo Vermelho; 20, Roxinho; 21, Jalo Mulato; 22, Bolinha; 23, Jalo Listras Pretas; 24, Carioca Pintado 2. The arrows indicate a DNA band of 1,790 bp.

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GENETIC DIVERSITY OF COMMON BEAN GERMPLASM COLLECTION OF THE NUPAGRI BY RAPD MARKERS

M.C. Gonçalves-Vidigal, P.S. Vidigal Filho, J.C. Molina, A. Gonela,
G.F. Lacanallo and V. L. Souza

Departamento de Agronomia, Universidade Estadual de Maringá, Brazil. E-mail:
mvidigal@pop.com.br

Introduction

The genetic diversity in gene banks enables plant breeders to choose the most suitable strategy for improvement programs (Lefort-Buson et al., 1988). In this sense, the availability of diverse common bean accessions represents a valuable source for the improvement of the species since co-adapted genes of different accessions can convey tolerance to various diseases (Harlan, 1975). The objective of this study was to characterize the genetic dissimilarity in common bean accessions belonging to the gene bank of the Núcleo de Pesquisa Aplicada à Agricultura (Nupagri) at the Universidade Estadual de Maringá using RAPD markers.

Material and Methods

The genetic diversity in 40 common bean accessions of the Common Bean Gene Bank (CBGB) from Nupagri (Center for applied agricultural research), obtained from pure lines, was evaluated using RAPD markers. The seeds were sown in pots with substrate and maintained in a greenhouse. A young leaflet in the V₃ stage was taken from the first trifoliolate leaves of one plant of each accession. Each leaflet was placed in an Eppendorf tube and immediately deep-frozen for follow-up DNA extraction. The DNA extracted from the 40 accessions was used as template for amplification reactions according to a methodology proposed by (Edwards, 1991) with modifications. The following primers were used for the amplification reactions: OPA18, OPSAS13, OPC08, OPF05, OPF06, OPF10, OPG19, OPH20, OPI03, OPM12, and OPX11, previously tested for their polymorphic profile in common bean.

The genetic divergence between common bean accessions was evaluated by a binary data matrix, based on the amplified polymorphic fragments. The underlying binary data matrix allowed an estimation of the genetic dissimilarity in the accessions by the arithmetic complement of the Jaccard's index (Jaccard, 1908). The unweighted pair-group method based on arithmetic averages (UPGMA) was used as clustering technique.

Results and Discussion

DNA of the accessions was extracted and amplified, using RAPD markers, which generated a total of 92 polymorphic bands.

Three groups were formed by the UPGMA method; group I consisted of three subgroups (Figure 1). It was observed that two subgroups (Ia and Ic) consisted of Mesoamerican accessions, while subgroup Ib contained only Andean accessions (Navy-UEM, Jalo Listras Pretas and Roxinho). On the other hand, group II comprised seven accessions, being all of them Andeans. Thus, the accessions were clustered according to their respective origin center. Similar results were obtained by Vilarinhos et al. (1995) studying the Mesoamerican and Andean of the set differential cultivars of anthracnose.

The Group III consisted of two subgroups that contained all accessions denominated CBGBs. The results showed that the most similar accessions were Carioca Pitoko and Iapar 31. However, the greatest dissimilarity was observed between accession CBGB9 and the pair Carioca Pitoko and Iapar 31 (Figure 1). Thus, the accessions were shown to represent a high potential of genetic variability for breeding programs.

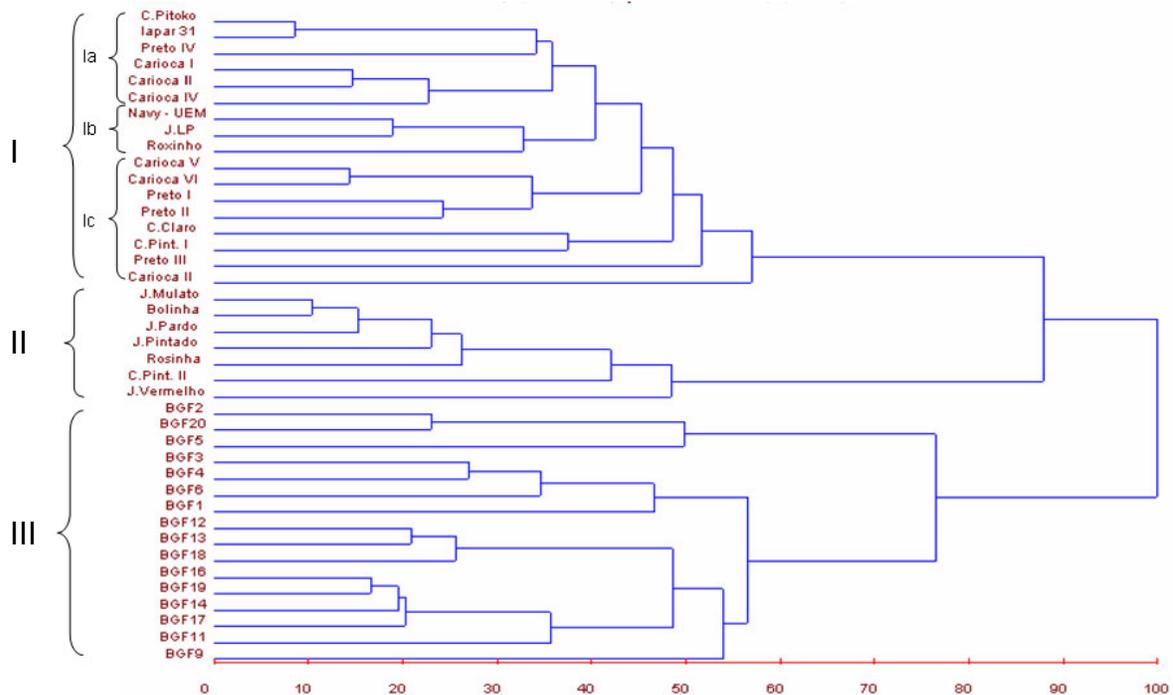


Figure 1 - Dendrogram of genetic divergence among 40 accessions of common bean obtained by UPGMA method using arithmetic complement of the Jaccard's index as measure of dissimilarity. JLP = Jalo Listras Pretas; C. Claro = Carioca Claro; C. Pint. I = Carioca Pintado I; C. Pint. II = Carioca Pintado II; J. = Jalo

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FUNCTIONAL MARKERS OF COMMON BEAN FROM DISEASE RESISTANCE GENE ORTHOLOGS

¹Zahirul I. Talukder, ²Phillip N. Miklas, ¹Thomas P. Gonnella, and ¹Khawaja G. Hossain
¹Division of Science and Mathematics, Mayville State University, Mayville, ND 58257;
²USDA-ARS, Prosser, WA 99350

Introduction

Disease infestation is a major constraint for subsistence production and economic yield of common bean (*Phaseolus vulgaris* L.). Development of cultivars with improved disease resistance is the primary goal of bean breeding programs throughout the world. Genetic linkage maps have been used to identify DNA markers tightly linked with resistance traits in common bean for purposes of marker-assisted selection. Several types of DNA markers have been used to tag and monitor disease resistance traits but very few of them are functional gene-based markers. Genes conferring resistance to different plant pathogens have been isolated from a variety of species (Baker et al., 1997; Hammond-Kosack & Jones, 1997). Comparing the predicted amino acid sequences of cloned resistance (R) genes, researchers were able to identify conserved motifs in R gene products. The majority of these genes encode cytoplasmic receptor like proteins that contain leucine-rich repeat (LRR) and nucleotide binding site (NBS) domains.

Compared to major crop species, the limited genomic resources in common bean has created a bottleneck in the development of functional markers. The availability of genomic databases in other important plant and animal species; however, has enabled application of genomic information across species. We have initiated a research project to develop functional molecular markers for localizing disease resistance genes in common bean using the sequence information of conserved NBS-LRR motifs of previously sequenced disease resistance genes in related legumes.

Materials and Methods

Parents of seven mapping populations known to segregate for different disease resistance traits were grown in the greenhouse. DNA was extracted and purified from leaves using the protocol supplied by the Dry Bean Breeding Group, Department of Plant Sciences, North Dakota State University, Fargo, ND. Disease resistance gene sequences in legumes were downloaded from NCBI GenBank database. Based on the predicted average intron size of 161 bp in *Medicago truncatula*, a model species for legumes, PCR-primers were designed using the PCR-primer designing program “Primer 3” to amplify common bean genomic DNA of ≤ 350 bp in size. For larger size gene sequences (>350 bp), overlapping primer pairs were designed. The screening of the primers was performed using a PCR program consisting of one cycle of 95°C for 5 min; 40 cycles of 95°C for 1 min, from 44 to 62°C for 1 min, and 72°C for 2 min; and one cycle of 72°C for 10 min. The amplified products were separated in 2% agarose gels at 70 volts for 4 hours or in 6% polyacrylamide gels at 200 volts for 3 hours and 40 minutes.

Results

A total of 48 primer pairs were designed from 27 disease resistance gene sequences from different legumes. Thirty primer sets amplified common bean genome, of which 11 primers produced single and five produced double bands with or without non-specific amplicons, and the rest amplified mainly non-specific multiple bands (Fig. 1).

Among the primer sets that produced single and double products, at least 15 primer sets showed polymorphism across parents of one or more of the seven mapping populations. A total of twelve primer pairs were selected in order to sequence the amplified products among the parents of the seven mapping populations. A generic cloning protocol is being used to sequence the amplified products. Sequence data will be assembled and analyzed to detect SNPs among the parents. The polymorphic markers will be localized in the linkage maps of the corresponding mapping populations to determine associations with known disease resistance genes and QTL.

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Acknowledgement

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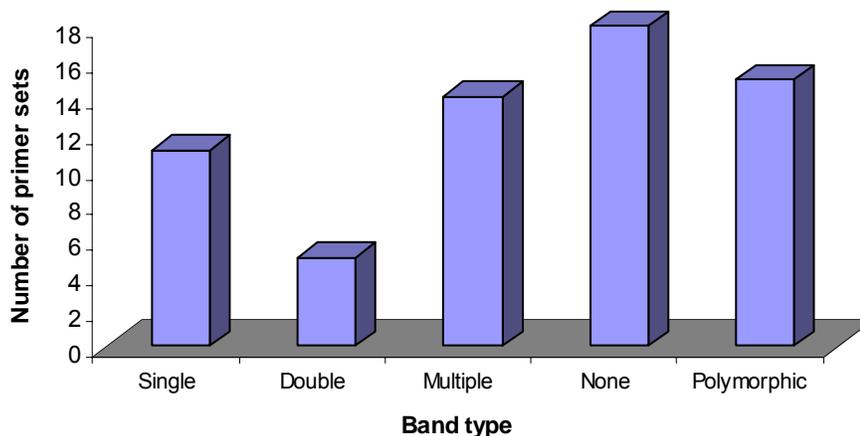


Figure 1. Amplification of disease resistant gene homolog primers in parents of common bean mapping populations

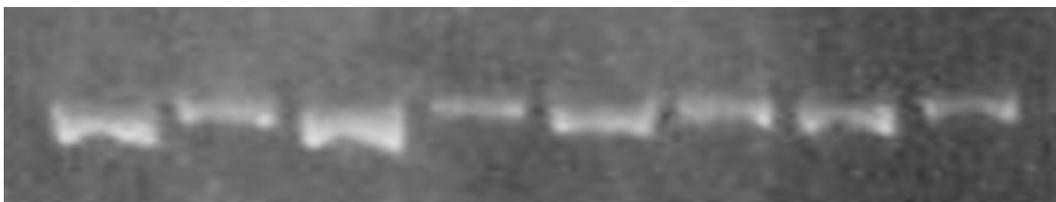


Figure 2. Primer 10 developed from sequence 16588612 showing polymorphism in BAT93 /Jalo EEP558 (Gradient PCR products separated on 6% polyacrylamide gel)

DEVELOPMENT AND GENETIC MAPPING OF A SCAR MARKER FOR THE BEAN GOLDEN YELLOW MOSAIC GEMINIVIRUS RESISTANCE GENE BGM-1

M.W. Blair¹, L.M. Rodriguez¹, F. Pedraza¹, F. Morales¹, S. Beebe¹, P. Miklas², J. Beaver³

¹CIAT, Cali, Colombia, ²USDA, Prosser, WA, ³Univ. of Puerto Rico, Mayaguez, PR

Introduction:

Bean golden yellow mosaic virus (BGYMV) is a whitefly-transmitted geminivirus of the Begomovirus family that causes important yield losses to common beans grown in tropical and sub-tropical countries of Latin America and the Caribbean. The disease is devastating in bean production areas below 1000 masl in many countries especially Dominican Republic, Guatemala, El Salvador, Haiti, Honduras and Nicaragua. BGYMV is also prevalent in parts of Costa Rica and southern Mexico and has been reported in Florida. Yield losses can be from 45 to 100% during epidemics and the disease continues to expand its range into new seasons and environments at higher altitudes and latitudes. A major resistance gene that has been widely deployed in this region is the recessive locus *bgm-1* that prevents the development of severe yellowing typical of the disease. In this study, our goal was to genetically map a sequence characterized amplified region (SCAR) marker that is tightly linked to the *bgm-1* resistance gene through comparative mapping using two genetic maps for the species. In addition we sought to map the SR2 marker relative to *bgm-1* in a segregating population of recombinant inbred lines from the resistant x susceptible cross of DOR476 x SEL1309.

Materials and Methods:

Population Development: A recombinant inbred line (RIL) population was developed from the cross of SEL1309 (susceptible) x DOR476 (resistant) through single seed descent from the F2 to the F5 generation. DOR476 contains the *bgm-1* resistance gene and the associated marker alleles derived from CIAT line, A429. The population was tested in the greenhouse in Colombia with a mechanically inoculated BGYMV–Guatemala strain and in the field in Puerto Rico with whitefly-transmitted natural infection. Ten plants were evaluated per treatment and the RILs were evaluated for overall symptom score and four separate characteristics (chlorosis, dwarfing, flower abortion and pod deformation) in greenhouse while field scores were given per row. In both cases a 1 to 9 scale was used where 1 was equivalent to resistant and 9 to susceptible. DNA was extracted from the population for use below.

SCAR marker development: SCAR development was carried out based on the RAPD band (R2) identified by Urrea et al. (1996). Briefly, amplification of the RAPD fragment was carried out in 25 volume reactions with the same reaction components as in this previous study. The polymorphic bands associated with resistance and susceptibility were cut out of 1.5% low melting point agarose gels and cleaned individually with the Wizard PCR prep purification system. The purified insert DNAs were cloned into the pPCR-Script Amp SK(+) plasmid vector and end sequenced with standard techniques and T7 and T3 primers. Specific primers were designed from the fragment ends and at an internal location using Primer 3.0 software and were tested for ability to amplify single-copy SCAR products as described below.

SCAR testing: PCR reactions for the SCAR markers were carried out in 25 µl reaction volumes containing 50 ng of genomic DNA, 0.2 µM each of forward and reverse primers, 20 mM of total dNTP, 1.5 to 2.5 mM MgCl₂ and 1 unit of Taq polymerase in 1X PCR buffer. The new SCAR was tested for amplification on a panel of genotypes representing parents of the test population described above and two mapping populations used for marker placement: DOR364 x G19833 and BAT93 x Jalo EEP558. Segregation for the SCAR was evaluated on the entire population of 100 individual RILs from the DOR476 x SEL1309 population where the marker was co-dominant. For the other two populations polymorphism was uncovered by CAPS marker analysis.

Results and Discussion:

The RAPD band was successfully converted into two SCAR markers named SR2 and SR21 (Figure 1). Polymorphism of the SR2 marker was shown to be based on a 37 bp insertion event in the allele associated with susceptibility compared to the allele associated with resistance. The SR2 marker was significantly associated with overall disease symptoms and with three of the four symptoms associated with the disease (yellowing or chlorosis, flower abortion, pod deformation) in a greenhouse trial in Colombia with the mechanically transmissible BGYMV–Guatemala strain and the mapped at a distance of 7.8 cM from the resistance gene *bgm-1* based on the chlorosis score. The SR2 marker was successfully converted into a polymorphic CAPS based marker for mapping in both the DOR364 x G19833 and BAT93 x Jalo EEP558 mapping populations (data not shown). In this mapping exercise, SR2 was located near the end of linkage group b03 (chromosome 5) suggesting a sub-telomeric position. Interestingly, the position of the *bgm-1* resistance gene was syntenic with that of *bc-1*, a strain-specific resistance gene for bean common mosaic virus (BCMV), based on linkage of SR2 with the SCAR marker SBD5 in the DOR364 x G19833 mapping population. It is fascinating to us that there is synteny between these two recessive resistance genes as this may suggest that there is an association between resistance genes for both begomovirus and potyvirus pathogens.

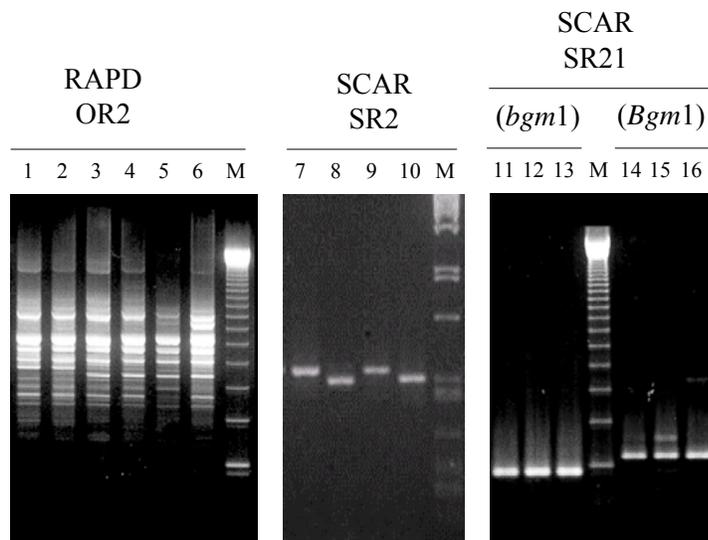


Figure 1. Conversion of RAPD marker OR2 (left panel) to SCAR markers SR2 (middle panel) and SR21 (right panel). Lane genotypes as in Blair et al (TAG 114: 261-271).

INTROGRESSION OF CMV RESISTANCE INTO SNAP BEAN

Michell E. Sass¹, Thomas German² and James Nienhuis¹

University of WI-Madison, Department of Horticulture¹ and Entomology²

Introduction: Recently, aphid-transmitted viruses have become common in snap bean production areas throughout the Great Lakes region, particularly Wisconsin. Research findings indicate that alfalfa mosaic virus (AMV), cucumber mosaic virus (CMV), and clover yellow vein virus (CIYVV) play important roles in this complex (Grau et al. 2001; 2002; German et al. 2004; Larsen and Eastwell 2004). While AMV and CMV occur most frequently in the Wisconsin snap bean landscape, CMV and CIYVV appear to be the most important due to economic losses related to pod quality including pod malformation and internal and external pod necrosis. Our research effort has focused on CMV (and to a lesser extent AMV) because CIYVV has not consistently been detected each year in Wisconsin. We have been unable to identify a source of immunity to CMV, but have identified lines that are consistently symptomless; however, a virus titer can be detected in these lines. The objective of this study is to determine the inheritance of the symptomless trait and the correlation with snap bean quality traits.

Materials and Methods: A RIL population consisting of 131 F₃ families derived from a cross between the snap bean cultivar MV185 and selection 2313.9.1000 was evaluated along with 29 checks at Hancock and West Madison Agricultural Research Stations (ARS) in 2006. At each location, spreader rows consisting of a 1:1 mix of an aphid susceptible soybean and the snap bean Hystyle were planted 14 days prior to the families. After planting the families, snap beans in the spreader rows were inoculated with CMV throughout the field in order to insure high CMV pressure once large aphid populations began migrating into the field. Because we were not seeing the virus symptoms we anticipated at West Madison ARS, we reinoculated snap beans within the spreader rows prior to flowering. We relied on natural inoculum sources and vectors for AMV infection.

Viral symptomatology ratings were taken during the growing season, at flowering and again at the green pod stage using a 1-5 scale. A “1” represented a completely healthy plant with no virus symptoms. A “2” was given to entries that did not have clear virus symptoms but appeared as if the plants were not completely healthy. A “3” was given to entries with obvious virus symptoms such as interveinal chlorosis, leaf distortion, leaf blistering, or minor pod symptoms. A “4” was given to entries whose virus symptoms were so great including severe plant stunting and that there would be a severe reduction in pod quality and yield as a result of virus infection. A “5” represented entries where virus infection was so severe that the entry was dead.

Prior to ELISA evaluation, 12 leaf samples from each location were harvested from symptomatic bean plants to determine which viruses were present. Total RNA was extracted using a Qiagen RNeasy Plant Mini Kit. RT-PCR was carried out using primers specific to AMV, CMV, and CIYVV detection. Controls included an uninfected snap bean as well as plants infected with single viruses including AMV, CMV, and CIYVV from the greenhouse.

At the green pod stage, a composite sample of ten leaves was harvested from each plot at both locations. Concentrated plant sap was collected from each composite, diluted, and prepared for CMV and AMV ELISA. AMV and CMV positive controls provided by the manufacturer (Agdia, Elkhart, IN) were included on each corresponding ELISA plate. In addition, controls including a sample of General Extraction Buffer only, sap of snap bean infected only with CMV and with AMV, and an uninfected and a known uninfected snap bean from the greenhouse were included.

Results and Discussion: RT-PCR virus specific primers amplified regions specific to CMV and AMV. Some samples were shown to be infected with only CMV, some only with AMV and some were infected with both viruses. CIYVV was not detected using the RT-PCR method.

Results indicate that all but 3 plots at Hancock ARS were positive for the presence of CMV. One entry in which we did not detect a CMV virus titer was PI 309881. This accession had previously been

identified by Phil Griffiths, Cornell University as being CMV resistant. Preliminary data indicates; however, that this line is susceptible to AMV and likely CIYVV as well. The other plots at Hancock that were CMV negative were of two different F3 families. It is likely that this result is due to sampling error or that these are escapes, however, seed was harvested from individual plants in each plot and will be screened at a later date in the greenhouse. CMV was detected in approximately 98% of the plots at Hancock ARS while AMV was detected in less than 5% of the plots. In contrast, although spreader rows at West Madison were inoculated with CMV twice during the summer, the ELISA results at West Madison indicate that approximately 40% of the plots were CMV positive and that more than 27% of the plots were infected with AMV.

We observed a low correlation between the symptoms at Hancock and symptoms at West Madison (Table 1). Although the spreader rows at both locations were inoculated with CMV and not AMV, the ELISA results emphasize the differences in aphid pressure at the two locations as well as the differences in the role each virus is playing at each location. It is unknown whether the aphids obtained the CMV from the spreader rows or if they were carrying CMV when they migrated into the plots. The data does indicate that visual symptoms at Hancock are positively correlated, though not highly, with the presence of CMV, but not correlated with the presence of AMV and that visual symptoms at West Madison are significantly correlated with the presence of both CMV and AMV (Table 1). Narrow sense heritability estimates were $h_2 = .40 \pm .08$ indicating that the symptomless phenotype is heritable.

Table 1. Correlation of field symptoms and the presence of CMV and AMV at West Madison and Hancock ARS.

	Symptoms	CMV+	AMV+
Symptoms	.13*	.31**	.28**
CMV+	.23**	-	-
AMV+	-.05 (ns)	-	-

*Significant, **Highly significant, and (ns) Not significant

Conclusion: Based on our preliminary data, we are optimistic that we can continue to select for the symptomless phenotype but are cautious in that we must use multiple location and replicated experiments as seed allows. We have also identified families that combine the symptomless phenotype and pod quality characteristics. Yield data will be taken on these lines in 2007.

Acknowledgement: This research was funded by the Midwest Food Processors Association and USDA-HATCH.

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BIOASSAYS TO DIAGNOSE SELECTED BEAN POTYVIRUSES

J. W. Shail Jr., A. G. Taylor and R. Provvidenti

Department of Horticultural Sciences and Plant Pathology, New York State Agricultural Experiment Station, Cornell University, Geneva, New York, USA 14456

A hot and dry growing season revealed many virus symptoms in commercial snap bean fields in upstate New York in 2005. Dr. Rosario Provvidenti suspected three potyviruses, Bean Yellow Mosaic Virus (BYMV), Clover Yellow Vein Virus (CYVV) and Watermelon Mosaic Virus (WMV) in field samples of 'Hystyle' snap bean in Geneva, New York. Unfortunately, common immunosorbent assays (ELISA) are not able to resolve specific potyviruses, and bioassays have not been reported to make distinctions between these three potyviruses. This report highlights the development of practical bioassays using specific indicator plants for diagnosing the potyviruses: BYMV, CYVV and WMV in the bean virus complex.

Pure cultures of each virus were obtained from the following sources:

BYMV – Richard Larsen USDA-ARS, Prosser WA

CYVV – Rosario Provvidenti collection, Geneva, NY

WMV- Rosario Provvidenti collection, Geneva, NY

The purity of these isolates was confirmed by RT-PCR at the New York State Agricultural Experiment Station (P. Griffiths, unpublished).

The bean genotypes used in this study as indicator plants consistently show distinct responses to virus infection under greenhouse conditions. A list of genotypes is provided below:

BT-1 and BT-2: Selections from a dry bean cultivar, Black Turtle were developed as diagnostic genotypes for WMV (Provvidenti, 1983). The original Black Turtle stock is referred to as BT-1 and the selection is BT-2. BT-1 carries a single dominant gene, *H_{sw}*, that confers resistance to WMV, while BT-2 is susceptible showing systemic yellow mottling and stunted growth.

B-21: Dry bean genotype developed by Provvidenti et al. (1989) that carries the *By-2* gene that confers resistance to BYMV. It is highly susceptible to CYVV infection showing apical necrosis and later death.

Black Knight: A dry bean variety developed from the Cornell breeding line CU M90 (Scully et al., 1991). It is highly resistant to CYVV and susceptible to BYMV with systemic yellow mosaic and stunting.

SP 17B: White seeded bean carrying resistance to BYMV and CYVV (Scully et al., 1995). Plants show a compact habit of growth and less vigor compared to other genotypes.

GN-1140 and USWK-6: White-seeded genotypes (GN-1140: seeds supplied by B. Scully and USWK-6: developed by P. Miklas et al, 2002) with slightly taller plant habits than other genotypes in this study. Both genotypes are resistant to CYVV and susceptible to BYMV infection with systemic yellow mosaic. They have the same responses as Black Knight with respect to BYMV and CYVV. USWK-6 carries the *bc-3* gene.

Clipper: A Navy bean developed in Ottawa, Ontario, Canada, supplied by Provvidenti. Highly resistant to CYVV and highly susceptible to BYMV with strong leaf mosaic and stunting. Clipper and Black Knight have similar responses to CYVV and BYMV.

Bioassay protocols for diagnosis of BYMV, CYVV and WMV. All protocols include inoculating plant extracts onto the first true leaves of the indicator bean plants.

BYMV - If BYMV is present, Black Knight is susceptible with stunted growth and yellow leaves, while B-21 is resistant.

CYVV - If CYVV is present B-21 is susceptible with the virus killing the growing point and later the whole plant dies. In contrast Black Knight is resistant. In the event that both CYVV and BYMV are present, then SP-17B is resistant to both viruses.

WMV - If WMV is present BT-2 is susceptible with stunted plants and chlorotic leaves, in contrast BT-1 is highly resistant.

Index of Genotype Responses to Viral Infections

Diagnostic Plant	BYMV	CYVV	WMV
BT-1	S _M	S _N	R
BT-2	S _M	S _N	S _N
B-21	R	S _N	R
Black Knight	S _M	R	NT
SP 17B	R	R	R

R= Resistant with no symptoms; SM = susceptible with systemic mosaic or mottling; SN = Susceptible with leaf distortion and apical necrosis; NT = not tested

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REPRODUCTION OF SOYBEAN CYST NEMATODE ON DRY BEAN CULTIVARS

S. POROMARTO AND B.D. NELSON, Department of Plant Pathology, North Dakota State University, Fargo, ND

INTRODUCTION

Soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe) was discovered in North Dakota in 2003 and is now established in two counties in the Red River Valley (RRV). It has also been found in Minnesota in the RRV. The North Dakota and northwestern Minnesota region is a large dry bean production area with about 324,000 ha in production. SCN has been known to reproduce on *Phaseolus* spp. since the 1950's where it was studied in Japan. Recent studies have shown SCN reproduces on *P. vulgaris* and that differences exist between bean classes and cultivars in the amount of reproduction (Melton et al., 1985; Smith and Young, 2003). Reproduction on dry bean cultivars in the ND/MN region has implications both for dry beans and soybean. These crops are grown in the same area and can be in the same rotations. Since SCN is a parasite on roots, this nematode may pose a new disease threat for the dry bean industry in the region. The objective of this research was to measure SCN reproduction on representative cultivars of dry bean classes grown in the ND/MN area.

MATERIALS AND METHODS

Twenty four dry bean cultivars representing four bean classes were used in this study: Pinto cultivars Winchester, Topaz, Remington, Rally, Othello, Maverick, GTS-900 and Buster; Navy cultivars Vista, Seahawk, Premiere, Norstar, Navigator, Mayflower, Ensign and Cirrus; Black cultivars Eclipse, Jaguer, Condor and T-39; and Kidney cultivars Red Hawk, Montcalm, Chinook and Cal Early. The susceptible soybean check was Lee74. Untreated seed was pre-germinated in rag dolls for 3-4 days. Conetainers (20.5 cm tall; 140 ml volume) filled with pasteurized river sand were placed in plastic pots and packed in with sand. The plastic pots were immersed in a water bath at 27 C. A 3 cm deep hole was made in the sand, a germinated seed was placed in the hole and 2000 SCN eggs (HG 0; ~race3) were added around the seed. Plants were grown under high-pressure sodium lights ($1,000\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) for 30 days in the greenhouse. There were 4 replications (one plant per replication) in a randomized complete block design. Pinto and Navy cultivars were each in separate experiments while Black and Kidney cultivars were tested together. Experiments were all repeated.

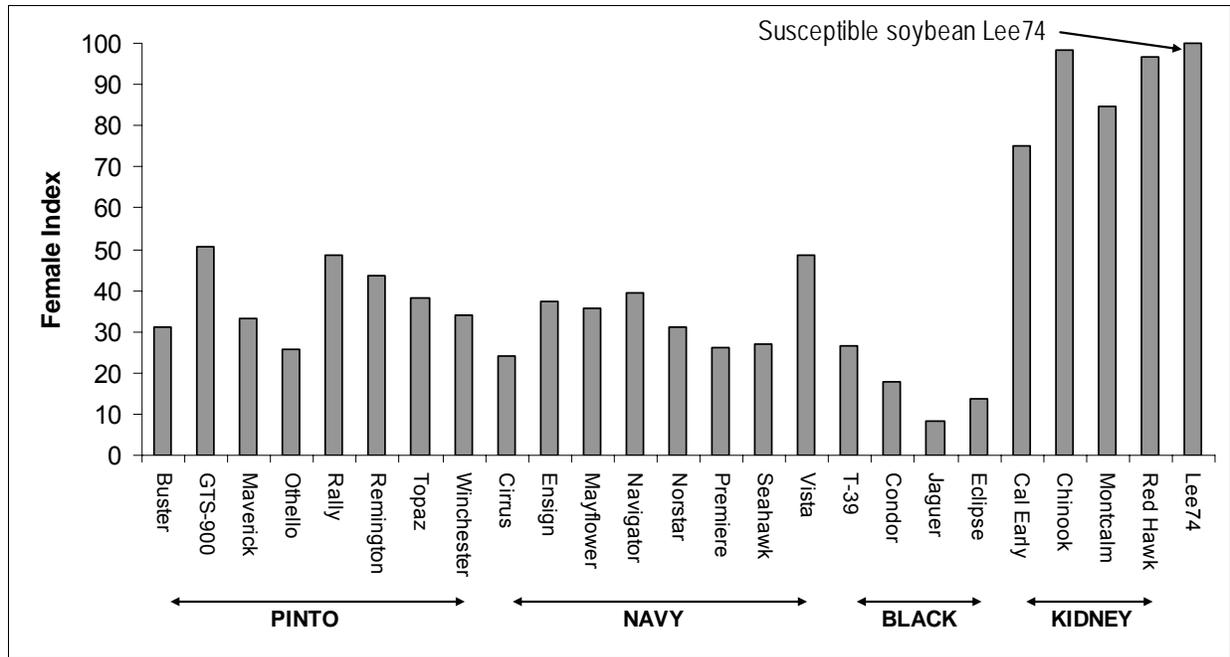
Plants were extracted from the sand and a hard stream of water passed over the roots to remove the females onto an 18 mesh screen nested over a 60 mesh screen. The sand was also washed through the sieves to remove females that had fallen off the roots. Females from each plant were then counted under a dissecting microscope and the mean number of females per plant for each cultivar determined.

RESULTS

SCN reproduced on all bean cultivars with kidney beans showing the greatest reproduction and black beans the least. The data is shown in Figure 1 as the Female Index (FI): $N_x/N_s \times 100$ where N_x is the average number of females on the test cultivar and N_s is the average number on the susceptible check Lee74. The levels of reproduction on these dry bean cultivars should concern the bean industry. Unfortunately, there has been minimal research on the effects of SCN on dry bean. There is only one report (Abawi and Jacobsen, 1984) that attempted to

measure the effects of SCN and that study was in the greenhouse with kidney bean. No effect was found on bean growth but the study was only run for 35 days, which we believe is insufficient time to adequately measure the effects on growth. In soybean, an FI of 31 or greater is considered moderately susceptible to susceptible (Niblack, 2005). Only eight of the twenty-four dry bean cultivars in our study had FI's less than 31. Research is needed to determine the effects of SCN on dry bean growth, yield and quality to know if SCN is a threat to bean production in the northern dry bean production areas.

Figure 1. Female index for reproduction of soybean cyst nematode on dry bean cultivars.



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VARIABILITY IN COLLETOTRICHUM LINDEMUTHIANUM FROM ECUADOR AND GUATEMALA

Halima Awale¹, Esteban Falconi², Julio Cesar Villatoro³ and James D. Kelly¹

¹Crop and Soil Sciences, Michigan State University, East Lansing MI 48824

²INIAP, Quito, Ecuador. ³ICTA, Chimaltenango, Guatemala

Nineteen isolates of *Colletotrichum lindemuthianum* collected primarily in growers' fields in Ecuador and Guatemala were characterized using the standard binary system based on 12 differential cultivars (Balardin et al., 1997). Fourteen races of anthracnose, six from Ecuador and eight from Guatemala were identified (Table 1) and only races 5 and 9 were found in both countries. Previous studies have identified races 3, 4, 256, 260 and 1346 (Falconi et al., 2003) and races 0, 1, 4, 5, 6, 9, 15, 65, 128, 129, 133, 256, 261 and 1153 in Ecuador (Muhuku et al. 2004). In Guatemala previously identified races included 9, 73, 1025, 1545, 1549, 1645 (Muhuku et al., 2004) and races 5, 585, 641, 1032, 1088, 1572, 1609, 1929 (Muhuku, pc). Often breeders are bewildered by the large number of anthracnose races and feel that individual resistance genes are needed to control individual races. The most effective strategy is to select those genes and/or alleles with the broadest resistance base. When assessing genetic variability of *C. lindemuthianum* illustrated by different races of the pathogen, it is important to group them by the number of similar/dissimilar virulence genes they possess. For example races 521 and 1545 seem quite distant based on their binary code, yet they differ for only one virulence gene, *Avr-Co-6* that defeats the AB-136 differential (1024). In this example the same gene can provide resistance to both races. In contrast races 2047 and 2048 appear similar but they differ for thirteen virulence genes *Avr-Co-1*, *I*^{2,3,4,5}, 2, 3, 4, 4³, 5, 6, 9, 11 in race 2047 and the three virulence genes *Avr-Co-4*², *Co-5*², *Co-7* present in race 2048. As a result very different gene combinations will be required to provide resistance to each race. To facilitate the selection of the most effective gene combinations in breeding for resistance, we suggest grouping races by the major virulence (*Avr-*) genes they possess to simplify the choice of the best resistance genes. Where multiple alleles exist at the *Co-1*, *Co-3*, *Co-4* and *Co-5* loci, breeders need to choose the most effective allele at that locus. In many cases the choice is easy, for example alleles *Co-1*², *Co-4*² are recognized for their broad resistance (Balardin et al., 1997), but alleles at the *Co-3/Co-9* and *Co-5* loci are less clear so the choice needs to be specific to the races present in the local region (Kelly and Vallejo, 2004; Muhuku et al., 2004). In addition, breeders need to consider all studies past and present on pathogen variability in their production region. As illustrated in the present study, the absence of previously reported races in this study may just be an escape in sampling and not necessarily the nonexistence of the race in the production area. The results of this study show that the two countries contrast in term of the variability among the races detected. A breeding strategy for Ecuador should include the deployment of Mesoamerican genes into elite local cultivars. The best choice would be to pyramid *Co-1*² and *Co-4*² genes from different gene pools as neither gene was defeated in Ecuador. Despite the differences in pathogenic variability between countries, the choices in breeding for resistance in Guatemala are similar to the recommendations for Ecuador. All races from Guatemala identified in this study were virulent to Mesoamerican cultivars that carry the *Co-2*, *Co-5* and *Co-6* genes. The same *Co-1*² and *Co-4*² gene combination provides resistance to most races with the exception of races 1572, and 1645. Since breeders need to make a choice among the different alleles at the Andean *Co-1* locus, the combination of *Co-1* and *Co-4*² provides resistance to the two races 1572 and

1645. This most effective gene pyramid combines the *Co-1²* and *Co-4²* genes and is available in advanced black bean lines from the MSU breeding program.

Table1. Reactions of common bean differential cultivars to 19 races of *Colletotrichum lindemuthianum* from Ecuador and Guatemala

Ecuadorian Isolates	Race^a	A^b (1)	B (2)	C (4)	D (8)	E (16)	F (32)	G (64)	H (128)	I (256)	J (512)	K (1024)	L (2048)
EC 1	1031	+	+	+	-	-	-	-	-	-	-	+	-
EC 2	4 ^{cd}	-	-	+	-	-	-	-	-	-	-	-	-
EC 3	4 ^{cd}	-	-	+	-	-	-	-	-	-	-	-	-
EC 4	13	+	-	+	+	-	-	-	-	-	-	-	-
EC 5	7	+	+	+	-	-	-	-	-	-	-	-	-
EC 6	5 ^d	+	-	+	-	-	-	-	-	-	-	-	-
EC 7	12	-	-	+	+	-	-	-	-	-	-	-	-

Guatemalan Isolates	Race^a	A^b (1)	B (2)	C (4)	D (8)	E (16)	F (32)	G (64)	H (128)	I (256)	J (512)	K (1024)	L (2048)
GU 1	520	-	-	-	+	-	-	-	-	-	+	-	-
GU 2	1024	-	-	-	-	-	-	-	-	-	-	+	-
GU 3	1024	-	-	-	-	-	-	-	-	-	-	+	-
GU 4	1097	+	-	-	+	-	-	+	-	-	-	+	-
GU 5	9 ^d	+	-	-	+	-	-	-	-	-	-	-	-
GU 6	1024	-	-	-	-	-	-	-	-	-	-	+	-
GU 7	1024	-	-	-	-	-	-	-	-	-	-	+	-
GU 8	1545 ^d	+	-	-	+	-	-	-	-	-	+	+	-
GU 9	521	+	-	-	+	-	-	-	-	-	+	-	-
GU 10	520	-	-	-	+	-	-	-	-	-	+	-	-
GU 11	1544	-	-	-	+	-	-	-	-	-	+	+	-
GU 12	648	-	-	-	+	-	-	-	+	-	+	-	-

^a Designation of races based on the binary nomenclature system. Sum of + binary numbers = race number

^b Common bean differentials used to identify races of *C. lindemuthianum* followed by the binary number (), and the *Co*-resistance genes present in each cultivar: A= Michilite,(1), *Co-11*; B = Michigan Dark Red Kidney, (2), *Co-1*; C = Perry Marrow,(4), *Co-1³*; D = Cornell 49242, (8), *Co-2*; E = Widusa, (16), *Co-1⁵*; F = Kaboon, (32), *Co-1²*; G = Mexico 222, (64), *Co-3*; H = PI 207262, (128), *Co-4³* and *Co-9*; I = TO, (256), *Co-4*; J = TU, (512), *Co-5*; K = AB136, (1024), *Co-6*; L = G2333, (2048), *Co-4²* and *Co-5²*, and *Co-7*. ^c Previously reported by Falconi et al., (2003).

^d Previously reported by Muhuku et al. (2004); ^e Previously reported by Muhuku personal communication

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INHERITANCE OF RACE-SPECIFIC RESISTANCE TO ANTHRACNOSE IN THE DIFFERENTIAL CULTIVAR AB136

Campa A.¹, E. Pérez Vega¹, R. Giraldez², J.J. Ferreira¹

¹ Área de Cultivos Hortofrutícolas, SERIDA, Villaviciosa, Asturias, Spain ² Área de Genética, Dpto. Biología Funcional, University of Oviedo, Spain

Anthracoze, caused by fungus *Colletotrichum lindemuthianum* (Sacc. and Magn.) Scrib., is one of the most important diseases of common bean (*Phaseolus vulgaris* L.). This fungus shows numerous pathogenic variants or races (Mahuku and Riascos, 2004). A set of twelve differential cultivars of common bean (Pastor-Corrales, 1991) is currently used to identify the pathogenic variants. AB136 is one of these twelve differential cultivars. Two independent genes have been reported in this cultivar (see Kelly and Vallejo, 2004): the dominant resistance gene *Co-6*, indirectly located in B7 linkage group using the OPZ04₅₆₀ marker, and the recessive *co-8* gene. The objective of this work was to investigate the inheritance of resistance to four races of anthracnose present in AB136.

Resistance to races 81, 357, 449 and 453 was independently evaluated on a total of 74 F_{2:3} families (at least 16 plants per F_{2:3} family) obtained from the cross AB136 x Michelite. The anthracnose evaluations were carried out according to standard methods (Pastor Corrales *et al.*, 1994). In order to determine the position of the resistance genes, the segregations of several markers located on linkage group B7 (SCARs SZ04 and SCARZ20, microsatellites BM185 and BM210 and the gene P_p) were analyzed. Amplifications were performed according to the instructions of the respective authors (Queiroz *et al.*, 2004; Blair *et al.*, 2003). Linkage analysis was carried out with the aid of JOINMAP V3.0 (van Ooijen and Voorrips, 2001) using a minimum LOD score of 3.0.

Table 1 shows the observed segregations for the resistance to races 81, 357, 449 and 453 in the F₂ population AB136 x Michelite. Segregations for resistance to races 81 and 449 fitted the expected ratio for single dominant genes (1R: 2H: 1S). Segregations for resistance to races 357 and 453 fitted the expected ratio for two independent dominant genes (7R: 8H: 1S).

Table 1. Segregation analysis for resistance to races 81, 357, 449 and 453 in the F₂ population AB136 x Michelite. R = F_{2:3} families with all plants resistant; H = F_{2:3} families showing resistant and susceptible plants; S= F_{2:3} families with all plants susceptible.

Race	Parents		Observed ratio				Expected ratio		χ^2	p
	AB136	Michelite	R	H	S	total	R : H : S			
81	R	S	19	45	10	74	1:2:1	5.65	0.06	
357	R	S	28	35	7	70	7:8:1	1.80	0.41	
449	R	S	15	43	16	74	1:2:1	1.97	0.37	
453	R	S	38	29	3	70	7:8:1	3.24	0.20	

The linkage analysis (Figure 1) indicated that the gene conferring specific resistance to race 81 is linked at a distance of 6.6 cM from the gene conferring specific resistance to race 449, on the B7 linkage group.

Resistance to races 357 and 453 are determined by two dominant genes and cannot be directly mapped. Contingency tests were carried out to determine whether the segregations for resistance to races 357 and 453 were independent of that for resistance to race 449. The corresponding chi square values (races 449-357: $\chi^2=28.87$, 0.01 >p; races 449-453: $\chi^2=15.96$; p< 0.05) indicate that segregations are not independent, suggesting that one of the genes conferring specific resistance to race 357 and one of the genes conferring specific resistance to race 453, are located in the region including the genes for resistance to races 81 and 449.

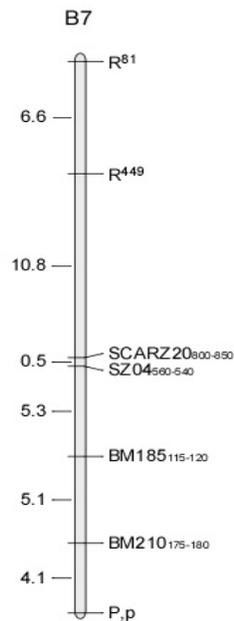


Figure 1: Relative positions of the genes conferring resistance to races 81 and 449, the molecular markers SCARZ20, SZ04, BM185 and BM210, and the P,p gene, within linkage group B7. Map distances are expressed in centimorgans (Kosambi map function).

The markers SCARZ20 and SZ04 have been described as linked to *Co-6* anthracnose resistance gene (Queiroz *et al.*, 2004). The present results agree with the existence of a cluster of anthracnose race-specific resistance genes, located in B7, that would correspond to gene *Co-6*. This situation is also in agreement with the organization in clusters recently proposed for *Co-2* and *Co-3/Co-9* anthracnose resistance genes (Rodríguez-Suárez *et al.* 2007). On the other hand, the present results indicate that AB136 carries at least a second dominant gene (or cluster), independent from *Co-6*, conferring resistance to races 357 and 453.

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ANTHRACNOSE RESISTANCE IN STEM AND LEAVES OF COMMON BEAN IS CONFERRED BY ASSOCIATION OF DOMINANT AND RECESSIVE GENES

Ana Lilia Alzate-Marin^{*1,2}, Ivan Schuster³, Maurilio Alves Moreira^{1,4} and Everaldo Gonçalves de Barros^{1,5}.

¹Instituto de Biotecnologia Aplicada à Agropecuária - BIOAGRO, Universidade Federal de Viçosa (UFV), 36570-000 Viçosa, MG, Brazil, ²Departamento de Genética, Universidade de São Paulo (USP), Ribeirão Preto, SP, Brazil, ³COODETEC, Caixa Postal 301, CEP 85.818-660, Cascavel-PR, ⁴Departamento de Bioquímica e Biologia Molecular, UFV, Viçosa, MG, Brazil, ⁵Departamento de Biologia Geral, UFV, Viçosa, MG, Brazil.

*Corresponding author: anaalzate@rge.fmrp.usp.br

Anthracoze, caused by the ascomycete *Colletotrichum lindemuthianum* (Sacc. & Magn.) Lams-Scrib., is one of the most important diseases of the common bean (*Phaseolus vulgaris* L.) in Brazil and in other bean growing regions of the world. Typical symptoms of common bean anthracnose are observed in the leaf and in pods. However, if the environment favors the development of fungus, injuries in the stem can also be observed. This may weaken the stem and impair its capacity to support the plant. Differential symptoms in leaves and stem were observed in segregating populations derived from backcrosses involving common bean Brazilian cultivar AN 910408 (Carioca x [carioca (Rio Tibagi x Guanajuato 31)]), resistant to races 64, 67, 73 and 83 of *C. lindemuthianum*, with susceptible recurrent parent cultivar Rudá. This led to the hypothesis that different genes might be involved in resistance in leaves and stem. Thus, this work aimed to study the genetic mechanisms of the resistance in the leaf and stem in segregating populations from backcrosses involving resistant cultivar AN 910408 and susceptible cultivar Rudá.

MATERIAL AND METHODS. BC₄F₂ plants derived from crosses between cultivars Rudá (recurrent) and AN 910408 (donor) were used. These crosses are part of a common bean backcross breeding program assisted by molecular markers conducted at the Universidade Federal de Viçosa, Viçosa, MG, Brazil. One hundred and sixty BC₄F₂ plants and twelve plants of each genitor were sown in the greenhouse. Fourteen days after sowing the first expanded trifoliate leaf of each plant was inoculated with spore suspensions of *C. lindemuthianum* race 83 (1.2 x 10⁶ spores/ml). Spore suspensions were applied with a horse-hair paint brush according to Pio-Rivero and Chaves (1975). The plants were then incubated for seven days in a mist chamber, which was maintained at 20 - 22 °C and 100% relative humidity. After this period, each plant was scored visually for disease symptoms using a 1 - 9 scale (Rava et al., 1993) in which 1 (one) is attributed to plants with no visible symptoms and 9 (nine) to severely diseased or dead plants. For evaluation of anthracnose symptoms in the stem, plants with no symptoms or with very small dark brown lesions were evaluated as resistant. Plants with severe symptoms showing depressed and obscure cankers leading or not to stem breakage were considered susceptible. The observed values of resistant and susceptible plants were compared with the expected values, for each tested hypothesis, through the Chi-square test.

RESULTS AND DISCUSSION. Previous inheritance studies showed that AN 910408 possesses one resistance gene to *C. lindemuthianum* race 73 (Paula Jr. et al., 1997). Our results indicate that two genes which interact epistatically, one dominant and one recessive, are involved in the genetic control of leaf anthracnose resistance when this cultivar was inoculated with *C.*

lindemuthianum race 83 (Table 1). As for stem anthracnose resistance, two genes also epistatic, one dominant and one recessive, explain the resistance to *C. lindemuthianum* race 83 (Table 1). The combined analysis of anthracnose symptoms in leaves and stems in a BC₄F₂ population (Rudá x AN 910408) showed that 21 plants did not present any symptoms in leaves or stems, whereas 11 showed symptoms only in stems, 3 showed symptoms only in leaves, and 125 showed symptoms in both organs (Table 2). Although these two characteristics present the same genetic control when analyzed separately (segregation 3:13, Table 1), the hypothesis that both are controlled by the same genes was rejected, because there were individuals that presented leaf resistance but stem susceptibility, and the opposite was also true. Our analyses indicate that the recessive gene is the same for leaf and stem resistance, however, the dominant genes are distinct and independent from each other (Hypothesis 2 - Table 2). These resistance genes could be part of a complex cluster that confers resistance to other races of *C. lindemuthianum* in cultivar AN 910408.

Table 1 – Separate analyses for common bean leaf and stem anthracnose resistance to *C. lindemuthianum* race 83

Hypothesis ^a	Population	Organ	Observed ratio		Expected ratio		Expected Frequency		χ^2 ^b	Probability
			R	S	R	S	R	S		
3:13 (R:S)	Rudá x AN 910408	Leaf	32	128	30	130	3/16	13/16	0.106	68.54
3:13 (R:S)	Rudá x AN 910408	Stem	24	136	30	130	3/16	13/16	1.477	22.42

^a3:13 = Resistance is only conferred when one dominant and one recessive genes are present

^b Chi-square value (χ^2)

Table 2 – Hypothesis test for combined inheritance of common bean leaf and stem anthracnose resistance in cultivar AN 910408

			Hypothesis 1 ^a				Hypothesis 2 ^b			
Reaction		Observed Number	Expected frequency	Expected number	χ^2 ^c	P ^d	Expected frequency	Expected number	χ^2 ^c	P ^d
Leaf	Stem									
S ^e	S	125	43/64	107.5			49/64	122.5		
R ^f	S	11	9/64	22.5	49.93	0%	3/64	7.5	4.48	21.37%
S	R	3	9/64	22.5			3/64	7.5		
R	R	21	3/64	7.5			9/64	22.5		

^a Hypothesis 1: Anthracnose resistance in leaf and stem is given by A_ *bbcc*; leaf resistance is given by A_ *bb* and stem resistance by A_ *cc*.

^b Hypothesis 2: Anthracnose resistance in leaf and stem is given by A_ *bb C_*; leaf resistance is given by A_ *bb* and stem resistance by *bb C_*.

^c Chi-square value (χ^2); ^d Probability; ^e Resistant, ^f Susceptible

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TRANSFER OF ANGULAR LEAF SPOT RESISTANCE GENE FROM ‘CORNELL 49-242’ TO THE CARIOCA CULTIVAR RUDÁ

Jeziel D. Damasceno¹, Demerson A. Sanglard¹, Bruno P. Balbi¹, Everaldo G. de Barros^{1,2} and Maurilio A. Moreira^{1,3*}

¹Instituto de Biotecnologia Aplicada à Agropecuária (BIOAGRO), Universidade Federal de Viçosa (UFV), 36571-000, Viçosa, MG, Brazil. ² Departamento de Biologia Geral. ³ Departamento de Bioquímica e Biologia Molecular.

*Corresponding author: moreira@ufv.br

Angular leaf spot occurs in almost all regions where beans are cultivated, especially under mild temperature conditions and high relative humidity. The use of susceptible cultivars in such environmental conditions favors the occurrence of the disease, thus leading to great yield losses. The development of new cultivars resistant to this disease is hindered by the pathogenic variability of its causative agent, *Phaeoisariopsis griseola* (SARTORATO and ALZATE-MARIN, 2004; SARTORATO, 2006). The BIOAGRO/UFV Bean Breeding Program has been using the backcrossing method for the introgression of resistance genes to this disease. Simultaneous backcrossing programs are conducted for each resistance source. Allelism studies carried out by CAIXETA et al. (2005) detected one single gene named *Phg-3* in cultivar Cornell 49-242, confirming the results obtained by NIETSCHKE et al. (2000). The resistance conferred by this gene is complementary to those of other sources that are presently being used in the BIOAGRO/UFV Bean Breeding Program. So, its use would increase the resistance spectrum of the cultivars released by the Program.

Aiming to transfer the angular leaf spot resistance gene from ‘Cornell 49-242’ to the carioca cultivar Rudá, three cycles of backcrossing were conducted. During this process, inoculations with the pathogen, molecular marker and fingerprinting analyses were used. Crossings between cultivars Cornell 49-242 (donor parent) and Rudá (recurrent parent) were carried out, and 32 F₁ seeds were obtained. They were planted in the greenhouse and the corresponding F₁ plants were used as pollen donors in the crossing with the recurrent parent Rudá. Fifty-one BC₁F₁ plants were obtained, which were inoculated with *P. griseola* race 63.23. The gene *Phg-3* present in ‘Cornell 49-242’ confers resistance to this race. Twenty-one resistant BC₁F₁ plants were identified and submitted to molecular fingerprinting analysis, aiming to identify those with the largest recovery of the recurrent parent genome. For this analysis, 14 randomly chosen RAPD primers led to the amplification of 41 monomorphic and 39 polymorphic bands. From these data, a genetic similarity matrix was generated. The genetic similarity of the BC₁F₁ individuals in relation to cultivar Rudá varied from 56% to 79%. Three plants with 79% genetic similarity in relation to ‘Rudá’ were selected to originate the BC₂F₁ population (Figure 1). BC₂F₁ plants resistant to *P. griseola* race 63.23 were crossed with Rudá “R”, a bean isolate which already harbors five resistance genes: one for rust (*Ur-ON*), one for angular leaf spot (*Phg-1*) and the other three for anthracnose (*Co-4*, *Co-10*, and *Co-6*) (RAGAGNIN et al., 2005).

The F₁ plants were selfed and different molecular markers linked to the genes of interest, including marker SCAR N02_{950a}, linked to *Phg-3* (3.2 cM), will be used to assist the pyramiding process.

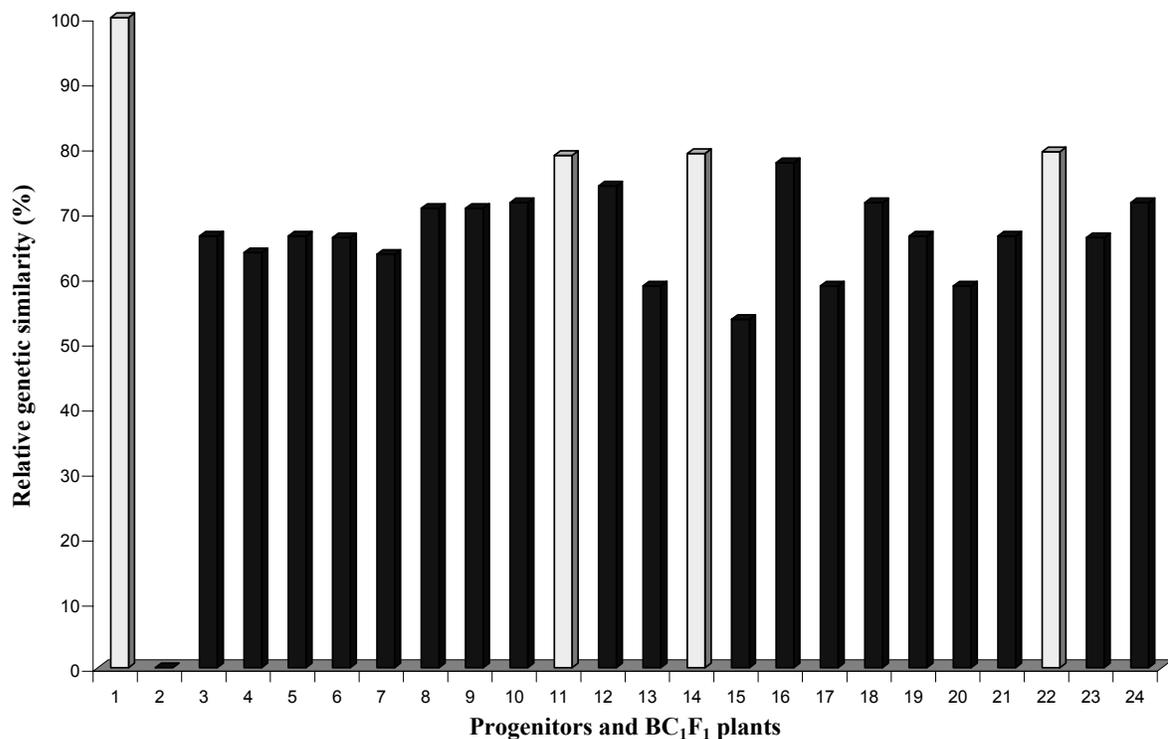


Figure 1. Relative genetic similarity (%) between the resistant parent Cornell 49-242 (2) and BC₁F₁ plants (3-24) in relation to the recurrent parent Rudá (1). Plants 11, 14 and 22 were genetically closest to the recurrent parent Rudá.

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GENETIC DIVERSITY IN PHAEOSARIOPSIS GRISEOLA BASED ON ISSR MOLECULAR MARKERS

Geraldina E. Fermoselle , Sebastian A. Stenglein y Pedro A. Balatti.

Instituto de Fisiología Vegetal. Facultad de Ciencias Agrarias y Forestales. Universidad Nacional de la Plata, Argentina

Angular leaf spot is a disease of bean (*Phaseolus vulgaris*) caused by the fungus *Phaeoisariopsis griseola* (Sacc.) Ferraris which occurs in tropical and subtropical cultivated areas around the world, causing important economic losses in Latin America where 20% of the world beans are produced. In Argentina, beans are mainly grown in the northwestern provinces of Salta, Tucumán and Jujuy, where the disease can provoke losses as high as 80%.

Pastor-Corrales and Jara (1995) defined within the pathogen two major groups: Andean and Mesoamerican. According to these authors, these two groups might have co-evolved with Andean and Mesoamerican beans, respectively. Pastor Corrales et al. (1998), Busogoro et al. (1999), Stenglein and Balatti (2006) identified variability between isolates of *P. griseola* not only at DNA level but also in the interaction with bean cultivars. Genetic diversity of the pathogen, *P. griseola* is crucial to develop a breeding program aimed at obtaining common bean cultivars tolerant to angular leaf spot.

In this study, we continued our analysis of diversity both on a larger collection and also on several isolates from a confined area, to understand how diversity arises within representative of this pathogen.

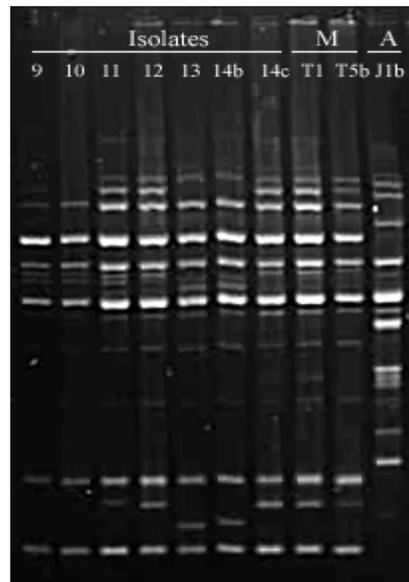
Seven isolates were obtained from bean leaves collected in a farmer's field in Zarate, province of Tucumán. ISSR markers have been used successfully to detect genetic variation in fungi and plant (Meng and Chen 2001). Di, tri and tetra-nucleotide anchored or non-anchored ISSR primers have been used to fingerprint the genome of fungal isolates. Among fourteen primers only five generated polymorphic fingerprint (Table 1). A total of 44 bands were generated. All the isolates showed amplification patterns similar to the Mesoamerican control and 18% of the amplified bands were found to be polymorphic within Mesoamerican isolates (Figure 1)

Analyzing 72 isolates from different producer areas (22°-28° S latitude and 62°-68° W longitude) we found diversity between Andean and Mesoamerican isolates and also within both groups. Polymorphic bands were generated with the ISSR primers tested. From a total of 155 amplified bands, 85% were polymorphic. We are currently examining a larger number of ISSR primers to support these results.

Table 1. ISSR polymorphic primer sequences used for the analysis of *Phaeoisariopsis griseola* with primer annealing temperature, number of bands amplified and number of polymorphic bands amplified.

Name	Primer sequence	Annealing temp °C	Number of bands amplified	Number of polymorphic bands
AA5	CAG(AAC) ₅	48°C	7	2
JA5	TA(AG) ₈	48°C	7	1
IA5	ACA(CAA) ₅	48°C	12	2
AN	(CAA) ₅	48°C	10	2
G	GAG(GAA) ₅	48°C	8	1

Figure 1. Amplification patterns of Zárate isolates, with Mesoamerican and Andean isolates of *P. griseola* used as control.



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VIRULENCE OF 6 ISOLATES OF PHAEOSARIOPSIS GRISEOLA (SACC) [FERR] UPON 41 COMMON BEAN LANDRACES IN TANZANIA

N.C. Fivawo and S. N. Msolla

Crop Science department, Sokoine University of Agriculture

P.O. Box 3005 Morogoro Tanzania

Introduction Angular leaf spot disease caused by *Phaeoisariopsis griseola* (Sacc)[Ferr] is the most devastating disease of common bean in Tanzania (Hillocks et al 2006). *P. griseola* is a genetically variable pathogen, however use of genetically resistant common bean cultivars can control angular leaf spot (ALS) disease in common bean (*Phaseolus vulgaris* L) effectively. Evaluation of the common bean germplasm for ALS disease resistance is essential when searching for new sources of resistance to angular leaf spot disease. The objective was to screen the 41 common bean landraces and 4 resistant cultivars from CIAT against 6 *P. griseola* isolates prior to breeding for improved yield and angular leaf spot disease resistance.

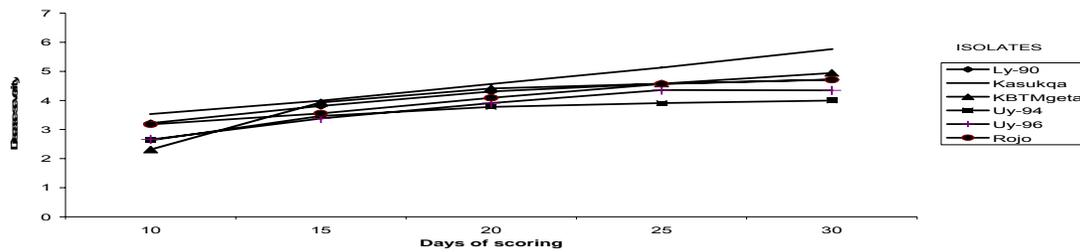
Materials and Methods Forty-one common bean landraces from the highland, eastern and northern zones of Tanzania and 4 angular leaf spot resistant cultivars from CIAT were screened against 6 *Phaeoisariopsis griseola* isolates. The isolates were designated as 59/23, from the northern zone, 59/67, 59/74 and 59/35 from the highland zone and 59/65 & 59/87 from the eastern zone. Twelve day-old common bean seedlings with fully developed first trifoliolate leaves were inoculated with conidia suspension for each of the 6 *P. griseola* isolates using a hand sprayer. Conidia concentration was adjusted to 2×10^4 ml using a haemocytometer. The inoculated plants were incubated in green house for 72 hours at >95% relative humidity and 22°C temperature, they remained in the green house until angular leaf spot symptoms were established. Then the plants were evaluated for their disease reaction using the CIAT rating scale of 1-9 five times starting from 10 days after inoculation then at interval of 5 days until at thirty days after inoculation.

Results and Discussion The overall mean virulence of the 6 isolates upon the 45 cultivars was 4.5 (CIAT scale 1-9), generally the 6 isolates exhibited different virulence pattern when inoculated on the 45 cultivars. Isolate 59/67 gave the most severe disease reaction on average followed by isolate 59/35. Some of the isolates collected from the same location showed differences in their pattern of virulence e.g. isolate 59/67, 59/74 and 59/35 collected from Uyole (Table 1). Results of disease evaluation five times at 10, 15, 20, 25, 30 days after inoculation showed that there was significant ($P=0.05$) increase in disease severity as days advanced from the day of inoculation (Figure 1). Among the 45 common bean genotypes tested against the 6 *P. griseola* isolates, only landraces, Nkanamna, Masukado, Beti-10, Nanka, Gonka and Nanavala showed good levels of resistance to all the 6 isolates in addition to the 4 introduced resistant cultivars, G 5686, Amendoim, BAT 332 and Mexico 54 (Table 1). This study has identified some landraces that can possibly be used as sources of resistance and suggests that there is a need to advance search for resistance sources to angular leaf spot among landrace cultivars in Tanzania. In addition, the virulence of the 6 isolates has been determined which is an important knowledge in developing bean cultivars with more durable ALS resistance.

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Mean ALS disease development with advancing days



Mean scores of 45 cultivars screened against 6 *P. griseola* isolates (CIAT scale of 1-9).

Cultivar	59/23	59/67	59/65	59/74	59/35	59/87	Means
1 Ly-90	4	6	5	4	6	5	4.6
2 B/Shamba	4	7	6	7	7	7	5.8
3 Uyole 94	5	4	6	3	5	5	4.6
4 Nyayo	5	6	7	6	6	5	5.8
5 Salunde	6	7	6	7	6	6	6.3
6 Jesca	6	7	6	7	6	5	6.1
7 G 5686	1	2	1	1	2	1	1.3
8 N/mhanga	7	6	7	5	7	7	5.1
9 Spenjeli	6	6	6	4	5	5	5.3
10 C/wonder	7	6	6	6	7	5	6.1
11 Msafiri	7	6	7	5	6	7	6.3
12 Wanja	6	4	5	4	5	6	5.0
13 Uyole 98	6	5	6	4	4	5	5.0
14 Kasuka	7	5	7	6	5	7	6.1
15 Amendoim	1	2	1	1	2	1	1.3
16 Uyole 96	5	5	4	4	4	4	4.3
17 Sua 90	5	6	4	3	5	4	4.8
18 Yangi	6	5	5	4	5	5	5.0
19 Masusu	7	6	6	5	7	6	6.1
20 Rojo	5	6	4	5	5	3	4.6
21 kombati	6	6	5	4	5	5	5.1
22 KBT imp	6	6	4	5	5	4	5.0
23 Ilomba	2	3	2	2	3	2	2.3
24 Kabanima	5	5	6	4	3	5	4.6
25 Selian 94	6	7	6	6	6	4	5.8
26 KBT Arusha	7	7	7	7	7	7	7.0
27 Karanga	3	7	5	6	7	6	5.6
28 KBT Mgeta	7	7	6	6	7	7	6.6
29 Maini	6	6	6	7	7	7	6.5
30 Chipukupuku	6	6	5	5	4	5	5.1
31 BAT 332	1	2	1	1	2	1	1.3
32 Nanavala	3	3	3	3	2	3	2.8
33 Ly 85	4	6	4	5	4	5	4.6
34 KBT L.U.	7	6	7	7	6	7	6.6
35 Fimwitu	4	4	4	5	5	7	4.3
36 Kibwebwe	3	4	3	2	5	3	3.3
37 Beti 10	2	3	2	2	2	3	2.3
38 Gonka	2	3	3	3	3	3	2.8
39 Mexico 54	1	2	1	1	2	2	1.5
40 Mkeredu	3	4	3	3	3	3	3.1
41 Nanka	2	2	3	3	3	3	2.7
42 Selian 97	3	6	5	4	4	4	4.3
43 Nkanamna	1	2	2	2	2	2	1.8
4 Kichumba	2	4	3	3	4	5	3.5
45 KBT Iringa	7	7	7	7	7	5	6.3
Mean	4.6	5.1	4.4	4.4	4.8	4.6	4.6
SE=0.05	0.23	0.61	0.22	0.38	0.67	0.93	
CV%	10.48	15.40	10.01	14.04	17.17	21.10	

IMPACT OF ANGULAR LEAF SPOT ON GRAIN YIELD OF COMMON BEAN LINES

Magno Antonio Patto Ramalho¹, Ângela de Fátima Barbosa Abreu², João Bosco dos Santos¹

¹Universidade Federal de Lavras, Lavras, MG, P. O. Box 3037, e-mail: magnoapr@ufla.br; jbsantos@ufla.br; ²Embrapa Arroz e Feijão/Universidade Federal de Lavras, Lavras, MG, P. O. Box 3037, e-mail: afbabreu@ufla.br

Our objective was to estimate the effect of angular leaf spot in 36 common bean lines evaluated in 15 experiments in the state of Minas Gerais, Brazil, in 2005 and 2006. The disease severity was evaluated using a 1 to 9 score scale, where 1 indicates no symptoms and 9 totally infected plants. After harvest the grain yield was estimated in kg/ha. Joint analyses for grain yield were performed considering all environments or only the environments with occurrence of angular leaf spot or those where the disease was not found.

In six of the fifteen environments, the occurrence of *P. griseola* was more severe and the lines differed in susceptibility. In these environments the estimates of the linear regression coefficient and the correlation between pathogen severity scores and grain yield were different from zero and negative, indicating that the stronger the disease severity, the greater the grain yield loss. In the mean of the six environments, the grain yield reduced 130.9 kg/ha for the increase of each unit of disease severity, which explained 52% of the grain yield variation. This confirmed that the pathogen can cause considerable yield losses, as mentioned above (Jesus Júnior et al., 2001).

However, the objective was to verify the effect of angular leaf spot severity in the identification of lines to be selected for VCU trials. Considering the mean of all environments, three groups were formed. Eight lines were classified as the most productive (Table 1). In the environments infected with angular leaf spot, four groups were formed and seven lines presented high performance. Of these seven, six ranked among the best in the overall evaluation. In the absence of the pathogen three groups were formed, but 18 lines were classified among those of best performance. It was concluded that the pathogen occurrence allowed a clearer discrimination and a greater safety for better line selection.

The lines with the abbreviation MA were part of a recurrent selection program targeting stronger *P. griseola* resistance; they generally confirmed the success of the ongoing selection (Amaro et al., 2007). The lines with the initials CVII had been taken from a recurrent selection program for grain yield. The *P. griseola* resistance was mostly weak. In the absence of the pathogen several lines were classified in the most productive group. The lines designated BP and RP were selected for best plant architecture and presented weak pathogen resistance. The others were commercial controls.

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Table 1. Mean score of angular leaf spot severity (1 to 9) and grain yield (kg/ha) of the lines in environments with and without disease, and in the mean of all environments.

Lines	Angular leaf spot	Grain yield		
		With disease	Without disease	Mean
Ouro Negro	2.9	2535 A	2421 A	2467 A
MAII-2	2.3	2659 A	2268 A	2424 A
MAII-16	3.0	2460 A	2252 A	2336 A
MAII-8	2.7	2402 A	2246 A	2308 A
MAII-22	2.0	2532 A	2136 A	2295 A
MAII-14	2.6	2332 B	2264 A	2292 A
MAII-5	2.2	2390 A	2211 A	2283 A
CVII-16	4.6	2278 B	2251 A	2262 A
MAII-10	2.8	2225 B	2247 A	2238 B
CVII-119-4	3.9	2080 C	2309 A	2217 B
RP-2	4.4	2079 C	2292 A	2207 B
MAII-3	2.5	2415 A	2063 B	2204 B
CVII-85-11	4.5	2138 C	2236 A	2197 B
CVII-45-5	4.8	2079 C	2225 A	2167 B
CVII-215-10	3.8	2209 B	2127 A	2160 B
RP-1	3.4	2240 B	2102 B	2157 B
BP-31	6.8	1952 C	2267 A	2141 B
RP-5	2.6	2242 B	2060 B	2133 B
CVII-85-11	5.0	2025 C	2183 A	2120 B
CVII-55-3	4.1	2035 C	2134 A	2094 C
CVII-39-18	5.6	1913 C	2156 A	2059 C
CVII-55-14	3.8	1987 C	2090 B	2049 C
BP-28	5.7	1964 C	2061 B	2022 C
BP-30	5.2	1949 C	2050 B	2009 C
Carioca MG	6.2	1942 C	2043 B	2003 C
Pérola	4.2	1962 C	2006 B	1988 C
BP-24	6.4	1837 D	2085 B	1986 C
RP-4	3.8	2059 C	1919 C	1975 C
BP-16	5.0	1921 C	2004 B	1971 C
BP-34	5.4	1792 D	2078 B	1964 C
CVII-85-17	3.8	2120 C	1818 C	1938 C
MAN-1	4.2	1805 D	2015 B	1931 C
BRSMG Talismã	5.5	1963 C	1874 C	1910 C
MAII-17	2.0	1995 C	1775 C	1863 C
Carioca	5.9	1796 D	1896 C	1856 C
RP-3	4.1	1605 D	2022 B	1855 C
Mean	4.1	2109	2116	2113

¹Means followed by the same letter belong to the same group by the Scott-Knott test (1974), at the level of 5% probability.

INTENSITY OF ANGULAR LEAF SPOT AND ANTHRACNOSE AND ANTHRACNOSE TRANSMISSION BY SEEDS OF BEANS CULTIVATED IN THREE CROPPING SYSTEMS

Trazilbo J. de Paula Jr.¹, Rogério F. Vieira¹; Hudson Teixeira¹, Clibas Vieira²

¹Epamig, Vila Gianetti 47, Viçosa-MG, 36571-000 Brazil; ²*In memoriam*

Introduction – In the State of Minas Gerais, Brazil, common beans are cultivated in many cropping systems. In general, monocrop is the most common system. On small farms, beans are usually planted in association with maize and other crops. Monocrop of bean grown on trellises is made for climbing beans, especially for snap beans. It is not clear yet the influence of cropping systems on severity of angular leaf spot (ALS) caused by *Phaeoisariopsis griseola*. Normally, anthracnose (ANT), incited by *Colletotrichum lindemuthianum*, is more intense in monocrop than on bean intercropped with maize, but the difference among systems is not well quantified. The purpose of this research was to evaluate the intensity of ALS and ANT on pods and the transmission of ANT by seeds of beans cultivated in three cropping systems.

Material and Methods – Trials were carried out in the spring-summer, in Coimbra, MG, Brazil, using the climbing common beans Preto 1379, P.I. 282.063, P.I. 310.740, and Compuesto Negro Chimaltenango; the semiclimbing Ouro Negro and Pérola; and the bush bean Trujillo 2. They were cultivated as monocrop, intercrop with maize, and monocrop grown on trellises. In monocrop, beans were planted 0.5 m apart. In intercrop, beans were planted simultaneously with maize and in its line. This cereal was sown 1.0 m apart with 4 plants per meter. Trellises were set up with 1.8 m high bamboos and beans were sown 0.65 m apart. Twelve seeds per meter of beans were used. Three independent trials were installed close to each other. Pods with symptoms of ALS and ANT and number of lesions on pods of these diseases were obtained from 50 pods in maturation took from each plot. Seeds of the most susceptible cultivar to ANT were germinated in sand and in conditions of high humidity to evaluate % of seeds transmitting the fungus. Four replications of 100 seeds were used. Methodology details of the research on field and yields achieved can be found in Vieira et al. (2003). For data which distribution were not normal, means were transformed by $(x + 0.5)^{1/2}$ before analysis of variance.

Results and Discussion – In monocrop, Pérola had 46 % of the pods with symptoms of ANT, 2.58 lesions per pod (Table 1), and 11 % of seeds transmitting the fungus (data not showed). In monocrop of beans grown on trellises, Pérola had 11.5 %, 1.34, and 9.1 %; when planted intercropped with maize, 5 %, 1.08, and 4.4 %, respectively. In general, the number of pods infected with ALS and ANT was lower when beans were intercropped with maize, compared with the other systems, especially with monocrop. In relation to number of lesion per pod the tendency was similar to that of number of pods infected. The lower bean population used in the intercrop with maize and in the monocrop grown on trellises, compared with monocrop, and the physical barrier of stems and leaves against fungi dispersion in the association bean-maize might help to explain these results.

Table 1 - Intensity of ALS and ANT on pods of bean cultivars in three cropping systems¹

Genotype	Monocrop	Monocrop grown on trellises	Intercrop with maize
	Number of pods infected with angular leaf spot		
Ouro Negro	41.0 a	30.5 ab	38.0 a
Pérola	9.0 c	14.7 c	10.0 d
Trujillo 2	28.2 b	34.2 ab	26.0 b
Preto 1379	29.2 b	27.5 ab	19.2 bc
P.I. 282.063	39.7 a	38.7 a	23.0 b
P.I. 310.740	25.0 b	24.0 bc	20.0 bc
C.N. Chimaltenango	11.2 c	15.0 c	11.2 cd
<i>Mean</i>	26.2	26.4	21.1
	Number of lesions per pod of angular leaf spot		
Ouro Negro	2.20 (4.34) a	1.95 (3.33) a	2.04 (3.72) a
Pérola	1.33 (1.26) c	1.41 (1.49) b	1.33 (1.27) c
Trujillo 2	1.66 (2.26) b	1.89 (3.10) a	1.54 (1.89) bc
Preto 1379	1.69 (2.39) b	1.55 (1.89) b	1.46 (1.62) c
P.I. 282.063	2.28 (4.73) a	1.94 (3.31) a	1.77 (2.68) ab
P.I. 310.740	1.62 (2.14) b	1.63 (2.16) b	1.50 (1.76) bc
C.N. Chimaltenango	1.39 (1.43) c	1.60 (2.08) b	1.63 (2.20) bc
<i>Mean</i>	1.74 (2.65)	1.71 (2.48)	1.61 (2.17)
	Number of pods infected with anthracnose		
Ouro Negro	0.71 (0.00) d	0.71 (0.00) c	0.71 (0.00) d
Pérola	4.82 (23.00) a	2.38 (5.75) a	1.70 (2.50) b
Trujillo 2	2.22 (4.75) b	1.81 (3.00) ab	2.26 (5.00) a
Preto 1379	1.10 (0.75) cd	0.93 (0.50) bc	1.05 (0.75) cd
P.I. 282.063	1.54 (2.00) c	1.91 (4.00) ab	1.48 (1.75) bc
P.I. 310.740	0.71 (0.00) d	0.97 (0.50) bc	1.40 (1.50) bc
C.N. Chimaltenango	0.71 (0.00) d	0.93 (0.50) bc	0.84 (0.25) d
<i>Mean</i>	1.68 (4.36)	1.38 (2.04)	1.35 (1.68)
	Number of lesions per pod of anthracnose		
Ouro Negro	0.71 (0.00) d	0.71 (0.00) c	0.71 (0.00) c
Pérola	1.75 (2.58) ab	1.35 (1.34) abc	1.26 (1.08) ab
Trujillo 2	1.39 (1.45) bc	1.79 (2.87) a	1.43 (1.57) a
Preto 1379	1.18 (1.00) c	0.88 (0.37) bc	0.97 (0.50) bc
P.I. 282.063	1.89 (3.31) a	1.52 (2.07) ab	1.56 (2.08) a
P.I. 310.740	0.71 (0.00) d	1.22 (1.25) abc	1.40 (1.50) a
C.N. Chimaltenango	0.71 (0.00) d	0.84 (0.25) bc	0.84 (0.25) c
<i>Mean</i>	1.19 (1.19)	1.19 (1.17)	1.17 (1.00)

¹ Numbers in parentheses refer to original data. Fifty pods per plot were evaluated. Average of four replications. Means followed by the same letter in a column are not significantly different (DMRT 5 %)

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INTROGRESSION OF ANGULAR LEAF SPOT RESISTANCE GENES IN COMMON BEAN ISOLINES

Demerson A. Sanglard¹, Jeziel D. Damasceno¹, Bruno P. Balbi¹, Everaldo G. de Barros^{1,2} and Maurilio A. Moreira^{1,3*}

¹Instituto de Biotecnologia Aplicada à Agropecuária (BIOAGRO), Universidade Federal de Viçosa (UFV), 36571-000, Viçosa, MG, Brazil. ²Departamento de Biologia Gerla. ³Departamento de Bioquímica e Biologia Molecular.

*Corresponding author: moreira@ufv.br

In Brazil, until the end of the 1980's, angular leaf spot caused by *Phaeoisariopsis griseola* was considered a disease of minor economical impact. According to VIEIRA (1998), the disease was not seen as an important one because it only occurred at the end of the culture cycle, with little effect on productivity. However, in the last years, angular leaf spot has been causing great losses, with severe defoliation in some cultivars. The existence of physiological races and the constant evolution of the pathogen demand a permanent search for new resistance sources. The pyramiding or association of resistance genes in the same cultivar has been proposed as a strategy to achieve long-term and broad spectrum resistance (KELLY et al., 2003). In the BIOAGRO/UFV Bean Breeding Program, carioca type isolines have been developed containing the following genes: *Ur-ON* and *Co-10*, which confer resistance to rust and anthracnose, respectively, from the Ouro Negro; *Co-4* and *Co-6*, which confer resistance to anthracnose, from the TO and AB 136, respectively; *Phg-1*, which confer resistance to angular leaf spot, from AND 277. RAGAGNIN et al. (2005) intercrossed these materials pyramiding these genes into four isolines which were named Rudá "R". Thus, aiming to introgress new angular leaf spot resistance genes into Rudá "R" crossings were carried out between this line and three other isolines derived from México 54, MAR-2 and BAT 332. These three isolines all had the carioca genetic background. Molecular markers linked to the resistance genes were used and inoculations were performed. According to allelism studies carried out by CAIXETA et al. (2005), and validation tests of the molecular markers using the isolines available until now, the possible and best combinations of genes were defined aiming to introgress the largest number possible of angular leaf spot resistance genes in the same background. Table 1 describes the isolines used in this work. Initially, the crossings were performed in two separate schemes, one starting with crosses between the lines MAR-138A-1-11-4 and BAT-67-15-8 and the other between MEX-37-3-6-3 and Rudá "R". In the first scheme, *P. griseola* race 63.19 was used to confirm the hybrid nature of the F₁ plants. DNA from resistant plants was extracted and amplified with molecular markers OPE04_{500a} and OPAO12_{950a} (Table 2). F₁ plants which presented the molecular markers were crossed with Rudá "R". In this way, F₁ plants (triple hybrid) were obtained. These plants were inoculated with *C. lindemuthianum* race 65 and monitored with molecular markers: SCAR F10_{1050a} (*Ur-ON* and *Co-10*), SCAR BA8_{560a} (*Ur-ON* and *Co-10*), SCAR AZ20_{845a} (*Co-4*), SCAR Y20_{830a} (*Co-6*), SCAR H13_{520a} (*Phg-1*), OPE04_{500a} (*Phg-4* and/or *Phg-5*²) and OPAO12_{950a} (*Phg-6*²) (Table 2). The F₂ generation segregated for all these loci, but plants bearing all the markers were obtained. In the second crossing scheme, F₁ plants (MEX-37-3-6-3 x Rudá "R") were selfed up to the F₃ generation. The resistance genes were also monitored by inoculation with *C. lindemuthianum* race 65 and the molecular markers mentioned above except that OPE04_{500a} and OPAO12_{950a} were replaced by OPE04_{650a} (*Phg-2* and/or *Phg-5* and/or *Phg-6*) (Table 2). Phenotypic evaluation of the lines obtained in both crossing schemes is underway. They will be used as a source of resistance genes in our breeding program and can also be released as new cultivars depending on their agronomical performance.

Table 1. Characteristics of angular leaf spot resistant isolines used in this work.

Isolines	Genealogy	Generation	Resistance Genes
MAR-138A-1-11-4*	Rudá/MAR-2	BC ₂ F _{2:6}	<i>Phg-4</i> and/or <i>Phg-5</i> ²
MEX-37-3-6-3*	Rudá/Mexico 54	BC ₂ F _{2:6}	<i>Phg-2</i> and/or <i>Phg-5</i> and/or <i>Phg-6</i>
BAT-67-15-8*	Rudá/BAT 332	BC ₃ F _{2:5}	<i>Phg-6</i> ²
Rudá "R"	Rudá/AND 277/TO/ AB 136/Ouro Negro	F ₇	<i>Phg-1</i> , <i>Co-4</i> , <i>Co-6</i> , <i>Co-10</i> and <i>Ur-ON</i>

* Isolines obtained by OLIVEIRA et al. (2005).

Table 2. Molecular markers linked to disease resistance genes in common bean.

Marker*	Distance [#] (cM)	Resistance Genes	Sources of Resistance	Reference
SCAR-Y20 _{830a}	1.20	<i>Co-4</i>	TO	QUEIROZ et al. (2004b)
SCAR-AZ20 _{845a}	7.10	<i>Co-6</i>	AB 136	QUEIROZ et al. (2004b)
SCAR-BA08 _{560a}	2.20	<i>Co-10</i> and <i>Ur-ON</i>	Ouro Negro	CORRÊA et al. (2000)
SCAR-F10 _{1050a}	6.50	<i>Co-10</i> and <i>Ur-ON</i>	Ouro Negro	CORRÊA et al. (2000)
SCAR-H13 _{520a}	5.60	<i>Phg-1</i>	AND 277	QUEIROZ et al. (2004a)
OPE04 _{500a}	5.80	<i>Phg-4</i> and/or <i>Phg-5</i> ²	MAR-2	FERREIRA et al. (2000)
OPE04 _{650a}	11.80	<i>Phg-2</i> and/or <i>Phg-5</i> and/or <i>Phg-6</i>	México 54	SARTORATO et al. (1999)
OPAO12 _{950a}	5.83	<i>Phg-6</i> ²	BAT 332	CAIXETA et al. (2003)

*a: coupling #cM: genetic distance (centiMorgan) of the molecular markers in relation to the resistance genes.

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COMMON BEAN GENOTYPES RESISTANT TO ANGULAR LEAF SPOT, RUST AND ANTHRACNOSE

Aloísio Sartorato

Embrapa Arroz e Feijão, P.O. Box 179, 75375-000 Goiania, GO, Brazil.

E-mail: sartorat@cnpaf.embrapa.br

Introduction

Angular leaf spot, anthracnose and rust, caused by the fungus *Phaeoisariopsis griseola*, *Colletotrichum lindemuthianum* and *Uromyces appendiculatus*, respectively, are the three most important air borne diseases of bean in Brazil. There are several ways to control these diseases including cultural practices, chemical control and genetic resistance. Although cultural practices are mentioned in the literature as a method of controlling these diseases, they are effective only against anthracnose. Chemical control is, nowadays, the most effective way to control most of air borne bean diseases. Nevertheless, the fungicide cost makes it a very expensive control practice to small farmers. As a result, genetic resistance becomes the less expensive and more practical way of controlling these diseases. However, their control by this method has been complicated by the fact that their causal agents present different pathotypes what makes the development of new resistant cultivars more difficult.

The objective of this paper is to report results obtained in the pre-breeding program of Embrapa Rice and Beans, mainly for bean angular leaf spot resistance.

Materials and Methods

A total of 78 bean genotypes were tested to 8 (Table 1), 4 and 15 isolates of *P. griseola*, *U. appendiculatus* and *Colletotrichum lindemuthianum*, respectively. For all experiments, plants were sown in aluminum pots, containing approximately 2,0 kg of soil, at a rate of 5 seeds per pot. *P. griseola* spores for inoculation were obtained by culturing the fungus on bean leaf-dextrose-agar medium in a BOD chamber at $24 \pm 2^\circ\text{C}$. *U. appendiculatus* spores, of each isolate, were obtained by inoculating the cultivar Rosinha G-2. *C. lindemuthianum* spores were obtained culturing the fungus in a sterilized bean pod in a test tube for 10 days. The spore suspension for *P. griseola* and *U. appendiculatus* was adjusted to 2×10^4 conidia/mL and for *C. lindemuthianum* the spore suspension was adjusted to $1,2 \times 10^6$ conidia/mL. For *P. griseola* and *U. appendiculatus*, bean plants were inoculated 14 days after planting and for *C. lindemuthianum* 8 days after planting. The inoculated plants were incubated in a moist chamber (>95% RH) for 36 h. *P. griseola* inoculated 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 to 3 (non-sporulating lesions) were considered resistant and 4 to 9 (sporulating lesions) as susceptible (CIAT, Cali, 1987, 54pp.; Sartorato, A., J. Phytopathology 152:385-390, 2004). *U. appendiculatus* and *C. lindemuthianum* inoculated plants were transferred to a temperature controlled chamber (22°C). Symptoms evaluation, in a 1 to 9 scale, occurred 14 and 10 days after inoculation for *U. appendiculatus* (Stavely et al., Ann. Rep. Bean Improv. Coop. 26:4-6, 1983) and *C. lindemuthianum* (Rava, et al., Fitopatol. bras. 18:388-391, 1993), respectively. For both diseases plants rating 1 to 3 were considered resistant and 4 to 9, susceptible.

Results and Discussion

The overall results of the test showed that 36 (46,0%), 17 (21,8%), 12 (15,4%) and 8 (10,3%) genotypes were susceptible to 8, 7, 6 and 5 isolates of *P. griseola*, respectively. Cultivars CNFC 10432 and CNFM 08080 (2,6%), CNFE 10815 and IPA 6 (2,6%) and BRSMG Majestoso (OPNS 0331) (1,3%) were resistant to 4, 5 and 8 isolates of *P. griseola*, respectively (Table 1).

The genotype OP-NS 0331, the most resistant cultivar tested, was recently released as BRSMG Majestoso. It was selected from a cross between Ouro Negro, a ALS resistant black bean cultivar and Perola, a ALS susceptible carioca grain type cultivar. Crosses were performed at the Biology Department of the University of Lavras, state of Minas Gerais.

Besides of being resistant to bean angular leaf spot, the cultivar BRSMG Majestoso is also resistant do 4 *Uromyces appendiculatus* isolates (2.1.3.1, 2.2.3.1, 1.1.1.1 and 4.1.1.4). To 3 of these isolates cultivar Majestoso has shown resistant/susceptible plants. In relation to antracnose, this cultivar was tested to 15 isolates showing complete resistance to 8 of them. For five isolates it showed some resistant and some susceptible plants and was completely susceptible to 2 isolates.

The fact that cultivar Majestoso had shown plants with resistant/susceptible reactions to some specific isolates of *U. appendiculatus* and *Colletotrichum lindemuthianum* was expected since this cultivar had never been exposed to bean rust or bean anthracnose.

Table 1. Bean genotypes resistant to 3, 4, 5 and 8 isolates of *Phaeoisariopsis griseola*.

Genotype	I S O L A T E (Ig CNF#)							
	60.4	525.4	584.3	709	784	786	809	874
	Resistant to 3 isolates							
CNFC 07812	S	R	S	R	S	S	R	S
PONTAL	S	R	S	R	S	S	R	S
CNFC 08013	S	S	S	S	R	R	S	R
GRAFITE	S	R	S	R	S	S	R	S
CNFR 07858	S	R	S	R	S	S	R	S
CNFR 10522	S	R	S	R	S	R	ND ¹	S
	Resistant to 4 isolates							
CNFC 10432	R	S	R/S ²	S	R	S	R	S
CNFM 08080	S	R	S/R ³	R	S	S	R	S
	Resistant to 5 isolates							
CNFE 10815	S	S	S	R	R	R	R	R
IPA 6	S	R	S	R	R	R	R	S
	Resistant to 8 isolates							
Majestoso (OP-NS 0331)	R	R	R	R	R	R	R	R

¹ND = no data available.

²R/S = mixture of disease reaction with more resistant than susceptible plants.

³S/R = mixture of disease reaction with more susceptible than resistant plants.

THE DILEMMA OF RECURRENT BACKCROSSING FOR INTROGRESSING COMMON BACTERIAL BLIGHT RESISTANCE IN COMMON BEAN

Robert W. Duncan¹, Shree P. Singh², Robert L. Gilbertson¹.
¹University of California, Davis, CA, ²University of Idaho, Kimberly, ID.

Common bacterial blight (CBB) caused by *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye and *X. campestris* pv. *phaseoli* var. *fuscans* causes severe yield and quality losses in common bean (*Phaseolus vulgaris* L.). Resistance to CBB is quantitatively inherited, with low to moderately high heritability. Recurrent backcross breeding has been used to increase levels of CBB resistance in common bean (Mutlu et al. 2002; Fourie and Herselman 2001). These studies suggested that CBB resistance could be retained, even after five backcrosses. The objective of the current research was to assess the effect of one and two backcrosses on the introgression of CBB resistance.

A double-cross (Wilkinson 2 / DRK 2 // DRK 1 / VAX 3) to introgress resistance to CBB was made in the spring of 2004. The double-cross F₁ of 707 plants was assessed in the fall of 2004 using direct CBB screening. Trifoliolate leaves of the F₁ were inoculated with an *Xcp* concentration of 1x10⁸ cfu/ml using the razor blade method of inoculation. Selected resistant plants were then backcrossed onto DRK 1 to produce 2188 BC₁F₁ seeds. The BC₁F₁ was assessed with the same direct CBB screening protocol and selected resistant plants were again backcrossed onto the recurrent parent to produce 1080 BC₂F₁ seeds.

The BC₁F₂ (43 F₂ families each derived from CBB-resistant F₁ plants) and BC₂F₁ (36 F₁ of distinct parental combinations) were assessed as single rows in a CBB field nursery in the summer of 2005. The nursery was inoculated with *Xcp* at the V3 – V4 and the R5 – R6 growth stages. Fifteen families with the highest levels of CBB resistance were selected from each of the BC₁F₂ and BC₂F₁. A single seed was taken from each plant within a family to form a balanced-bulk. From each balanced-bulk, 12 seeds were randomly taken to advance to the next generation in the greenhouse. Direct selection was employed to select one plant per family with the highest level of CBB resistance.

The four parents, 15 BC₁F₄ and 15 BC₂F₃ families were evaluated for CBB reaction in the greenhouse using a randomized complete block design with four replicates. A 1 – 9 rating scale (1<4 = resistant, 4<7 = intermediate, 7-9 = susceptible) was used to assess the CBB development 21 days post-inoculation. DRK 1 and DRK 2 were susceptible to CBB, whereas VAX 3 and Wilkinson 2 were resistant and intermediate, respectively. The mean disease severity index (DSI) of the 15 families was 4.3 for the BC₁F₄ and 5.7 for the BC₂F₃. The range for the fifteen families within each backcross was similar, but the BC₁F₄ families had lower minimum and maximum CBB scores.

After one backcross to DRK 1, 9 families retained a resistant mean CBB score, whereas 5 were intermediate and only one was susceptible. In contrast, following the second backcross, only one family retained a resistant mean CBB score, whereas 13 were intermediate and one was susceptible. Thus, after a single backcross (i.e., with the 75% genetic contribution of the CBB

susceptible recurrent parent), the majority of families remained resistant to CBB. However, with the second backcross, and 87.5% genetic contribution of the susceptible parent, only one family had a CBB-resistant mean.

Our findings suggest that, to recover a higher frequency of families with a high level of CBB resistance in a given market class, a single backcross with a relatively large BC₁F₁ population may be preferable over multiple backcrosses.

Table 1. The mean and range of the common bacterial blight disease severity index (DSI) for parents, BC₁F₄ and BC₂F₃ families as determined by direct screening for resistance.

CBB score	DRK 1	DRK 2	VAX 3	Wilkinson 2	BC₁F₄	BC₂F₃
Mean DSI	7.5	7.2	2.5	4.6	4.3	5.7
Range	7.0 – 9.0	6.0 – 8.0	2.0 – 3.7	2.3 – 5.0	2.9 – 7.0	3.4 – 7.2

Table 2. The total number of families with resistant, intermediate, and susceptible mean CBB scores as determined by direct common bacterial blight screening.

CBB score	DRK 1	DRK 2	VAX 3	Wilkinson 2	BC₁F₄	BC₂F₃
Resistant	0	0	1	0	9	1
Intermediate	0	0	0	1	5	13
Susceptible	1	1	0	0	1	1

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TWO CYCLES OF RECURRENT SELECTION FOR PYRAMIDING COMMON BACTERIAL BLIGHT RESISTANCE IN COMMON BEAN

Robert W. Duncan¹, Shree P. Singh², Robert L. Gilbertson¹.

¹University of California, Davis, CA, ²University of Idaho, Kimberly, ID.

Common bacterial blight (CBB) is one of the most important diseases of common bean (*Phaseolus vulgaris* L.) and is caused by *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye and *X. campestris* pv. *phaseoli* var. *fuscans*. More than 20 quantitative trait loci, a few with major effect, but most with minor contributions to CBB resistance, are distributed across the genome (see Kelly et al. 2003). Resistance to CBB has been introgressed into common bean from the tepary (*P. acutifolius* A. Gray) and scarlet runner (*P. coccineus* L.) bean. Backcross and pedigree methods or their modifications have commonly been used to breed for CBB resistance. While recurrent selection (RS) in common bean has been used to improve plant architecture (Kelly and Adams 1987), seed yield (Beaver and Kelly 1994; Singh et al. 1999) and white mold resistance (Lyons et al. 1987), it has not been evaluated for improving CBB resistance. The objectives of this study were to 1) assess the number of RS cycles required to pyramid high levels of CBB resistance and 2) determine the effect of intermating genotypes with differing levels of CBB resistance for RS.

A double-cross (Wilkinson 2 / DRK 2 // DRK 1 / VAX 3) for resistance to CBB was made in the spring of 2004 (C₀S₀). Trifoliolate leaves of the F₁ were inoculated with *Xcp* at a concentration of 1x10⁸ cfu/ml using the razor blade technique. Plants with resistant or intermediate CBB scores (on a 1 – 9 rating scale, where 1<4 = resistant, 4<7 = intermediate, and 7-9 = susceptible), 21 days post-inoculation, were used for intermating each RS cycle. Selected resistant plants were intermated (R x R). Similarly, resistant by intermediate (R x I) and intermediate by intermediate (I x I) plants were intermated, to generate three subpopulations within each RS cycle. All plants that were selected for intermating were also used to produce RSC₀S₁ seed. From each RSC₀S₁ plant, 12 seeds were screened and the plant with the highest level of CBB resistance within a family was used to produce the RSC₀S₂ seed.

Thus, the first RS produced three subpopulations within the RSC₁S₀ (R x R - 12 cross-combinations, R x I – 7 cross-combinations and I x I – 10 cross-combinations). From each cross-combination, the plant with the highest CBB resistance was intermated to a plant with the highest CBB resistance from another cross-combination (using chain-crossing). All selected plants were also used to produce the RSC₁S₁ and RSC₁S₂ as described above. The second RS cycle also generated the three subpopulations within the RSC₂S₀. The plant with the highest CBB resistance in each cross-combination within each of the three subpopulations was used to produce the RSC₂S₁ and RSC₂S₂ as described above.

The four parents, and 12 R x R, 7 R x I and 10 I x I subpopulations for each RS cycle (RSC₀S₂, RSC₁S₂ and RSC₂S₂) were evaluated for CBB reaction in the greenhouse using a randomized complete block design with 4 replications. DRK 1 and DRK 2 were susceptible to CBB; whereas VAX 3 and Wilkinson 2 were resistant and intermediate, respectively. Regardless of subpopulation (e.g., R x R, R x I and I x I), CBB resistance improved with each RS cycle (Table 1). Thus, the RSC₂S₂ had the highest mean CBB resistance, and levels were similar between the R x R and R x I subpopulations. The RSC₂S₂ also had the lowest minimum and maximum mean

CBB scores in comparison to the RSC₀S₂ and RSC₁S₂ (Table 2). Relatively smaller differences in the minimum and maximum mean CBB scores were found among the three subpopulations in the RSC₂S₂. The greatest number of resistant recombinant cross-combinations were produced in the RSC₂S₂ R x R subpopulation, followed by RSC₂S₂ R x I and I x I, and RSC₁S₂ R x R and I x I subpopulations (Table 3).

In summary, each cycle of RS produced a greater frequency of resistant genotypes. At least one cycle of recurrent selection was needed to produce a family with a mean CBB-resistant score. Additionally, it was also possible to pyramid a high level of CBB resistance using RS among initial recombinants with intermediate CBB resistance.

Table 1. The mean common bacterial blight scores for the R x R, R x I and I x I subpopulations in the recurrent selection cycles RSC₀S₂, RSC₁S₂ and RSC₂S₂.

Subpopulation	RSC ₀ S ₂	RSC ₁ S ₂	RSC ₂ S ₂
R x R	5.6	5.1	4.1
R x I	6.4	4.9	4.0
I x I	6.1	4.9	4.7

Table 2. The range for common bacterial blight mean scores for the R x R, R x I and I x I subpopulations of the recurrent selection cycles RSC₀S₂, RSC₁S₂ and RSC₂S₂.

Subpopulation	RSC ₀ S ₂	RSC ₁ S ₂	RSC ₂ S ₂
R x R	4.7 – 6.8	3.3 – 7.4	2.9 – 5.4
R x I	5.8 – 7.7	3.7 – 6.5	3.0 – 5.3
I x I	4.7 – 7.5	3.6 – 6.2	3.4 – 5.8

Table 3. The total number of recombinants with resistant (R), intermediate (I), and susceptible (S) mean common bacterial blight scores selected in the recurrent selection cycles RSC₀S₂, RSC₁S₂ and RSC₂S₂.

Subpopulation	RSC ₀ S ₂	RSC ₁ S ₂	RSC ₂ S ₂
R x R	12I	3R, 8I, 1S	6R, 6I
R x I	6I, 1S	1R, 6I	3R, 4I
I x I	6I, 4S	3R, 7I	3R, 7I

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IMPROVING RESISTANCE TO CBB IN ANDEAN COMMON BEAN USING MAS

S. Fernandez*, A. Ibeas, C. Asensio and M.C. Asensio-S.-Manzanera
ITACyL, Ctra. Burgos Km 119 47071 Valladolid (Spain) [*ferlobso@itacyl.es](mailto:ferlobso@itacyl.es)

Introduction

Common Bacterial Blight (CBB), caused by *Xanthomonas campestris* pv. *phaseoli* (Smith) (XCP), a seed-borne disease, is one of the major production constraint worldwide. There are >20 CBB resistance QTL with large or small effects distributed across all 11 chromosomes introduced into *P. vulgaris* breeding lines by intra or interspecific hybridization. Phenotypic selection following inoculation with the pathogen was used in the ITACyL bean breeding program to introduce resistance to CBB into Spanish commercial landraces. Several markers were developed for use in MAS for CBB resistance including SAP6, SU91 and BC420. The objective in this project was to test the effectiveness of these markers out of their mapped population as a tool to complement phenotypic selection by inoculation methods.

Material and Methods

Two double crosses and their reciprocal were used in this research:

Cross 1: Beluga/MCA-40-4//Tremaya/4D-50-1

Cross 2: Tremaya/4D-50-1// Beluga/MCA-40-4

Cross 3: Cueto/MCA-82-3//ZJ-1192/ITA-485-1-22

Cross 4: ZJ-1192/ITA-485-1-22// Cueto/MCA-82-3

The parents used and their characteristics appear in Table 1. Genetic resistance was provided by 4D-50-1, MCA-40-4, MCA-82-3 and ITA-485-1-22; these genotypes are advanced breeding lines that came from the gamete selection for introgression and pyramiding for resistant to bacterial blight diseases (Asensio- S.-Manzanera et al. 2005, 2006). All parents and the F₁ were inoculated in the trifoliolate leaf by the multiple-needle technique in the greenhouse with XCP isolate #659, at a concentration of 5 x 10⁸ cfu. Disease evaluation was made from 14 to 21 days after inoculation, using a rating scale from 1 to 9. Plants with scores from 1 to 3 were considered resistant (R), 4 to 7 intermediate (I) and 7 to 9 susceptible (S). Young tissue from an unexpanded trifoliolate leaf was taken for DNA extraction and screened for the presence or absence of the three SCAR markers in a single multiplex PCR (Miklas et al. 2000). All plants were harvested individually for progeny test, and the next generation (F_{1:2}) evaluated for CBB reaction in the field with the same XCP isolate using the spray-inoculation. The disease severity index (DSI) and F₁/F₂ correlation were calculated.

Results and Discussion

Crosses #1 and #2 had higher proportion of CBB resistant plants than crosses #3 and #4 (Table 2). Similar results were found in the F_{1:2}. These results could be expected because of the CBB resistance genes present in the parents. Progeny tests were positive and highly significant demonstrating effectiveness of direct selection for resistance to CBB in F₁.

The results of molecular marker assays showed that SAP6 and SU91 were more frequent in these populations (Table 3). The marker group most frequent was SAP6 +SU91, and no individual had all three markers. The presence of markers was associated with lower DSI and it was more evident when SAP6+SU91 were present. These results showed the effectiveness of MAS, although 14,4% of F₁ plants and their 8,7% of F_{1:2} without any markers were resistant. We concluded that, in our populations, as reported by Duncan et al. (2006), direct selection was more effective. It is necessary to develop additional molecular markers for QTL of CBB resistance in Andean populations.

Table 1. Origin and CBB reaction of parents used in double-crosses.

Parents	Origin	CBB reaction	Genepool
Beluga	Michigan State University	Susceptible	Andean
MCA-40-4	ITACyL Advanced breeding line	Resistant	Recombinant
Tremaya	ITACyL Breeding cultivar	Intermediate	Recombinant
4D-50-1	ITACyL Advanced breeding line	Resistant	Recombinant
Cueto	ITACyL Landrace	Susceptible	Andean
MCA-82-3	ITACyL Advanced breeding line	Resistant	Recombinant
ZJ-1192	Comercial Cultivar	Susceptible	Andean
ITA-485-1-22	ITACyL Advanced breeding line	Resistant	Recombinant

Table 2. The number of plants and the mean disease severity index (DSI) of two double-crosses (and their reciprocals) for CBB.

Cross	F ₁	R [1, 4]	I [4,7]	S[7,9]	Total	F _{1,2}	R [1, 4]	I [4,7]	S[7,9]	Total
1	N	64	12	36	113	N	40	36	41	117
	DSI	1.75	5	8.53	4.27	DSI	1.27	5.14	7.39	3.27
2	N	28	16	24	68	N	23	22	28	73
	DSI	1.96	4.75	8.33	4.86	DSI	1.26	5.54	7.5	4.94
3	N	9	9	31	49	N	2	21	32	55
	DSI	2.11	5	8.48	6.67	DSI	1	5.33	7.28	6.30
4	N	15	11	24	50	N	5	31	16	52
	DSI	2.06	4.63	8.5	5.72	DSI	1.8	4.83	7.18	5.27

Table 3. The number of plants and the mean disease severity index for the presence or absence of CBB resistance SCAR markers (SAP6, SU91 and BC420) according to classes determined by phenotypic evaluation.

Cross		SAP6+	SAP6 -	SU91+	SU91-	BC420+	BC420 -	SAP6 +SU91	SAP6 - SU91- BC420-
1	R	43	21	21	44	1	66	19	18
	I	8	4	2	9	0	7	2	4
	S	24	12	9	28	0	37	8	11
	Total/DSI	75/4.32	37/4.19	42/3.90	81/4.43	1/2	110/4.25	29/3.83	33/4.24
2	R	15	13	4	24	0	28	4	13
	I	8	8	3	13	1	13	2	7
	S	7	17	2	22	0	24	2	17
	Total/DSI	30/ 3.97	389/5.58	9/3.77	59/5.05	1/4	65/5.20	8/3.63	37/5.59
3	R	3	6	1	8	3	6	0	3
	I	2	7	0	9	1	8	0	6
	S	5	26	2	29	2	29	1	23
	Total/DSI	10/5.7	39/6.92	3/5.67	46/6.74	6/4.16	43/7.02	1/3.83	32/7.38
4	R	7	8	1	14	0	15	1	8
	I	4	7	0	11	1	10	0	6
	S	5	19	0	24	1	23	0	18
	Total/DSI	16/4.56	34/6.26	1/3	49/5.77	2/6.5	48/5.68	1/3.63	32/6.26

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PHYSICAL MAPPING OF A MAJOR QTL CONDITIONING COMMON BACTERIAL BLIGHT ON CHROMOSOME 1 IN COMMON BEAN

Shuyu Liu, Kangfu Yu, Margaret Haffner, and Soon J. Park
Agriculture Agri-Food Canada, Greenhouse and Processing Crops Research Center, Harrow ON
N0R 1G0, Canada. Yuk@agr.gc.ca

Abstract

A major QTL conditioning common bacterial blight (CBB) resistance in bean lines HR45 and HR67 was derived from XAN159 whose resistance was inherited from tepary bean line PI319443. Four markers have been found tightly linked to this major CBB resistance QTL. A BAC library was constructed from high molecular weight DNA of HR45 and has 5.7-fold bean genome coverage. We screened BAC pools using these markers. Two to eight BAC clones were identified from each marker. Two clones were found to have both markers PV-ttcc001 and STS183. Preliminary contigs covering this major QTL were constructed. This is the first report for physical mapping of a major QTL for CBB resistance in common bean.

Introduction

BAC library is stable, rarely chimeric, and easier to manipulate, which makes it popular with researchers (Shizuya et al. 1992). Common bean (*Phaseolus vulgaris* L.), a food legume crop with the smallest genome (about 600 Mb), is very important because it serves as the major plant protein source for people in developing countries. There is an international effort to work on phaseomics (<http://www.phaseolus.net/>). Its smaller genome size and fewer repetitive sequences attract researchers' efforts to study its important traits using genomics. BAC libraries have been constructed using varieties from common bean and lima bean (*Phaseolus lunatus*). Common bean lines involved are Sprite, BAT98, G02771, and G21245. BAC clones from Sprite hybridizing with markers flanking the nuclear fertility restorer gene, Fr (Vanhouten et al. 1999), an anthracnose resistance gene *Co-4*² (Melotto et al. 2001), and the *I* gene conditioning bean common mosaic virus resistance (Vallejos et al. 2006) have been identified. The arcelin-phytohemagglutinin- α -amylase (APA) families of seed proteins were analyzed by studying four BAC libraries constructed from cultivars with different genotypes, (Kami et al. 2006). A major QTL conditioning common bacterial blight (CBB) resistance on chromosome 1 of common bean is found in HR45 and HR67. It is one of the major sources of CBB resistance in breeding programs worldwide. Its genomic analysis will facilitate its usefulness in genetic studies and breeding programs. The objectives of this research are to: 1) screen BAC pools using tightly linked markers, 2) map this CBB resistance QTL physically using BAC clones.

Materials and Methods

BAC library screening: DNA was extracted from mixed clone cultures (40 μ l per clone) of plate pools (PPs), column pools (CPs) and row pools (RPs). All four markers tightly linked to this major CBB resistance QTL were used to identify positive PP, CP and RP. All combinations of target PP, CP and RP from each marker were screened to identify all single target BAC clones of that marker.

BAC fingerprinting and contig assembly: DNA from each BAC clone was digested using *Hind*III and run on 0.8% agarose gel. The standard band size from each gel was estimated using Kodak Digital Science 1D Image Analysis Software (Rochester, USA) and then used to set up the standard file for Image 3.10 (<http://sanger.ac.uk/Software/Image>). The vector band was excluded from the data. Then the output files were further analyzed using FPC 4.8 (Soderlund 2000) to assemble the contigs.

BAC end sequencing and primer design: Forward and reverse BAC ends of most single positive BAC clone were sequenced. The primers were designed using Primer 3 (<http://frodo.wi.mit.edu/cgi-bin/primer3>) and were used to confirm those overlapping from the BAC digestion.

Results

The insert size distribution: *Not*I digestion of 100 random clones from the library indicated that all of the clones analyzed have an insert and the insert size averaged at 107 kb with a range from 30 kb to 280 kb. Based on the bean genome size of about 637 Mb, the HR45 BAC library contains about 5.7 haploid genome equivalents. Among 100 random clones, 65% do not have endogenous *Not*I sites, 26% have one *Not*I site and 9% have two or three *Not*I sites. Because leaf nuclei were used as the source for high-molecular-weight DNA, contamination of the library with organelle sequences should be low (Vanhouten et al. 1999).

Use of markers to screen the BAC pools: All four molecular markers tightly linked to this major CBB resistance QTL were used to screen the PPs, CPs and RPs. Single BAC clones were confirmed using PCR. Two, four, five, and eight BAC clones gave positive results to UBC420, PV-tttc001, STS333 and STS183, respectively.

BAC contig assembling: All 17 target BAC clones were digested with *Hind*III and the band were analysed and the contigs were assembled. There are six BACs on the minimum tiling path with a size of about 800 kb. This is subject to further confirmation.

BAC end sequencing and primer design: Both ends of target BAC clones were sequenced and the primers were designed to amplify a band from 350 to 600 bp. The polymorphic markers were mapped back to the QTL region.

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SEED QUALITY OF DRY BEAN PRODUCED AT A SEMIARID LOCATION IN THE HIGHLANDS OF MEXICO

R. Navarrete-Maya¹, D. Brena-Gómez¹, J. A. Acosta-Gallegos², J. Navarrete-Maya¹, I. Cuellar-Robles², and F. J. Ibarra-Pérez²

¹UNIGRAS, FES-C, UNAM, km. 2.5 carr. Cuautitlán-Teoloyucan, México, 54700, ²INIFAP Serapio Rendón 83, Col. San Rafael, México, D.F., 06770, ²Campo Experimental Bajío, INIFAP, Apdo. Postal 112, Celaya, Gto., 38010. rosa_navarrete@hotmail.com

The state Durango is the second in area planted to dry bean during the summer season and the use of certified seed is low since most growers use for sowing the grain they produced. Common bacterial blight (CBB) (*Xanthomonas campestris* pv. *phaseoli*) (*Xcp*) is among the four most important bean diseases in the highlands of Mexico (3), and it has been reported that one seed infected by *Xcp* among 10,000 could induce an epidemic disease in the next growing cycle (5). The internal infection of the seed is the main source of disease dissemination and primary inoculum (1, 4). High quality seeds are those that have cultivars and physical purity, high percentage of germination, high vigor and are free of pathogens. Therefore is important to know the status of the seed produced in the state of Durango, including the health, to avoid the development of seed-borne diseases.

In this research the evaluation of the seed quality of 36 bean cultivars, previously classified as 18 tolerant and 18 susceptible to *Xcp*, is reported. Cultivars were sown during the spring-summer season of 2005 at Valle del Guadiana Experimental Station of INIFAP, located near Durango, a site of semiarid climate, 412 mm yearly precipitation and 18 °C mean temperature. Seed evaluation was performed at the lab at the FES-Cuatitlan, UNAM; following measurements were made: 1) physical test: considering color, size, and weight of 100 seeds; 2) physiological test: standard germination and vigor using rolled towels and a temperature of 25°C during 8 days; and 3) health test with emphasis in the detection of *Xcp* in a sample of seeds from each cultivar, for that the seed was washed with sterile water and an aliquot was striated on potato dextrose agar media (PDA). A second sample of seeds was washed with NaOCl 1% during 1 min, and sown on PDA. Seeds were incubated during 7 days at 25°C. After three days the bacterial suspicious colonies were sub cultured on nutritive agar (NA) and three days later on yeast extract, calcium carbonate and agar (YDC) media (2, 4). All tests were made with three replicates and the statistical analysis was as a completely random design.

Out of the 36 cultivars analyzed, AFN, Azufrado Namiquipa, Flor de Mayo Anita, Negro Vizcaya, Negro Durango, and Flor Mayo Sol showed good seed quality when disinfected with NaOCl. However, when they were washed only with water their health quality was deficient (Table 1). Deficient health quality means that different kind of pathogenic fungi and bacteria were recovered from the seeds. It is well known that the pathogens can be externally associated to the seeds as contaminant or are internally allocated as potential pathogens (1, 4).

Nineteen cultivars from the whole set did not have *Xcp* in the seed, eight that were previously classified as tolerant and eleven as susceptible. Of those cultivars whose seed was free of *Xcp*, only two, A-774 and Bayo Andrade showed good seed quality. On the contrary Negro Vizcaya and Pinto Zapata that were classified as susceptible cultivars in the field, were free of bacteria, but their physical, physiological and health quality was heterogeneous (Table 1). There was not

evidence that cultivars showing susceptibility in the field produced seed of low quality since seeds of some cultivars like Pinto Bayacora (highly susceptible), with seed infected with *Xcp*, showed good physical, physiological and health quality (without and after being externally cleaned with NaOCl). On the other hand, Pinto PS 99, a tolerant line from the USA, with seed infected with *Xcp*, showed good physical and physiological quality, but its health quality was deficient.

Results indicated that cultivars: Azufrado Namiquipa, AFN, Negro Vizcaya, Flor Mayo Sol and A-774, can be suitable for seed production in this region since its seed was of good quality and free of diseases. However, the seeds of other cultivars such as Pinto Bayacora must be produced in other region or environments with less pressure from seed transmitted diseases. Also the use of a seed disinfectant (NaOCl) improved the healthy of some cultivars, particularly of Negro Durango and G21212. The use of NaOCl is a relatively cheap treatment that could also be recommended for seed production.

Table 1. *Xanthomonas campestris* pv. *phaseoli* seed contamination and seed quality of dry bean cultivars growth at Durango, Dgo., 2005.

Cultivar	Previous reaction ¹	<i>Xcp</i> ²	S e e d q u a l i t y ³			
			Physical	Physiological	NaOCl	Water
AFN	T	-	G	G	G	D
Azufrado Namiquipa	T	-	G	G	G	D
FMA nita	T	-	G	G	G	D
Pinto 98001	T	+	G	G	D	D
Pinto PS99	T	+	E	G	D	D
A 774	S	-	G	G	G	G
Bayo Andrade	S	-	G	E	G	G
Negro Vizcaya	S	-	G	G	G	D
N. Durango	S	-	E	G	E	D
N. Sahuatoba	S	+	D	E	G	G
FMSol	S	-	G	G	G	D
97RS101	S	-	G	G	D	G
G 21212	S	+	G	G	E	D
Pinto Mestizo	S	-	G	D	G	G
Pinto Bayacora	S	+	G	G	G	G

¹S: susceptible, T: tolerant, ²presence (+) or absence (-) of *Xcp* in the seed, NaOCl: sodium hypochlorite 2%, ³D: deficient, G: good, E: excellent.

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TOWARDS THE IDENTIFICATION OF COMMON BACTERIAL BLIGHT RESISTANCE GENES IN PHASEOLUS VULGARIS

G.E. Perry, Y. Reinprecht, J. Chan and K.P.P.Pauls

Department of Plant Agriculture, University of Guelph, Guelph Ontario, Canada

Introduction

Common bacterial blight (CBB) is endemic to all regions of the world where dry beans (*Phaseolus vulgaris*) are cultivated, and represent a significant barrier to crop production. The disease is caused by the bacterium, *Xanthomonas axonopodis* pv. *Phaseoli*, and results in reduced seed yield and the contamination of future seed. (Broughton *et al.*, 2003).

CBB resistance has been studied for a number of years, and has led to the development of several lines which have demonstrated resistance to *X. axonopodis* pv. *Phaseoli*. Recently, a CBB-resistant cultivar, OAC-Rex (registration no. 5491), tested as OAC 95-4, was derived from a cross between HR20-728 and MBE 7 made in 1988. Another CBB-resistant line, HR67, was produced by a series of crosses between Centralia, HR13-621, OAC Rico and XAN159 (Yu *et al.* 2000).

To aid in the identification of lines possessing CBB-resistance related genes, a number of molecular markers have been identified for various lines, including HR67 (Yu *et al.* 2000, Yu *et al.* 2004) and OAC-Rex (Tar'an *et al.* 2001). Although these markers represent a useful tool for breeding CBB-resistant lines, the actual genes involved in resistance are not yet known. The objectives of this project are to develop BAC libraries for two important CBB-resistant *P. vulgaris* lines (HR67 and OAC-Rex) and to identify genes associated with CBB resistance.

Materials and Methods

DNA Isolation and Library Construction

High molecular weight (HMW) DNA from the fully expanded leaves of OAC-Rex and HR67, was extracted and encapsulated according to an established protocol (Zhang *et al.*, 1995). The encapsulated DNA was partially digested with 5 units of *Bam*HI (Roche) for 15 min. and electrophoresed through a 1% (w/v) low melting point agarose gel using a pulse field gel electrophoresis unit (BioRad). The DNA fragments between 100-400 Kbp were excised from the gel, and the DNA released through an enzymatic digestion with Gelase (EpiBio) according to the manufacturers protocol.

The BiBac2 vector was prepared according to the protocol of Hamilton *et al.* (1996). The ligation reactions contained 50ng of insert DNA and 1 μ g of prepared vector with 5U of T4 DNA ligase (Invitrogen). The reaction mixtures were incubated overnight at 4°C, and 2 μ l were used to transform DH10 *Escherichia coli* (Invitrogen) cells according to the manufacturers' instructions. Colonies were selected using a GeneTAK G3 automated workstation and transferred to 96-well plates containing LB media with 50 mg L⁻¹ kanamycin. The plates were incubated overnight at 37°C.

Library Screening

The OAC-Rex and HR67 libraries were spotted onto nylon membranes in a 5-by-5 matrix using a Biomek 2000 automated workstation (Beckman) with a 96-pin high-density replication tool. The membranes were prepared according to the protocol of Olsen *et al.*, (1993). Hybridization with the DIG-labeled pvCTT001-derived probe was performed according to the manufacturers' instructions (Roche).

Clones that were identified by probe hybridization were characterized using a gel-based restriction fingerprinting method (Chang *et al.* 2001; Tao *et al.* 2001; Zhang and Wu 2000). The bands generated for each clone were used to assemble contigs using the FingerPrint Contig analysis software (AGCol) to align the fragments.

Results and Discussion

OAC-Rex and HR67 libraries with 31,776 clones and 22,560 clones, respectively, were constructed. The OAC-Rex library has an average insert size of 150 Kbp, providing a library depth of 5.6. The HR67 library has an average insert size of 300 Kbp, with a depth of 8.1. Initial screens of the OAC-Rex library with the pv-ctt001 marker-derived probe identified 23 positive clones. These results were confirmed by PCR using primers for the pv-ctt001 marker (data not shown).

After separation by electrophoresis (Figure 1a) the bands from each clone were analyzed and they were aligned into a contig (Figure 1b). The library will be re-probed with fragments at the extremes of the contig (Clone 3 and Clone 4) to begin walking down the chromosome and expanding the size of the contig.

To identify clones containing CBB-resistance genes, selected clones will be transformed into a susceptible *P. vulgaris* line (OAC-Seaforth) and infected with *X. axonopodis* pv. phaseoli. The size of the resulting lesions will be measured, and clones resulting in reduced lesion size will be sub-cloned and sequenced. The genes identified in this study will facilitate the development of future CBB-resistant lines.

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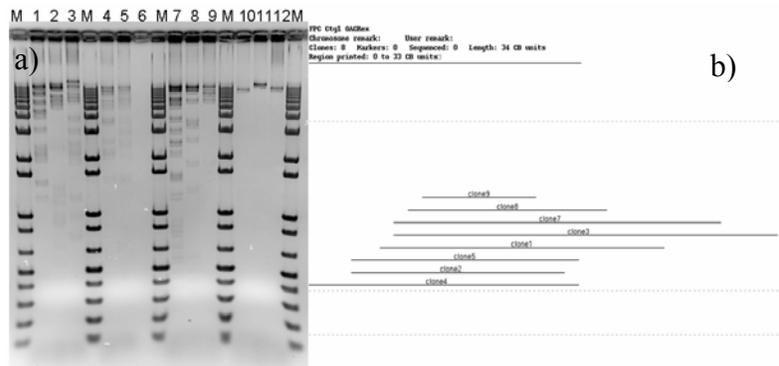


Figure 1: Contig construction from the OAC-Rex PV-ctt001 SSR marker. A) Clones identified from the membrane hybridizations were digested with *HindIII* and separated according to size. B) The band patterns were analyzed with FPC Contig (Sanger) software and aligned into a single contig.

FOLIAGE, POD AND INTERNAL SEED INFECTION OF SELECTED COMMON BEAN LINES WHEN INOCULATED WITH TWO STRAINS OF XANTHOMONAS AXONOPODIS PV. PHASEOLI

Mildred Zapata¹, James Beaver² and Tim Porch³

Departments of Crop Protection¹ and Agronomy and Soils², Univ. of Puerto Rico and USDA-ARS Tropical Agriculture Research Station³, Mayagüez, Puerto Rico 00681

Common bacterial blight (CBB) is an important foliar and seed-borne disease of common bean (*Phaseolus vulgaris* L.) grown in tropical, subtropical and temperate areas. The disease is caused by *Xanthomonas axonopodis* pv. *phaseoli*. Most common bean cultivars are currently susceptible to the bacterium. The bean breeding program at the University of Puerto Rico has developed CBB resistant breeding lines using VAX 6, VAX 3 and WBB-20-1 as sources of resistance. The objective of this study was to determine the level of resistance incorporated into these lines using strains of Xap prevalent in Puerto Rico and to identify lines with the most resistance in foliage, pods and seeds. Two strains from Puerto Rico of the common type of Xap (484 and 3353) were selected for inoculation at 1.0 A and 590 nm. Leaves were inoculated with 10^7 CFU/ml using multiple needles and scored at 9 days using the CIAT scale (1-9). Green pods (6-8 cm) attached to plants were inoculated before the expression of seed formation (flat) using pipette tips to place 5 μ l of inoculum on three sites on the pod surface without penetration. Lesion diameters were evaluated at 7 and 14 days using a 1-9 scale where 1 = 0 mm, 2 = 1.5 mm, 3 = 2 mm, 4 = 2.5 mm, 5 = 3 mm, 6 = 3.5 mm, 7 = 4 mm, 8 = 4.5mm, and 9 \geq 5mm. A selected number of pods were harvested at maturity and maintained at 5°C until used to determine the percentage of internal bacterial seed infection. Eight susceptible lines for each bacterial strain and their respective non inoculated controls were evaluated as well as eight resistant genotypes and their respective non-inoculated genotypes using individual pods and seeds. The cultivar 'Morales' was included as a susceptible check. For bacterial internal detection 100 μ l of 10^{-1} dilutions of seeds previously disinfested were placed on yeast dextrose calcium carbonate agar.

No significant difference in virulence was detected between the strains when inoculated on leaves and pods (Table 1). However, line PR 0443-38 showed resistance to Xap 484 and susceptibility to Xap 3353 on pods which suggests a differential reaction on the pods to the strains. A significant difference in the ability of the strains to infect internal seed tissues was observed. Internal seed infection was detected only when pods were inoculated with Xap 484. The level of seed infection on susceptible lines ranged from 16.6 to 100.0% (Table 1). One hundred percent of the Morales seed was infected when inoculated with Xap 484, whereas no seed of Morales was infected with Xap 3353. None of the bean lines with susceptible reactions to Xap 3353 on the pod surface had internal seed infection. All of the breeding lines with resistant pods were also resistant to internal seed infection for both strains. Xap 3353 was less compatible on the seed tissue than in the parenchyma pod tissues. This observation is supported by the reactions of other susceptible breeding lines that were also resistant to internal seed infection by Xap 3353 strain. The most important difference between the two strains of Xap is their ability to colonize internal seed tissue. In practice, it is very difficult to distinguish between the strains and both were previously determined to belong to the same rDNA ribogroup using EcoR1. Survival of the strains under natural conditions may differ. Primary inoculum for Xap 484 may depend on seed infection whereas Xap 3353 may depend mainly on seed surface contamination and contact with infected residues for primary inoculum. The resistant reaction on leaves, pods and internal seed infection has a very important implication in the reduction

of bacterial inoculum under field conditions. The most promising lines with resistant reactions on both leaves, pods and no internal seed infection were PR0443-17, derived from the cross ‘VAX 5 /// DOR 483 / BelNeb RR2 // MUS 83 / DOR 483’; PR0443-73, derived from the cross ‘VAX 6 /// DOR 483 / BelNeb RR2// MUS 83 / DOR 483; and PR0443-3, derived from the cross ‘DOR 364 / WBB-20-1 // DOR 482 /// VAX 6’. These white-seeded bean breeding lines have the SR-2 SCAR marker for the *bgm* gene and the SW12 QTL for Bean Golden Yellow Mosaic Virus resistance, the SW13 SCAR marker for the *I* gene resistance to Bean Common Mosaic Virus and the SAP 6 QTL for CBB resistance. Only PR0443-17 has the SU-91 SCAR marker for CBB resistance. These breeding lines produce seed yields equal to or better than Morales, the preferred white bean cultivar in Puerto Rico.

Table 1. Mean leaf and pod reactions and percentage of seed infection of common bean lines inoculated with two strains of the common type of *Xanthomonas axonopodis* pv. *phaseoli*

Interaction	Pod reaction ¹		% seed infection ²	Leaf reaction ³	
	7DAI	14 DAI			
Xap 484 Group 1					
Compatible lines		Xap 484	Xap 484	Xap 484	Xap 3353
Morales	3.00	4.05	100.0	7.47	7.90
PR0443-110	2.75	4.05	16.6	5.00	4.65
PR0443-7	2.51	2.70	60.0	3.17	2.90
PR0313-95	3.50	4.20	50.0	7.47	7.20
Incompatible lines					
PR0443-17	1.0	1.0	0.0	1.07	1.20
PR0443-7	1.0	1.0	0.0	3.17	2.90
PR0443-38	1.0	1.0	0.0	2.25	2.33
PR0443-73	1.0	1.0	0.0	1.76	1.30
Xap 3353 Group2					
Compatible lines		Xap 3353	Xap 3353	Xap 484	Xap 3353
Morales	3.26	4.80	0.0	7.47	7.90
PR0443-38	2.25	3.00	0.0	2.25	2.33
PR0443-110	2.25	2.55	0.0	5.00	4.65
PR0309-4	2.25	4.50	0.0	3.20	1.90
Incompatible lines					
PR0443-3	1.00	1.00	0.0	1.20	1.10
PR0443-105	1.00	1.00	0.0	3.40	3.67
PR0443-103	1.00	1.00	0.0	4.30	3.20
W-BB-20-1	1.00	1.00	0.0	3.30	3.80

¹Pod reaction refers to mean severity scores observed on pod surfaces at 7 and 14 days after inoculation (DAI) under greenhouse conditions where 1 = no symptoms and 9 = lesion size \geq 5 mm.

²Seed % infection refers to the percentage of internal seed infection detected under *in vitro* conditions.

³Mean leaf reactions to two Xap strains at nine days after multiple needle inoculation. The CIAT 1-9 scale was used where 1 = no symptoms and 9 = severe symptoms on the leaves.

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RUST RESISTANCE GENE PRESENT IN COMMON BEAN CULTIVAR OURO NEGRO (UR-ON) DOES NOT CORRESPOND TO UR-3+

Thiago Lívio Pessoa Oliveira de Souza¹, Suelen Nogueira Dessaune¹, Demerson Arruda Sanglard¹, Maurilio Alves Moreira² & Everaldo Gonçalves de Barros^{3*}

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

Rust, incited by the fungus *Uromyces appendiculatus*, can cause serious damage to common bean (*Phaseolus vulgaris* L.) crops in humid tropical and subtropical areas of the world. Resistance gene pyramiding has been used as a strategy to overcome this problem. Identification of different rust resistance genes with wide resistance spectra and determination of the allelic relationships among them are basic steps for works aiming at developing new bean commercial cultivars with durable resistance.

Cultivar Ouro Negro is the main rust resistance source used in Brazil (Faleiro *et al.*, 2004). It is resistant to several pathotypes of *U. appendiculatus* collected in central, northern and southern Brazil (Rios *et al.*, 2001; Souza *et al.*, 2005). The temporary symbol *Ur-ON* was assigned to the rust resistance gene present in 'Ouro Negro' because it has not been fully characterized. Allelism studies reported by Alzate-Marin *et al.* (2004) showed that *Ur-ON* does not correspond to genes *Ur-5* (cv. Mexico 309) or *Ur-11* (line Belmidak RR-3). To aid the characterization of the rust resistance locus present in 'Ouro Negro', in this work we determined the allelic relationships between *Ur-ON* and the gene *Ur-3*⁺ from cultivar Mexico 235. Initially, the reactions of 'Ouro Negro' and 'Mexico 235' to eight selected races of *U. appendiculatus* from the state of Minas Gerais (central Brazil) were determined. In addition, the presence/absence of SCAR and RAPD markers reported as linked in coupling phase to the gene *Ur-ON* was tested in 'Mexico 235'. Finally, we analyzed the inheritance of rust resistance in an F₂ population derived from crosses between 'Mexico 235' and 'Ouro Negro'.

Ten days after sowing the primary leaves of ten plants each of cultivars Ouro Negro, Mexico 235 and U.S. Pinto 111 (susceptible control) were inoculated with spore suspensions (2.0 x 10⁴ spores/ml) of eight selected races of *U. appendiculatus* (Table 1). The plants were then incubated for two days in a mist chamber kept at 20-22°C and 100% relative humidity (Souza *et al.*, 2005). Fifteen days after inoculation the plants were scored visually for the disease symptoms using a 1-to-6 scale (Stavelly *et al.*, 1983). Resistance reaction was assigned to plants with no or limited symptoms (grades 1 to 3), whereas plants graded 4 or greater were considered to be susceptible. Leaf DNA from plants of these three cultivars was extracted by a procedure based on Doyle & Doyle (1990). Amplification reactions were according to Faleiro *et al.* (2000) for the RAPD marker OPX11₅₅₀ and according to Côrrea *et al.* (2000) for SCAR markers SF10₁₀₅₀ and SBA08₅₆₀.

Eighty-one F₂ seeds (Mexico 235 x Ouro Negro) were obtained and sowed in the greenhouse. Using the same inoculation and disease symptom evaluation procedures described the F₂ plants and ten plants each of cultivars Ouro Negro, Mexico 235 and U.S. Pinto 111 were inoculated with *U. appendiculatus* race 63-3. The phenotypic frequencies observed in the F₂ population were tested for goodness-of-fit to theoretical ratios with chi-square test.

Cultivars Ouro Negro and Mexico 235 presented a similar resistance spectra. 'Ouro Negro' was resistant to all eight races and 'Mexico 235' was susceptible only to race 29-15 (Table 1). These results did not allow an accurate distinction between the resistance loci present

in these two cultivars, but they show that both are good resistance sources to races from the state of Minas Gerais. Two of the three tested molecular markers were polymorphic between ‘Ouro Negro’ and ‘Mexico 235’ (Table 1). This indicates that the resistance allele of the locus *Ur-ON* is not present in cultivar Mexico 235. It also shows that the two polymorphic markers (OPX11₅₅₀ and SF10₁₀₅₀) can be useful for monitoring the pyramiding of *Ur-ON* and *Ur-3⁺* in the same genetic background.

The results of the allelism test are shown in Table 2. The segregation ratio was of 15 resistant to 1 susceptible plant in the F₂ population (Mexico 235 x Ouro Negro) indicating that two independent genes govern resistance in this population. These results confirm that the gene (or complex gene locus) present in ‘Ouro Negro’ does not correspond to gene *Ur-3⁺*. Thus, cultivars Ouro Negro and Mexico 235 can be used simultaneously as rust resistance sources in common bean breeding programs in Brazil.

Table 1. Phenotypic and molecular characterization of cultivars Ouro Negro (*Ur-ON*), Mexico 235 (*Ur-3⁺*) and U.S. Pinto 111 (susceptible control) regarding the resistance to selected races of *U. appendiculatus* and the presence/absence of RAPD (OP) and SCAR (S) markers linked in coupling phase to the rust resistance gene *Ur-ON*

Cultivar	Gene	Reaction to races of <i>U. appendiculatus</i> ^a								Markers (<i>Ur-ON</i>) ^b		
		21-3	29-3	29-15	53-3	53-19	61-3	63-3	63-19	OPX11	SF10	SBA08
Ouro Negro	<i>Ur-ON</i>	R	R	R	R	R	R	R	R	1	1	1
Mexico 235	<i>Ur-3⁺</i>	R	R	S	R	R	R	R	R	0	0	1
U.S. Pinto 111	-	S	S	S	S	S	S	S	S	0	0	0

^aResistance (R) and susceptibility (S). ^bPresence (1) and absence (0) of molecular markers.

Table 2. Evaluation of resistance to race 63-3 of *U. appendiculatus* in the F₂ population derived from crosses between cultivars Mexico 235 (*Ur-3⁺*) and Ouro Negro (*Ur-ON*)

Genetic material	Locus tested	Nº of plants	Expected ratio (R:S) ^a	Observed ratio (R:S) ^a	χ^2	P(%) ^b
Ouro Negro	<i>Ur-ON</i>	10	1:0	10:0	-	-
Mexico 235	<i>Ur-3⁺</i>	10	1:0	10:0	-	-
F ₂ population	<i>Ur-ON</i> and <i>Ur-3⁺</i>	81	15:1	75:6	0.185	66.7

^aResistant (R) and susceptible (S) plants. ^bProbability in percentage.

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IDENTIFICATION OF POTENTIAL CANDIDATE GENETIC MARKERS IN PHASEOLUS VULGARIS FOR RESISTANCE TO PHAKOPSORA PACHYRHIZI

C. M. Bellato¹, R. D. Frederick², J. Bowers², D. Zhang³ and M. Pastor-Corrales¹
¹Vegetable Laboratory, PSI, USDA/ARS-BARC-W, 10300 Baltimore Ave Bldg 010A, Beltsville, MD 20705-3000; ²Foreign Disease-Weed Science Research Unit, USDA-ARS-NAA, 1301 Ditto Avenue, Ft Detrick, MD, 21702-5023; ³Sustainable Perennial Crop Laboratory, PSI, USDA/ARS-BARC-W, 10300 Baltimore Ave, Bldg 001, Beltsville, MD 20705-3000

Introduction: *Phakopsora pachyrhizi*, the fungus that causes the Asian soybean rust (ASR) disease, is a very aggressive pathogen that can significantly reduce soybean yield (up to 80%). The host range of *P. pachyrhizi* is broad, with more than 90 plant species including common beans (*Phaseolus vulgaris* L.) (Miles et al., 2007). *P. pachyrhizi* was first observed in Louisiana, USA, in 2004, and within two years, it was found in 274 counties in 15 states (<http://www.sbrusa.net>; USDA, 2007). ASR poses a major threat to North American soybean production since none of the U.S. soybean commercial cultivars are resistant to *P. pachyrhizi* (Miles et al., 2003). Recently, Miles et al. (2007) identified *P. vulgaris* cultivars [Compuesto Negro Chimaltenango (CNC), Aurora, PI 181996 and Pinto 114] that were resistant to six isolates of *P. pachyrhizi* from Asia, Africa and Latin America. These cultivars had lower disease severity, less sporulation and consistent reddish-brown (RB) lesions, which is associated with resistance in soybean. These findings suggest that these four cultivars have genes for resistance to *P. pachyrhizi* that might be a source of protection for common bean and soybean against ASR. The current study was undertaken to identify molecular markers in these common bean cultivars that are associated or linked to ASR resistance. Here, we report preliminary findings on potential molecular markers using an F₂ population derived from a cross between the susceptible and resistant *P. vulgaris* cultivars, Mexico309 and CNC, respectively (Miles et al., 2007).

Material and Methods: Cultivars Mexico 309 (susceptible) and CNC (resistant) were crossed to produce the F₂ population used in this study. Young leaf tissue was collected from both parental cultivars and 117 F₂ plants, prior inoculation with the *P. pachyrhizi* isolate BZ01-1. Tissue samples were frozen in liquid nitrogen and stored at -80°C. Each sample was ground to a fine powder in the presence of liquid nitrogen, and genomic DNA was extracted from 100 mg using a DNeasy Plant Mini kit (Qiagen). Primers were based on SSR, plant defense-related genes, and conserved regions associated with resistance (R) genes. Selected sequences were blasted against *P. vulgaris* ESTs, *Glycine max* and other leguminous plants for primer design. Their length ranged from 17 to 26 nucleotides, and the forward primer had a WellRed-D4 dye label coupled at its 5' end. Thirty oligonucleotide primer sets (herein designated Pri1 through Pri30) were initially tested with Mexico 309 and CNC. Those that produced signals representing polymorphic fragments between the parents were subsequently tested with the F₂ population. PCR was conducted using 3 ng DNA in 12 µL, which contained 0.2 mM dNTPs, 0.5 mM MgCl₂, 500 nM primer, 1 x Qiagen *Taq* buffer and 0.6 U of HotStar*Taq* DNA polymerase (Qiagen). The annealing temperature, depending on the primer set tested, varied from 48 to 58°C. Amplicons were analyzed in the CEQ™ 8000 Genetic Analysis System / Beckman Coulter (Fullerton, CA). Disease symptoms on Mexico309, CNC, the F₂ population and soybean cultivar Williams 82, a positive control, were rated in the USDA-ARS FDWSRU Biosafety Level 3 Containment

Greenhouse at Fort Detrick, MD according to Miles et al. (2007). Chi-square (χ^2) was used to test for goodness-of-fit of observed to the expected 3:1 and 9:2 ratios in the F₂ population.

Results and Discussion: Signals representing polymorphic DNA fragments were obtained with primers #13 (CNC had a 664-bp fragment not found in Mexico309); # 14 (CNC had a 694-bp fragment not present in Mexico309) and #27 (Mexico 309 had a 272-bp fragment not found in CNC). The phenotypic data for the F₂ population (117 plants) were 69 resistant and 48 susceptible plants, suggesting that resistance was controlled by a putative two-gene segregation ($\chi^2 = 0.35$). The genotypic results for the F₂ plants, based on these primers, and associated with the phenotypic data are as follow:

Primer 13

Genotype of the F ₂ plants	Phenotype	# Plants	Total	Ratio	χ^2
with 664 bp	Resistant	51	86	3:1	0.01712
with 664 bp	Susceptible	35			
without 664 bp	Resistant	18	31		
without 664 bp	Susceptible	13			

Primer 14

Genotype of the F ₂ plants	Phenotype	# Plants	Total	Ratio	χ^2
with 694 bp	Resistant	50	82	3:1	0.93103
with 694 bp	Susceptible	32			
without 694 bp	Resistant	19	34		
without 694 bp	Susceptible	15			

Primer 27

Genotype of the F ₂ plants	Phenotype	# Plants	Total	Ratio	χ^2
with 272 bp	Resistant	30	44	9:7	1.59606
with 272 bp	Susceptible	14			
without 272 bp	Resistant	38	72		
without 272 bp	Susceptible	34			

The 3:1 ratios suggest that a putative gene is involved, while the 9:7 ratio suggests a putative two-gene segregation. Although none of the markers showed a cosegregation that completely matched the resistance/susceptible phenotypes, these preliminary findings are encouraging for tagging resistance to ASR in *P. vulgaris*. About 150 additional F₂ plants will be tested to confirm these findings. Other primers based on known genomic regions are being evaluated.

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EVALUATION OF COMMON BEAN CULTIVARS FROM THE UNITED STATES FOR THEIR REACTION TO SOYBEAN RUST UNDER FIELD CONDITIONS IN BRAZIL AND SOUTH AFRICA

M. A. Pastor-Corrales¹, A. Sartorato², M. M. Liebenberg³, M. J. del Peloso², P. A. Arraes Pereira^{2,4}, J. Nunes Junior⁵, H. Dinis Campos⁶

¹Vegetable Laboratory, USDA-ARS, Beltsville, MD, 20705-2350, USA, ²EMBRAPA Arroz e Feijao, C.P.179, CEP 75375-000, San Antonio de Goias, GO, Brazil, ³ARC-Grain Crops Institute, Private Bag X1251, Potchefstroom, 2520, South Africa, ⁴Embrapa/Labex, USDA, ARS, OIP, Beltsville, MD 20705-5141, USA, ⁵Centro Tecnológico de Pesquisa Agropecuária Ltda., C. Postal 533, CEP 74001-970, Goiânia, GO, Brazil, ⁶Universidade Federal de Rio Verde, Rio Verde, GO, Brazil. Corresponding author: pastorm@ba.ars.usda.gov

Introduction. The host range of the soybean rust pathogen (*Phakopsora pachyrhizi*) is broad with at least 95 leguminous species, including dry and snap beans (*Phaseolus vulgaris*). This pathogen was initially reported in the Americas infecting mostly soybean (*Glycine max*) but also kudzu (*Pueraria spp.*). However, recently *P. pachyrhizi* has been reported infecting dry beans in South Africa, the U.S., Argentina, and Brazil. However, little is known about the capacity of *P. pachyrhizi* to infect and cause damage to most common bean cultivars under field conditions. There is speculation about the possible negative effect that soybean rust may have on common bean, especially where dry bean and soybean are planted in adjacent fields, as it occurs in the U.S., Canada, Brazil, Argentina, South Africa and other countries. We report here the reaction of common bean cultivars belonging to important commercial classes in the U.S. and Canada, to a natural infection of the soybean rust pathogen under field conditions of Brazil and South Africa.

Material and Methods. Twenty seven dry bean cultivars from nine commercial market classes were evaluated in three locations in the state of Goias, Brazil: 1. Senador Canedo (four evaluations), 2. Goiania (three evaluations), and 3. Rio Verde (three evaluations). Bean cultivars were planted adjacent to fields with soybeans naturally infected with *P. pachyrhizi*. In all three locations soybean varieties were included as checks. Leaves from top, middle and lower parts of the common bean plants were collected and evaluated for soybean rust severity using a 0-100 % scale. In South Africa, bean cultivars were planted in Cedara, KwaZulu-Natal province (two evaluations) in unreplicated single rows, with an inter-row spacing of 75cm. The side rows and every second row contained a mixture of highly susceptible early and late soybean cultivars. To ensure a constant supply of inoculum, one half of these rows were planted one month before and the rest at the same time as the trial entries. Disease severity was rated on five plants per row, 82 and 89 days after planting using a 1-9 scale where 1 corresponded to no visible soybean rust symptoms and 9 to very severe symptoms that resulted in severe premature defoliation.

Results and Discussion. In Brazil, all common bean cultivars in all three locations had very mild soybean rust symptoms compared to the soybean check cultivars (Table 1). Conversely, the soybean plants had very severe soybean rust symptoms in Senador Canedo (100%) and Goiania (78%), and mild (20%) in Rio Verde. In South Africa, all common bean cultivars had various degrees of soybean rust symptoms, but these were rather mild compared to the symptoms on the soybean cultivars. In a study conducted in South Africa, (Liebenberg et al 2007), it was found that soybean rust symptoms on common bean decreased significantly within 20 m from the inoculum source. Thus, it appears that the more severe soybean rust symptoms observed in common beans in South Africa (than in Brazil) was due to the close proximity of the common beans to heavily infected soybeans. We have included in the table data the common bean cultivars, PI 181996, CNC, Aurora, and Pinto 114 for comparison. These cultivars (highlighted in Table 1) were the resistant to six different isolates of the soybean rust pathogen from Africa,

Asia and South America under greenhouse conditions. Interestingly, they were also among the most resistant common bean cultivars under field conditions in Brazil and South Africa.

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Reaction of common bean cultivars to natural infection of the soybean rust pathogen (<i>Phakopsora pachyrhizi</i>) under field conditions in Brazil and South Africa								
Bean Cultivar (Market class)	Brazil ¹				South Africa ²			
	Aver. Dis. Severity (0-100%)			Average Disease Incidence	Disease Rating (1-9)			
	Senador Canedo	Goiania	Rio Verde		82 days		89 days	
				Lower	Upper	Lower	Upper	
Buster (Pinto)	0.69	4.37	0.0	1.72	(A)	-	-	-
California Early (LRK)	0.71	3.49	0.10	1.43	(R, A)	-	-	-
Montcalm (DRK)	0.29	1.65	0.20	0.71	(A, R)	-	-	-
Beryl (GN)	0.43	1.28	0.27	0.66	0.0	0.0	(R)	-
Norstar (Navy)	0.64	1.28	0.00	0.64	3.5	0.0	DF	3
Matterhorn (GN)	0.55	0.85	0.17	0.52	3.0	0.0	DF	3.5
Midnight (Black)	1.00	0.07	0.00	0.36	3.5	0.0	DF	2
Red Hawk (DRK)	0.19	0.77	0.10	0.35	(A)	-	-	-
Bill Z (Pinto)	0.57	0.22	0.13	0.31	(R)	0.0	-	-
Montrose (Pinto)	0.76	0.09	0.07	0.31	0.6	0.0	DF	0?
Chinook 2000 (LRK)	0.38	0.40	0.03	0.27	(A)	-	-	-
Jaguar (Black)	0.17	0.40	0.17	0.25	2.2	0.0	DF	3.75
SVM Taylor (Cranberry)	0.08	0.60	0.07	0.25	(A)	-	-	-
Brooks (Small Red)	0.18	0.36	0.13	0.22	3.0	0	(R)	-
Pink Panther (LRK)	0.15	0.30	0.17	0.21	(A,R)	-	-	-
Eclipse (Black)	0.01	0.21	0.17	0.13	4.2	2.0	DF	4
Otebo (White)	0.24	0.06	0.07	0.12	(R)	0.0	(R)	-
T-39 (Black)	0.09	0.07	0.17	0.11	4.0	0.0	5.0	2.4
Sedona (Pink)	0.10	0.07	0.13	0.10	0.0	0.0	DF	3.5
Merlot (Small Red)	0.19	0.08	0.00	0.09	0.0	0.0	(R)	-
Seahawk (Navy)	0.06	0.04	0.13	0.08	3.0	0.0	DF	4
UI 239 (Small Red)	0.12	0.04	0.03	0.06	(A;R)	-	-	-
Vista (Navy)	0.04	0.02	0.10	0.05	(R)	-	-	-
CNC	0.09	0.06	0.07	0.07	4.8	0	DF	2
Arthur (Navy)	0.00	NG ³	0.07	0.04	3.5	0.0	DF	4
Othello (Pinto)	0.04	0.05	0.00	0.03	(R)	-	-	-
Maverick (Pinto)	0.06	0.00	0.00	0.02	3.0	0.0	(R)	-
Condor (Black)	0.00	0.02	0.03	0.02	3.2	0.0	DF	2
Pinto 114	0.01	0.06	0.07	0.05	(R)	-	-	-
Aurora	0.01	0.00	0.10	0.04	(R)	-	-	-
PI 181996	0.00	0.00	0.03	0.01	nd	nd	5.0*	0.0*
Soybean variety (Check)	100.00	78.30	20.10		1 st : 7 2 nd : 5	1 st : 5 2 nd : 3	1 st : 9 2 nd : 9	1 st : 9 2 nd : 4

Soybean rust evaluation. In Brazil, leaves were evaluated using a 0-100 % scale. In South Africa, soybean rust was rated on five plants per row using a 1-9 scale, where 1 corresponded to no visible symptoms and 9 to very severe symptoms that resulted in severe premature defoliation. (A, R) = dead or defoliated due to angular leaf spot (A) or common bean rust (R). 1st = first soybean planting, one month before common beans; 2nd = second soybean planting, together with common beans); nd = no data; * = 2005 data; ³= Did not germinate.

ASIAN SOYBEAN RUST ON COMMON BEAN AND OTHER LEGUMES

MM Liebenberg¹, AJ Liebenberg¹ and ZA Pretorius²

¹ARC-Grain Crops Institute, Private Bag X1251, Potchefstroom, 2520, South Africa

²Department of Plant Sciences, University of the Free State, PO Box 339, Bloemfontein 9300, South Africa

¹LiebenbergM@arc.agric.za

INTRODUCTION: The fungus *Phakopsora pachyrhizi*, causal agent of Asian soybean rust, is a major cause of yield losses of soybean. It has also been reported to be pathogenic on a large number of legumes (Smith, 2004; Lynch et al., 2006) but nothing was known of its possible effect on these hosts in South Africa. In 2004 it was observed to cause severe symptoms and defoliation of the common bean under high disease pressure at Cedara Agricultural Research Station near Pietermaritzburg and in 2005/6 a study was undertaken to quantify this effect (Liebenberg et al., 2006). It was observed that, although serious damage to bean plants occurred when adjacent to soybeans, very little damage occurred in fields further removed from the inoculum source. In the present study, the effect of Asian soybean rust on the common bean and other legumes over increasing distance from infected soybeans, and their ability to sustain an epidemic in the absence of soybeans, was studied. The effect of one application of fungicide on the disease (common bean only) was also investigated.

MATERIALS AND METHODS: A legume trial (randomised block design) with three replications and containing six selected common bean (*Phaseolus vulgaris*) accessions {CAL 143, CNC, Mkuzi (A 286), NEP 2, Sederberg and Teebus-RR1} and one variety of each of five other legumes, namely *Vigna unguiculata* (cowpea: cv. PAN 311), *Pisum sativum* (pea: cv. Green Feast), *P. coccineus* (“large white kidney” bean: cv. GWK 1), *P. lunatus* (lima bean: Western Cape landrace) and *P. acutifolius* (teparty bean: line T19) were planted at Cedara in January 2006. Common bean accessions were chosen which were resistant to other diseases prevalent in the area. The legumes were planted in 20 m rows with one end adjacent to a mixture of early and late maturing, highly susceptible soybeans and the further end 20 m from the inoculum source. Relative area covered by sporulating pustules (PA) and necrotic flecking (NF) were rated in both the lower and upper canopies at 0, 10 and 20 m from the source at various times after planting. Ratings at 102 days after planting are reported here. Yield of the five plants closest to 0, 10 and 20 m from the source was recorded.

The fungicide trial (a factorial with three common bean accessions, namely Mkuzi, Sederberg and Teebus-RR 1) was laid out as a randomised block design with six replications.. One application of a carbendazim/flusilazole (250/125 g/l) fungicide at a rate of 400 ml/ha was applied 62 d after planting when the first soybean rust symptoms appeared on common bean. No fungicide was applied on the control plots or on the soybeans. A mixture of highly susceptible soybean cultivars was planted between all the plots. PA and NF were rated in both the lower and upper canopies at various times after spraying (ratings at 42 days are reported here) and yield recorded.

In both trials the soybeans were planted one month before the other legumes. By middle March they had become heavily infected and had defoliated by the second week of April. Ratings were subjected to ANOVAs and regression analyses using StatGraphics Plus version 5.0. LSD ($p=0.05$) was used for the comparison of means.

RESULTS: *Legume Trial:* Symptoms decreased with increasing distance from the source. Both PA and NF in the lower canopy differed significantly at 0 and 20 m from the source for all

accessions. For common bean, there was a positive correlation ($r = 0.88$) between PA and NF. Early senescence, marked necrosis and premature chlorosis occurred and decreased with increasing distance but were not significantly correlated with above, although they did appear to adversely affect the plants. Reaction in the upper canopy was similar to but generally less severe than that in lower canopy. Yield of common bean cultivars with an indeterminate growth habit increased significantly at 10 m above that at 0 m from the source. Those with a determinate growth habit were more seriously affected and for these accessions, there were no significant yield increases with increasing distance from the source. The lima bean and cowpea were the least affected. Both had very few pustules, the latter showing no yield increase with increasing distance from the source (the lima bean did not flower). PA on the cowpea and lima bean was significantly less than on other accessions although both showed a high degree of NF on the upper leaf surface at 0 m and to a lesser degree at 10 m and 20 m. The *P. coccineus* cultivar GWK 1, the pea cultivar Green Feast and the tepary bean (line T19) were the most seriously affected and increasing distance had less effect on symptoms. PA differed significantly between 0 and 20 m but not between 0 and 10 m or between 10 and 20 m. For GWN 1 and T-19 there was no yield increase over increasing distance (peas, a winter crop in SA, did not yield) and symptoms were also severe in the upper canopy.

Fungicide trial: The reactions of all three accessions were similar. Fungicide had a significant positive effect on both PA and NF and on mean yield, which increased by an average of 63% above that of the unsprayed control. The cv. Teebus RR-1, with a determinate growth habit, benefited significantly more from the fungicide than the other two (indeterminate) cultivars, probably because almost no new growth that could be infected appeared after the fungicide application.

CONCLUSIONS: All legumes tested were infected in the field by *P. pachyrhizi*. Increasing distance from infected soybeans generally results in lower infection levels and the common bean, cowpea cv. PAN 311 and lima bean (Western Cape landrace) cannot sustain an epidemic of Asian soybean rust without the close proximity of infected soybeans. However, general conclusions for cowpeas and lima beans as crops cannot be drawn, as differences in susceptibility may exist between accessions within the same species. *P. coccineus* cv. GWN 1, tepary line T19 and pea cv. Green Feast, once infected, may be able to sustain an epidemic of Asian soybean rust under favourable climatic conditions and may also serve as significant sources of inoculum for infection of soybean crops. The effect of pathogenic variability on the various legumes and viability of spores from these hosts needs to be determined. Experience in the greenhouse indicates that spores from secondary hosts may be less viable than those from soybeans. This may not be the case for those legumes apparently able to maintain the epidemic in the field.

One fungicide application, applied when symptoms appear, can give satisfactory control of Asian soybean rust on common bean but should only be necessary where the crop is planted adjacent to infected soybeans under conditions favourable for the disease. Follow-up trials have been planted to confirm the above conclusions.

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REACTION OF ONTARIO DRY BEANS TO ASIAN SOYBEAN RUST (*PHAKOPSORA PACHYRHIZI*) IN PRELIMINARY TRIALS IN SOUTH AFRICA

Soon J. Park¹, Merion M. Liebenberg² and Liezl C. van Jaarsveld³

¹ Agriculture and Agri-Food Canada Greenhouse and Processing Crops Research Centre, Harrow, Ontario, Canada N0R 1G0, ² ARC GRAIN Crops Institute, P/Bag X 1251, Potchefstroom, 2520, South Africa and ³ Department of Plant Sciences, University of the Free State, PO Box 339, Bloemfontein 9300, South Africa

Soybean rust caused by *Phakopsora pachyrhizi* is a new disease spreading from Asia through the Middle East, Zimbabwe, South Africa, across Atlantic Ocean to Brazil and Colombia, and it was found in the southeastern U.S. in November 2004. It has since been found as far north as Indiana in 2006. Although it will not overwinter in Canada, it may occasionally reach Canada by air currents similar to the patterns of common bean rust (*Uromyces appendiculatus*) and wheat rust (*Puccinia* sp). Therefore, we conducted a preliminary testing of Canadian dry bean cultivars in South Africa for their reaction to this new rust. Field and greenhouse trials were carried out during 2005-2007 with 13 dry bean cultivars/line developed in Ontario, Canada [5 navy beans (n), 3 kidney beans (rk), 2 pinto beans (p), 2 black beans (b) and a cranberry line (cr)] to examine their reaction to soybean rust.

A field trial was planted (07-12-2005) in unreplicated single row plots alternately with soybeans to spread rust spores to beans. Soybean rust infection was observed as premature leaf yellowing (yellow), sporulation on the lower (LW) and upper (UP) leaves on 12-04-2006 and a week later on 19-04-2006 in a scale of 0 to 9 (where 9 indicates very severe disease, leading to defoliation). The general effect of the soybean rust on the whole plot was also estimated using the 1 to 9 scale. All navy beans were susceptible to Asian soybean rust, and lower leaves defoliated. Other diseases, including angular leaf spot, bean rust, and Ascochyta blight complicated the results, causing defoliation and death of the plants, because all Ontario beans were highly susceptible (Table 1). Observations of whole plots on May 5, 2006 revealed some differences among the varieties: Nautica (3, low), Trident (5.5, ms), AC ELK, Pintoba and Harohawk (susceptible) and the rest were highly susceptible. In the field, indeterminate growth habit appeared to be an advantage as plants continued to produce fresh leaves which took some time to succumb to the disease.

Greenhouse trials were conducted with the same 13 entries by inoculating the primary leaves of seedlings planted in cones (2 seeds/cone x 3 reps) on 30-08-2006 (8 mg of soybean rust spores per 24 plants) on 13-09-2006. The rust was scored on Oct 2 and 9, 2006 for pustule size and quantity (using a scale of 0-9, which were each converted to a percentage of the maximum possible rating). Leaf reactions (necrotic flecking, premature chlorosis and premature defoliation) were also rated, each expressed as a percentage of the maximum. These measurements, together with an overall disease score that incorporates all measurements are shown in Table 2. Leaves of many plants were defoliated on the second observation. All navy beans and pinto beans were moderately susceptible and all other classes (red kidney, cranberry and blacks) highly susceptible. The trait "Quantity" was most in accordance with the field data. Nautica, AC ELK, AC Trident, and Pinoba, as well as Galley, had fewer pustules, whereas pustule size was moderate to large for all accessions.

Overall results from both field and greenhouse trials suggested that the dry beans tested were moderately to highly susceptible to soybean rust when inoculum levels are high. Also, most of the entries were susceptible to other leaf diseases such as Ascochyta blight, angular leaf spot and bean rust which affected the results.

Table 1. Reaction of Ontario Dry Beans to Asian Soybean Rust (*Phakopsora pachyrhizi*) in a Single Row Evaluation Trial in South Africa in 2006

Accession	12-04-2006 (0-9)			19-04-2006 (0-9)			General Rate (0-9)	General Comment*		
	Yellow	Low LF	Up LF	Yellow	Up LF	PP Rate		Asc	Als	Ua
AC Trident (n)	med	1.8	1.8	def	def	2.5	5.5		7	6
AC Compass (n)	med	2.4	2.4	"	"	3.0	7.0	7	7	3
Gallery (n)	med	3.6	3.8	"	"		7.9	7		5
Nautica (n)	med	2.0	0.0	"	M	4.8	3.0			5
OAC Rex (n)	med	3.0	0.0	"	L	5.5	5.5			8
AC Calmont (drk)	dead	-	-	def	def		7.5	S to all leaf diseases		
Majesty (drk)	med	0.0	0.0	dead	dead		8.0		7	8
AC ELK (lrk)	dead	-	-				6.0		9	7
AC Pintoba (p)	low	0.0	0.0	dead	dead		6.0		4	6
Pecos (p)	dead	-	-	dead	dead		8.0			9
AC Harblack (b)	low	3.0	0.0	dead	M	5.0	6.0			8
Harohawk (b)	low	0.0	0.0	dead	L	3.0	6.0			7
HR 163 (cran)	dead	-	-	dead	dead		7.5		9	8

* Ascochyta blight (Asc), angular leaf spot (Als), and bean rust (Ua).

Table 2. Results of Screening Ontario Dry Beans with Asian Soybean Rust in the Greenhouse in South Africa in 2006 showing mean percentages.

<i>Cultivar</i>	Pustule Size (%) (std error: 8%)	Quantity (%) (std error: 9%)	*Disease Score (%) (std error: 5%)
<i>AC Calmont</i>	78	67	60
AC Compass	78	33	46
AC ELK	93	9	56
AC Harblack	90	43	54
AC Pintoba	80	23	43
AC Trident	75	12	43
Gallery	70	20	40
Harohawk**	70	30	48
HR 163	87	37	60
Majesty	75	60	67
Nautica	85	6	47
OAC Rex	70	40	52
<i>Pecos</i>	72	27	43

*Disease Score = (0.3 * Pustule Size) + (0.3 * Quantity) + (0.2 * Necrotic Flecking) + (0.1 * Chlorosis) + (0.1 * Premature Defoliation). **Only one replicate.

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CORRELATIONS OF WHITE MOLD RESISTANCE IN SNAP BEAN (*PHASEOLUS VULGARIS* L.) AMONG FIELD EVALUATIONS, OXALIC ACID TEST, AND STRAW TEST

Yong Suk Chung, Michell E. Sass, and James Nienhuis
Department of Horticulture, University of Wisconsin – Madison

White mold is an important constraint of dry and snap bean production in the United States (Park et al., 2001). In snap bean, yield reductions due to white mold infection result in weakened stems which break along with attached pods during harvest and also due to discolored pods infected with fluffy mycelial growth (Kull et al., 2003). The objective of the research was to compare two types of greenhouse tests and field based methods to screen for resistance to white mold in snap bean.

Correlations between field and greenhouse evaluations were measured using two populations that share a common white mold resistant donor parent, G122. A recombinant inbred line (RIL) population, GA, was derived from a cross between G122 and Astrel, a small sieve snap bean cultivar. An independent inbred backcross population (IBC), GPP, was derived from a cross between G122 and PLS8088, a large sieve processing snap bean cultivar. Field evaluations were performed at the Arlington, WI Agricultural Research Station (ARS) in 2004 and 2005 using a blocks within replication design with three replications. Disease severity (DS) was calculated as the mean number of diseased branches per plant using eight plants sampled at random from each plot. Disease incidence (DI) was calculated as the percentage of plants with at least one infected branch using all plants within each plot. Greenhouse evaluations using a randomized complete block design were done using the oxalic acid test (OX) (Kolkman and Kelly, 2000) in 2005 and using a modified straw test (ST) (Petzoldt and Dickson, 1996) in 2005 and 2006.

Large positive correlations (0.87, 0.80) were observed between the two field evaluations based on DI and DS in the GA and GPP populations, respectively (Table 1). If the plants in a plot are genetically homogeneous as would be expected in inbred populations, then all plants would be expected to respond the same for white mold resistance. Thus, the deviation in the correlation between DI and DS is likely due to field heterogeneity and experimental error rather than due to differences in their disease reaction mechanisms.

The correlations between the field evaluation for white mold resistance based on DI and DS and the greenhouse evaluations based on ST and OX were low and mostly nonsignificant (Table 1). As previously reported in dry and snap bean populations, the correlations between field and greenhouse evaluation varied from low to moderate (0.24 to 0.45) in RIL populations derived from G122 and A55 (Miklas et al., 2001). The effect of variation in canopy density on the incidence and severity of field grown dry beans indicates that closed canopies promote greater plant-to-plant contact within and among the rows resulting in the infection of healthy plants by infected plants (Vieira et al., 2003). The IBC population (GPP) has a greater variation for canopy width compared to the RIL population (GA). Thus, the lower correlations observed between field and greenhouse evaluations of white mold resistance with the GPP population compared to the GA population may be due to the smaller architectural stature of the Astrel parent compared to PLS8088 parent.

The low correlations between the field and greenhouse methods to evaluate white mold resistance suggest that field and greenhouse methods may be associated with different components of white mold resistance based on physiological and avoidance mechanisms as well

as environmental fluctuation in the field. The magnitude of phenotypic correlations suggests that in spite of higher heritabilities associated with the greenhouse evaluation methods; selection based on ST and OX may not result in the expected correlated response for improved white mold resistance in the field.

Table 1. Spearman correlation coefficients among two greenhouse tests, and two field evaluations for resistance to white mold over years (GA is above diagonal, and GPP is below diagonal).

	DI	DS	OX	ST
DI	1.00	0.87*** (66†)	0.19ns (64†)	0.18ns (62†)
DS	0.80*** (66†)	1.00	0.20ns (64†)	0.31* (62†)
OX	0.06ns (66†)	0.10ns (66†)	1.00	0.37** (60†)
ST	0.26* (66†)	0.27 (66†)	0.00ns (67†)	1.00

*, **, and *** Significant at the 0.05, 0.01, and 0.001 probability level, respectively.

ns = Nonsignificant at $P < 0.05$.

† indicates number of entries.

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BULGARIAN LANDRACES AND LINES OF COMMON BEAN (*PHASEOLUS VULGARIS* L.) WITH PHYSIOLOGICAL RESISTANCE TO WHITE MOLD

Dimitar Genchev and Ivan Kiryakov
Dobroudja Agricultural Institute – General Toshevo 9520, Bulgaria,
E-mail: genchev@dai-gt.org

White mold (WM) caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is one of the most harmful diseases on a number of crops, including common bean. Its harmful effect on bean is affected by the growth habit, crop production practice and by the physiological resistance of the cultivars (Schwartz et al., 1987). Combining physiological resistance with mechanisms for prevention of infection and pathogen development (erect plant, open plant canopy) is the current breeding strategy for minimizing yield losses due to white mold in common bean (Miklas et al., 2000).

Material and Methods

219 accessions from Dobroudja Agricultural Institute – General Toshevo core collection were tested for physiological resistance to white mold (WM). Twenty plants from each accession were used for each test. Four weeks following sowing, plants were inoculated by the straw-test (Petzoldt and Dickson, 1996). Each plant was inoculated with a 3 day-old mycelial plug from a colony of *S. sclerotiorum* isolate SsPh-2 (bean isolate) grown in the dark on potato dextrose agar (PDA) at 20°C, in the field. Individual plants were rated for their reaction to WM on a 1 to 9 scale according to Petzoldt and Dickson (1996).

The accessions with physiological resistance to WM were scored to growth habit, common (CBB) and halo (HB) bacterial blight, anthracnose (ANT) and rust according to Singh (1982), Kiryakov (1999), Genchev (1983) and Stavely (1983), respectively.

Results and Discussion

Twelve out of the thirteen accessions were with compact and upright growth habit (I and II), and only one accession was with growth habit IV (Table 1). The growth habit is one of the reason for avoidance of the disease. Growth habit of I and II type facilitate air circulation and light penetration within the canopy to help reduce infection. I and II habit type ensured not only good airing of the crop, but also had well developed mechanisms providing the erect position of the plants. Probably physiological resistance is linked to the degree of development of the mechanical elements.

All accessions were susceptible to CBB (Table 1), seven accessions: IIRR 7585(3.0, 3.2), DS 186(3.0, 3.0), Grozdevo(3.7, 3.7), Padesh 1(2.0, 3.0), Hotovo 2(2.8, 3.0), DG 84-34-1(5.0, 4.2) and IIRR 1426(2.2, 4.5), showed resistant reaction of leaves to races 1 and 6 of HB; eight accessions showed resistant reactions of pod to race 1 of HB: DG 91-10-Lan(5.0), IIRR 7585(3.5), DS 186(3.0), Grozdevo(3.0), Padesh 1(1.0), Hotovo 2(2.3), DG 84-34-1(4.5) and IIRR 1426(3.0); three accessions were with resistance of pod to race 6 of HB: DS 186(3.0), Grozdevo(5.0) and IIRR 1426(4.5); Three accessions showed resistance to race 81 of ANT: IIRR 7585(2.0), Dounav 1(3.0) and Grozdevo(2.1); and ten accessions: DG 91-10-18(5.0), IIRR 7585(3.0), Dounav 1(3.0), Grozdevo(5.0), Padesh 1(4.0), Hotovo 2(3.0), DG 84-34-1(5.0), DG 85-2-6(4.0), Rouets(5.0) and IIRR 1426(3.0) showed resistant reaction to races 20-0, 20-2 and 20-3 of rust. The landrace Grozdevo is resistant to race 1 and 6 (leaf and pod) of HB, to race 81 of ANT and to races 20-0, 20-2 and 20-3. Resistant to races 1 and 6 (leaf and pod) of HB and races 20-0, 20-2 and 20-3 of rust was landrace IIRR 1426.

Future work should investigate the performance of the land races and breeding lines of common bean when exposed at different growth stages to different types of inoculum under varying environmental conditions (field and greenhouse). These resistant parents should be useful for future genetic improvement of multiple disease resistance of common bean.

Table 1. Scores for 13 Bulgarian landraces and breeding lines of common bean with physiological resistance to white mold evaluated for common (CBB) and halo (HB) bacterial blights, anthracnose (ANT), rust, growth habit (GH), 100 seed weight (SW) and days to physiological maturity (PM).

Accession	WM	Seed	GH	SW	PM	CBB		Halo blight				ANT	Rust
						Leaf	Pod	Leaf		Pod			
								Race 1	Race 6	Race 1	Race 6		
DG 91-10-18	3.0		II	23.4	96	9.0 ^Z	9.0	7.0	7.0	7.0	7.0	9.0	5.0
DG 91-10-Lan	3.0		II	21.8	93	9.0	9.0	6.0	6.0	5.0	6.0	9.0	7.0
IIRR 7585	3.0		I	54.8	84	8.2	8.0	3.0	3.2	3.5	5.7	2.0	3.0
DS 186	3.0		II	20.0	75	9.0	7.3	3.0	3.0	3.0	3.0	9.0	9.0
Dounav 1	3.7		I	37.4	83	8.0	8.0	7.0	7.0	7.0	7.0	3.0	3.0
Grozdevo	4.0		I	28.7	76	9.0	9.0	3.7	3.7	3.0	5.0	2.1	5.0
Padesh 1	4.0		I	37.1	79	8.0	9.0	2.0	3.0	1.0	6.0	9.0	4.0
Hotovo 2	5.0		I	24.1	82	9.0	8.0	2.8	3.0	2.3	5.3	9.0	3.0
Samoranovo 2	5.0		IV	46.7	89	9.0	7.0	8.0	9.0	5.3	5.7	9.0	9.0
DG 84-34-1	5.4		II	42.6	98	8.8	7.0	5.0	4.2	4.5	5.7	9.0	5.0
DG 85-2-6	5.5		II	30.9	91	8.0	8.3	6.0	5.8	7.0	7.0	9.0	4.0
Rouets	5.7		I	49.5	82	8.3	9.0	6.6	6.8	7.0	8.3	9.0	5.0
IIRR 1426	6.0		I	44.7	78	7.0	9.0	2.2	4.5	3.0	4.5	9.0	3.0

^ZResistant reaction: 1 – immune reaction and 9- very susceptible reaction.

^YGrowth habit: I – Determinate growth habit; II – Indeterminate growth habit, vegetative terminal bud on main stem and branches, both main stem and branches strong and upright; III – Indeterminate growth habit, branches relatively weak and open, semi-prostrate or twining.

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IDENTIFICATION OF PARTIAL RESISTANCE TO *SCLEROTINIA SCLEROTIORUM* IN COMMON BEAN AT MULTIPLE LOCATIONS IN 2006

L.K. Otto-Hanson and J.R. Steadman (University of Nebraska-Lincoln). Data from C. Kurowski (CA), R. Mainz (MN), J. Kelly (MI), P. Griffiths (NY), B. Schatz (ND), J. Myers (OR), P. Miklas (WA), H. Schwartz (CO), S. Singh (ID), and K. Kmiecik (WI).

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

Field tests consisted of two rows of each entry and a common susceptible genotype, resulting in a three-row plot 4.6m (15 ft.) long replicated three times in a randomized complete block design. There were 16 screening tests at 11 locations, eight field and eight greenhouse (straw test). The greenhouse screen tested 22 bean lines this year, and the field screen tested 12 bean lines. Due to lack of seed, Cornell 601 was used in OR, ID, and CA, while the other five locations received VA 19. The field test results were ranked from most resistant (1) to most susceptible (12) (Table 1). The straw tests were analyzed by using the mean rating of each entry from each location (1=most resistant, 9=most susceptible). Spearman and Pearson correlations were used to compare entry ratings in the greenhouse test, and entry rankings in the field test.

The highest positive field correlations were ND and MN ($r=0.779$, $p=0.0028$), and NE and WA ($r=0.732$, $p=0.0068$). The significant positive straw test correlations were CO and NY ($r=0.636$, $p=0.0015$), NY and OR ($r=0.807$, $p<0.0001$), NY and NE ($r=0.647$, $p=0.0011$), OR and NE ($r=0.596$, $p=0.0034$), OR and WI ($r=0.545$, $p=0.0087$), and WA and WI ($r=0.588$, $p=0.0040$). There was greater agreement in the straw tests than in the field tests.

When an ANOVA was used on field rankings with each test as a block and bean line (entry) as a treatment, differences among the lines were highly significant ($p<0.0001$). Highly significant differences ($p<0.0001$) were also found among the lines in the greenhouse straw test.

Cornell 603, 604, and 605 were identified as having white mold resistance in both the field and greenhouse tests. The multi-site field tests combined with greenhouse results helped to identify disease escape or avoidance in WM 55 that is similar to Bunsu. WM 55 was ranked among the susceptible lines in the greenhouse but was intermediate in the field.

The USDA has released AN 37 (USPT-WM-1) as a white mold resistant pinto bean line. P. Miklas. ARS-USDA. 29 August 2006. New pinto beans resist white mold. Plant Health Progress.

Table 1. Mean rankings of bean entries (lines) (1=most resistant) for white mold reaction in eight different field locations using ANOVA (Alpha=0.05, LSD=2.9).

Entry	NE	WA	MN	CA	ID	OR	ND	MI	Mean Ranking	t Grouping
Beryl	12	12	12	12	12	12	12	12	12.0	A
AN 37	10	5	7	9	10	8	11	10	8.8	B
B05001	9	11	11	4	5	7	5	9	7.6	C B
Bunsi	5	8	4	11	9	9	4	11	7.6	C B
IO1892-115M	11	10	6	5	2	6	8	7	6.9	C B
Cornell 603	6	4	9	8	1	10	9	6	6.6	C B
G122	4	2	9	10	11	3	9	4	6.5	C B
WM 55	8	9	5	6	5	5	2	5	5.6	C D
VA 19	7	6	7	.	.	.	5	2	5.4	C D
Cornell 601	.	.	.	1	4	11	.	.	5.3	C D
Cornell 606	1	1	3	7	2	1	5	8	3.5	E D
Cornell 604	1	7	2	3	5	4	3	3	3.5	E D
Cornell 605	1	3	1	1	5	2	1	1	1.9	E

Table 2. Mean straw test ratings of bean entries (lines) (1=most resistant) for white mold reaction in eight different greenhouse locations using ANOVA (Alpha=0.05, LSD=1.1).

Entry	CO	NY	OR	ID	MI	NE	WA	WI	Mean Rating	t Grouping
Beryl	6.2	5.0	5.9	8.0	7.9	8.3	5.8	3.0	6.3	A
WM 35	3.0	5.0	6.0	.	8.0	8.0	5.8	4.2	5.7	B A
Bunsi	4.5	4.9	5.8	3.5	8.0	7.7	6.0	4.9	5.7	B A
WM 54	3.9	4.6	6.6	.	7.8	7.0	4.8	4.8	5.7	B A
WM 32	3.0	4.8	7.4	.	8.4	5.8	6.4	3.7	5.6	B A
PS02-006D15	4.9	5.0	6.4	.	4.7	7.7	5.3	4.6	5.5	B A
WM 55	3.9	5.0	7.5	3.0	7.1	6.0	7.0	4.1	5.5	B A
IO1892-115M	4.2	5.0	6.4	2.0	8.1	5.1	8.0	4.0	5.3	B A
B05001	3.6	5.0	7.3	3.0	4.3	5.3	7.8	4.4	5.1	B C
PS02-006D-10	3.5	4.9	5.4	.	5.4	7.7	4.7	2.3	4.8	B C D
B05002	3.4	4.7	3.2	.	5.7	6.3	6.9	2.6	4.7	B C D
AN 37	4.4	4.2	5.2	4.0	8.0	4.5	4.6	2.2	4.6	B E C D
PS02-029C-20	4.3	3.6	3.3	.	7.1	4.0	4.8	2.4	4.2	F E C D
Cornell 604	2.7	2.8	4.2	3.0	7.6	6.2	4.8	1.3	4.1	F E C D
Cornell 606	2.5	2.5	4.0	2.0	6.9	5.8	5.4	3.5	4.1	F E C D
B05003	3.4	4.1	3.0	.	5.7	2.9	6.2	3.1	4.1	F E C D
PS02-029C-1	3.3	2.9	4.2	.	6.8	3.1	4.7	2.3	3.9	F E D
VA 19	2.9	2.5	3.9	.	4.8	4.6	5.2	3.0	3.8	F E D
A195	2.5	1.6	2.9	.	6.0	4.3	5.6	3.6	3.8	F E D
G122	3.8	3.3	3.7	2.2	4.8	5.1	4.3	3.0	3.8	F E D
Cornell 603	3.4	2.0	2.8	1.0	7.9	3.4	5.2	3.0	3.6	F E
Cornell 605	3.0	2.1	2.7	3.0	4.3	4.1	4.7	2.9	3.3	F

DEVELOPING WHITE MOLD RESISTANT INTERSPECIFIC BREEDING LINES FROM THE SECONDARY GENE POOL OF COMMON BEAN

Shree P. Singh¹, Henry Terán¹, Howard F. Schwartz², Kristen Otto², and Margarita Lema¹

¹Univ. of Idaho, 3793N 3600E, Kimberly, ID 83341-5076; ²Dept. of Bioagr. Sciences & Pest Management, Colorado State Univ., Fort Collins, CO 80523-1177.

Development of Interspecific Breeding Lines

Phaseolus coccineus (G 35171 and G 35172), *P. costaricensis* (S 33720) and *P. polyanthus* (G 35877) were crossed and backcrossed with small-seeded black bean 'ICA Pijao' at CIAT, Colombia in 1988-1989. *P. coccineus* accessions were selected for their resistance to BGYMV, *P. polyanthus* for Ascochyta blight, and *P. costaricensis* to determine crossability with the common bean. The four accessions have growth habit Type IV and a perennial tendency. Moreover, it took nearly two years and a change of location from the highlands near Popayan to Tenerife near Palmira for *P. costaricensis* to flower. Plant-to-plant pair-wise pollinations were made using ICA Pijao as the female. The inbred-recurrent and inbred-congruity backcrosses to both parents alternately, followed by four or five generations of inbreeding without selection for any trait were used. The 547 interspecific breeding lines (IBL) derived from the 12 populations (Table 1) were introduced from CIAT in 1998-1999. Only 423 were relatively insensitive and produced seed when first grown at Parma, Idaho in 2000. From 2000 to 2004 plants within each IBL were harvested in bulk. No selection for white mold (WM) resistance or any other trait was practiced in 2000 and 2001. Nonetheless, a majority of IBL had upright erect growth habit Type II, similar to ICA Pijao, very few were of growth habit Type III, and none were of Type I or Type IV.

Greenhouse Screening of Interspecific Breeding Lines

The 423 IBL, ICA Pijao, and WM susceptible and resistant checks were screened in the greenhouse at Fort Collins, Colorado using the straw-test from 2002 to 2006. The surviving IBL were also screened using the modified petiole-test in 2004 and the cut-branch method in 2005 and 2006 in the greenhouse at Kimberly, Idaho. Initially, WM was scored at 1 d, 2 d, and 7 d after inoculation (DAI) on a 1 to 9 scale, where 1 = symptomless, and 9 = severely diseased. Subsequently, WM was rated at 7, 14, and 28 DAI. In 2005 and 2006 while the bulk samples of each IBL were used for the field tests, in the greenhouse, only plants with resistant (1 to 3) or intermediate (4 to 6) WM scores were harvested individually for subsequent screening.

Field Screening of Interspecific Breeding Lines

For the field screening in Idaho in 2002 and 2003, each plot consisted of a single row 3.5 m long spaced 0.56 m apart without replicates. A randomized complete block design with two replicates, each plot of four rows, in 2004 and 2005 and three replicates in 2006 was used. The nursery was inoculated three times with the ascospores (in 2003), ascospores and mycelial culture (2004), or only mycelial culture (2005-2006). Reaction to WM was recorded on a 1 to 9 scale, where 1 = no visible WM symptoms on stem and pods, and 9 = severely diseased or dead plants. Each year, both field and greenhouse data were considered together, only IBL with the lowest combined white mold scores were advanced for testing the next year. Thus, only the five WM resistant IBL survived the greenhouse and field tests conducted from 2002 to 2006. These five of 423 IBL

were also characterized for growth habit, flower color, maturity, and seed characteristics in the field trials in Idaho in 2006. These five IBL along with the resistant and susceptible checks will be tested in 2007.

Results and Discussion

Of the 12 interspecific populations only one recurrent-backcross each with *P. costaricensis* S 33720 and *P. polyanthus* G 35877 produced one WM resistant IBL, whereas three resistant IBL were derived from a congruity-backcross with *P. coccineus* G 35172 (Table 1). The delayed field planting, three mycelial inoculations during flowering, relatively lower temperatures and evapotranspiration during the reproductive stage, and high humidity were conducive to WM development in the field in Idaho in 2005 and 2006.

Table 1. White mold screening and selection of interspecific breeding lines derived from the crosses of ICA Pijao with the three *Phaseolus* species of the common bean's secondary gene pool in the greenhouse and field in Colorado and Idaho from 2002 to 2006.

Genomic dosage†	Pedigree	Year						
		2000	2001	2002	2003	2004	2005	2006
2V1C	ICAPijao/G 35171//ICA Pijao	24	17	5	5	5	3	
2V2C	ICA Pijao/G 35171//ICA Pijao/3/G 35171	54	32	2	2	2		
3V2C	ICA Pijao/G 35171//ICA Pijao/3/G 35171/4/ICA Pijao	9	6					
1V1C	ICA Pijao/G 35172	7	3					
1V2C	ICA Pijao/G 35172//G 35172	11	9	2	2	2		
2V1C	ICA Pijao/G 35172//ICA Pijao	48	42	19	19	19	6	
2V2C	ICA Pijao/G 35172//ICA Pijao/3/G 35172	31	28	26	26	26	6	3
3V2C	ICA Pijao//G 35172//ICA Pijao/3/G 35172/4/ICA Pijao	11	8	2	2			
1V1P	ICA Pijao/G 35877	37	10	1	1			
2V1P	ICA Pijao//ICA Pijao/G 35877	127	119	62	62	62	28	1
1V1R	ICA Pijao/S 33720	43	16	1	1	1		
2V1R	ICA Pijao//ICA Pijao/S 33720	144	133	12	12	12	4	1
	Total	547	423	132	132	129	47	5

† V = *Phaseolus vulgaris*, C = *P. coccineus*, P = *P. polyanthus*, and R = *P. costaricensis*.

WHITE MOLD FUNGICIDE TRIALS ON DRY BEANS IN MICHIGAN

G. Varner¹ and K.A. Terpstra²

¹Michigan Dry Bean Production Research Advisory Board, Saginaw, MI; ²Department of Crop and Soil Sciences, Michigan State University, East Lansing, MI

Introduction

This study was undertaken to evaluate commercially available and experimental fungicides for control of white mold (*Sclerotinia sclerotiorum*) on dry beans (*Phaseolus vulgaris*) over a two year period, 2005 and 2006. White mold is a serious, yield limiting disease of dry bean in the Midwest. Michigan dry bean producers can spend upwards of one million dollars annually on fungicides to control white mold. Evaluating fungicides provides growers with valuable information in determining the most effective fungicides to use. Similar fungicide trials have been conducted by the Michigan Dry Bean Production Research Advisory Board since 1982.

Materials and Methods

This study was conducted at the Montcalm Research Farm in Montcalm County, Michigan in 2005 and 2006. Matterhorn great northern bean variety was planted June 10, 2005 and June 13, 2006 (Kelly et al., 1999). Matterhorn was chosen because of its known susceptibility to white mold. Four replications of 30 foot (10 m) four row plots with 20 inch (51 cm.) row spacing treated. The fungicide treatments were applied on July 22, 2005 and July 25, 2006. Plots were sprayed using a four-row CO₂ sprayer with one twin jet nozzle directly over each row. Treatments were applied at a pressure of 65 psi and a rate of 26 gallons per acre. In 2005 plots were irrigated with 0.5 inches of water twice a week, totaling 5 inches, from flowering to mid-pod fill to supplement the 4.8 inches of rainfall accumulated during the study. In 2006 plots were similarly irrigated to supplement the 11.8 inches of rain accumulated during the study. Plots were rated on September 1, 2005 and September 4, 2006 for white mold incidence and severity using a percentage scale for both ratings where 0% would indicate no white mold present and 100% would indicate complete crop loss from white mold. Plots were harvested on September 19, 2005 and September 15, 2006. The harvested area of each plot was 15 feet (5 m) of the center two rows. Samples were milled and yield and moisture data were recorded. The yield data presented in pounds per acre is expressed at 18% moisture.

Results and Discussion

Treatments that included Endura, Omega, Formula A, both Switch treatments, and Topsin-M at 30 oz. out yielded the untreated check plot at a statistically significant level ($p>0.05$) in 2005. In 2006, only the Topsin M at 30 oz. and the Formulation A+B treatments out yielded the untreated check plot with statistical significance ($p>0.05$). White mold severity was much higher in 2005 than in 2006. In 2005, all treated plots except Proline showed a significant reduction in white mold incidence and severity as compared to the untreated check. In 2006, five treatments, including Proline, Formula A, and Formula B did not show a significant reduction in white mold incidence and severity as compared to the check.

Omega was consistently at or near the lowest disease incidence and severity of the fungicides tested and, along with both Topsin M treatments, showed a statistically significant decrease in incidence and severity in both years ($p>0.05$). Omega is not yet labeled for use in the United

States on dry beans, but EPA approval is expected in 2007. Despite the superior performance of Endura, which is marketed as Lance in Canada, growers may remain reluctant to use Endura because of its relatively higher cost as compared to other available fungicides.

Table 1 – Results of the 2005 and 2006 White Mold Fungicide Trials in Montcalm County, Michigan.

2005 and 2006 WHITE MOLD FUNGICIDE TRIALS – MICHIGAN									
TRT.	CHEMICAL NAME	FORM	RATE	% INFECTION 2005		YIELD 2005 Lbs/A	% INFECTION 2006		YIELD 2006 Lbs/A
				DI	DSI		DI	DSI	
FORMULA A+B	EXPERIMENTAL	5 WD + 50 DW	1 OZ + 12 OZ				32	26	2456
T-METHYL	THIO-PHANATE METHYL	4.5 F	30 OZ	56	44	2088			
ENDURA	BOSCALID	70 WDG	8 OZ	39	31	2681	38	31	2312
FORMULA A	EXPERIMENTAL	50 WD	1.75 OZ				48	40	2288
FORMULA B	EXPERIMENTAL	50 DW	12 OZ				40	33	2281
TOPSIN-M	THIO-PHANATE METHYL	4.5 F	30 OZ	55	41	2254	21	16	2446
OMEGA	FLUAZINAM	4 SC	8 OZ	42	32	2609	21	15	2249
ABOUND	AZOXYSTROBIN	2.08 FL	0.2LB AI/A				42	35	2248
FORMULA A	EXPERIMENTAL	50 WD	0.877 OZ				48	41	2240
FORMULA A	EXPERIMENTAL	50 WDG	3.5OZ	53	45	2333	31	23	2238
TOPSIN-M	THIO-PHANATE METHYL	70 WDG	24 OZ	59	45	2026	27	18	2231
SWITCH	CYPRODINIL & FLUDIOXONIL	62.5 WDG	11 OZ	53	42	2308	36	26	2227
ENDURA	BOSCALID	70 WD	6 OZ				33	25	2190
CHECK				73	62	1663	39	34	2113
SWITCH	CYPRODINIL & FLUDIOXONIL	62.5 WDG	14 OZ	54	44	2202	24	24	2081
FORMULA B	EXPERIMENTAL	50 WDG	16 OZ	51	43	2019			
FORMULA B	EXPERIMENTAL	50 WDG	24 OZ	52	44	2011			
PROLINE + INDUCE	PROTHIOCONAZOLE + SURFACTANT		5.7 OZ + 1/4%				47	38	1863
PROLINE	PROTHIOCONAZOLE		5.7 OZ				36	32	1825
PROLINE	PROTHIOCONAZOLE	4 SC	5 OZ	65	58	1822			
	Mean			54.3	44.3	2168	35.2	28.6	2206
	LSD @ .05			8	6.9	585	9.3	8.1	230
	C.V. Value %			10.3	10.9	18.8	18.6	18.2	7.3

Trt = Treatment; Form = Formulation; OZ=Ounces; LB=Pounds; AI=Active Ingredient; % Infection on a 0-100% scale with 100% equal to total crop loss; DI = Disease Incidence; DSI = Disease Severity; Yield = Yield (pounds/acre) at 18% Moisture

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IDENTIFICATION OF ROOT ROT RESISTANCE SOURCES IN COMMON BEAN

Esteban Falconi¹, Ángel Murillo I¹., José Pinzón¹, Eduardo Peralta I¹, George Abawi².

¹Programa Nacional de Leguminosas y Granos Andinos (PRONALEG-GA). INIAP. Casilla 340. Quito-Ecuador. legumin@pi.pro.ec. ²Department of Plant Pathology, New York State Agricultural Experiment Station, 630 West North Street, Geneva, New York 14456, USA.

The identification of sources of resistance to root rot pathogens has been considered as a research priority by the Legume Program of INIAP – Ecuador. The latter conclusion was based on observing and documenting a high incidence and damage of root diseases on beans throughout the major production areas in Ecuador. Unfortunately, crop rotation practices to reduce root pathogens and their damage have not been well accepted by farmers, since there is limited land available and high demand for bean consumption. Additionally, seed treatment with effective fungicides is not frequently used by producers and is neither recommended by extension educators due to the high toxicity of available products, thus the potential health risk to handlers and consumers. Accordingly, this investigation was initiated to identify bean genotypes with high levels of resistance to the major root pathogens in Ecuador, *Fusarium solani* and *Rhizoctonia solani*.

Materials and Methods:

To identify root rot resistance sources, three field experiments have been conducted over previous three production seasons (2005 and 2006) in the Tumbaco Research Farm (TRF) located in the Province of Pichincha near Quito (0° 13'0S; 78° 24'0W; 2355 masl). The accessions evaluated in the experiments were obtained from the gene bank of INIAP, however the majority of the breeding lines has been developed by various international breeding programs. The germplasm evaluated included bean accessions from Mesoamerica and Andean regions (Tables 1 and 2). During the first two seasons of testing, bean germplasm were evaluated under natural infections of *F. solani* and *R. solani*. However in the third season, inoculum of *F. solani* was prepared and incorporated into the soil/bean row at planting (3000 conidia/g of soil). Individual plots were 1 to 4 rows and 4.0 m long. In the third season, a soil application of an insecticide (Lorsban ® 1cc/L) was made to avoid insect (stem borers) damage and interference with root rot evaluations. Root rot evaluation was performed at flowering time. Six to 10 plants were excavated from each plot and their roots were cleaned immediately with water. Root rot severity (RRS) was recorded on a scale of 1-9, where: 1= no visible disease symptoms and 9= > 75% of root tissues with lesions, severe rotting and reduction in size of roots (Abawi and Pastor-Corrales, 1990).

Results:

Highest levels of resistance was exhibited by the bean genotypes A55, Je.Ma, L88-63, DOR 446, Porrillo Sintético, AFR476, BAT477, RAB651 and NSL (Tables 1 and 2) which had a RRS of <4. However, RAB 651 exhibited a susceptible reaction in the 2006 test, which might be due to the additional inoculation with *F. solani*. Disease pressure was high and uniform in the three evaluations, as the susceptible Imbabello showed high RRS scores in all evaluations. The majority of the bean germplasm showing high levels of resistance was of Mesoamerica type, characterized by a small size seeds. The latter character will make breeding to transfer the resistance factor difficult, especially using a backcross strategy. Fortunately, among the resistant germplasm identified is the Andean-type cultivar Je.Ma, which is large seeded. High incidence

and damage of insects was observed in the second trial, which made it difficult to evaluate for root rot resistance. However, the third evaluation was free of insect damage due to the use of the insecticide Lorsban.

Conclusions:

Several sources for resistance to root rot pathogens (primarily *F. solani* and *R. solani*) have been identified and are available for breeding use in the Legume Program of INIAP. A number of these lines show resistance to both bean pathogens, whereas others appear to be resistant to only one of the two pathogens. The strategy would be to cross one or multiple genotypes possessing resistance to root rot pathogen(s) to a local or in-bred cultivars accepted by farmers and consumers in Ecuador. Of course, a backcross program might be needed to recover the commercial characteristics of the seed.

References: Abawi, G.S. and Pastor-Corrales, M.A. 1990. CIAT, Cali, Colombia. 114p.

Table 1. Reaction of bean genotypes to infections caused by *Fusarium solani* and *Rhizoctonia solani* evaluated under normal (Yp) and drought stressed (Ys) conditions in Tumbaco, Ecuador in 2005.

Genotype	RRS ¹	
	Yp	Ys
ABE4	5.3	5.7
ACE1	5.0	4.0
ACE2	4.7	4.3
AFR476	3.0	2.7
ARME2	4.3	4.0
C.BOLA 60	5.7	6.0
COCACHO	6.0	5.7
L88-63	2.7	2.3
Concepción	4.7	5.0
Paragachi	4.7	4.0
POA10	5.3	5.7
RAB651	2.7	2.7
RAB655	2.3	2.7
SEQ1016	5.0	5.0
Yunguilla	5.0	4.0
YxAs7	5.0	5.3
Promedio	4.5	4.3
LSD (0.05)	1.1	1.1
CV(%)	14.3	15.6

¹ Root rot rating on a scale of 1 (normal root/healthy) to 9 (>75% of root and stem tissues affected and decaying).

Table 2. Reaction of bean genotypes to infections caused by *Fusarium solani* and *Rhizoctonia solani* in Tumbaco, Ecuador in 2006.

Genotype	RRS ¹	Std Dev.	Causal agent ²
A 55	3.30	0.26	<i>F.s.</i>
L 88-63	3.50	0.52	<i>F.s.</i>
DOR 446	3.57	0.15	<i>F.s.</i>
Porillo Sintético	3.67	0.31	<i>F.s.</i>
NSL	3.77	0.38	<i>F.s.</i>
JE.MA	4.07	0.59	<i>F.s., R.s.</i>
BAT 477	4.37	0.80	<i>F.s.</i>
JAMAPA	4.40	0.46	<i>F.s.</i>
Paragachi	4.57	0.45	<i>F.s.</i>
ABE4	4.57	0.15	<i>F.s.</i>
Seahawk	4.67	0.55	<i>F.s.</i>
C03131	4.87	0.12	<i>F.s., R.s.</i>
Concepción	4.87	0.97	<i>F.s.</i>
C03155	4.90	0.60	<i>F.s.</i>
C03151	4.90	0.85	<i>F.s.</i>
ACE 2	4.93	1.14	<i>F.s.</i>
RAB 651	5.13	0.60	<i>F.s.</i>
C03121	5.13	0.64	<i>F.s., R.s.</i>
C03102	5.27	0.55	<i>F.s., R.s.</i>
Imbabello	6.50	0.00	<i>F.s.</i>
Average	4.50		

¹ Root rot rating on a scale of 1 (normal root/healthy) to 9 (>75% of root and stem tissues affected and decaying).

² Causal agents: *F.s.* = *Fusarium solani* and *R.s.* = *Rhizoctonia solani*.

**TILLAGE, HUMIDITY AND FERTILIZATION EFFECTS ON BEAN YIELD,
CHARCOAL ROT INCIDENCE AND PATHOGENICITY OF
*MACROPHOMINA PHASEOLINA***

Krystal Lira-Méndez¹, Arturo Díaz-Franco² & Netzahualcoyotl Mayek-Pérez¹

¹Centro de Biotecnología Genómica, Instituto Politécnico Nacional, Apdo. Postal 152, Reynosa, México ²INIFAP-Campo Experimental Rio Bravo, Apdo. Postal 172, Rio Bravo, México. E-mail: nmayek@ipn.mx

Charcoal rot is caused by the imperfect fungus *Macrophomina phaseolina* (Tassi) Goid. and provokes significant yield losses in important crops such as soybean, beans, sesame, maize, and sorghum. Crop rotation could constitute an appropriate management strategy, however can be not enough effective due the fungus is non specific and can attack a wide range of plant species. In addition, fungal microsclerotia can survive in crop debris and low water contents in soil. Soil management can help to reduce *M. phaseolina* population density due affects fungal biology and promote antagonism. No-tillage reduces charcoal rot progress in soybean, despite crop biomass also decreases up 30 % compared to conventional tillage. Conventional tillage also increases the microsclerotia density in soils (1). Irrigation can reduce charcoal rot incidence in soybean but can not prevent fungal infection and colonization under field conditions (2). We determined tillage, humidity and fertilization effects on pathogenicity of *M. phaseolina* isolates and grain yield and charcoal rot incidence in common beans growing in Río Bravo, Tamaulipas, México.

Four tillage (moldboard plow, subsoil-bedding, shred-bedding, no-tillage), two humidity (rainfed and irrigation) and two fertilization treatments (chemical, using 40-20-00 of NKP, and biological using vesicular-arbuscular mycorrhiza *Glomus intraradices*) were evaluated in Negro INIFAP common bean cultivar in Río Bravo, Tamaulipas, México (25° 57' N, 98° 01' W, 30 m above sea level) during 2005 and 2006. The sixteen treatments were sown using a split-plot treatment arrangement randomized in RCB design with three replications. At maturity, variable numbers of roots and stems were collected in each experimental unit (UE) and one *M. phaseolina* isolate per UE was obtained. Pathogenicity of 96 *M. phaseolina* isolates was measured in five common bean cultivars (Azufrado Tapatío, Bayo Madero, Flor de Mayo Bajío, Negro Altiplano, Negro INIFAP). Charcoal rot infection per seed was measured using an arbitrary visual scale which consisted on six values (from 0 to 5) where 0 = un-infected seed and 5 = > 80% of seed coat and cotyledons or roots infected by the pathogen. Grain yield (kg ha⁻¹) per UE was estimated at harvest as well as charcoal rot incidences. Data from field experiments were subjected to ANOVA using SAS 6.12 version.

No statistical differences in pathogenicity among isolates obtained in 2005 and 2006 were found, although isolates collected in 2006 from moldboard plow and shred-bedding tillage treatments showed the lowest pathogenicity in common bean seeds (Fig. 1). None humidity and fertilization treatments affected pathogenicity of *M. phaseolina* isolates. Under field conditions, opposite results were found due tillage treatments did not affect grain yield while irrigation (719 kg ha⁻¹) and mycorrhiza (638 kg ha⁻¹) increased grain yield compared to rainfed conditions (496 kg ha⁻¹) and chemical fertilization (567 kg ha⁻¹), respectively. Our results suggest that short-term tillage did not affect charcoal rot pathogenicity and common bean grain yield as been found in

soybean (4), although tillage treatments promote crop residues accumulation on soil surface improving soil sustainability indicators compared to conventional tillage (moldboard plow) (3). In addition, no-tillage reduces water stress negative effects and charcoal rot incidences in Brazil and could constitute as better choice to suppress charcoal rot than crop rotation due conserves soil moisture and reduces disease progress (1). Charcoal rot pathogenicity was reduced 10 % from 2005 to 2006 and we suggest long-term tillage could affect survival, microsclerotia densities and/or *M. phaseolina* pathogenicity. Further analysis of long-term tillage effects in common bean should be addressed to confirm this suggestion.

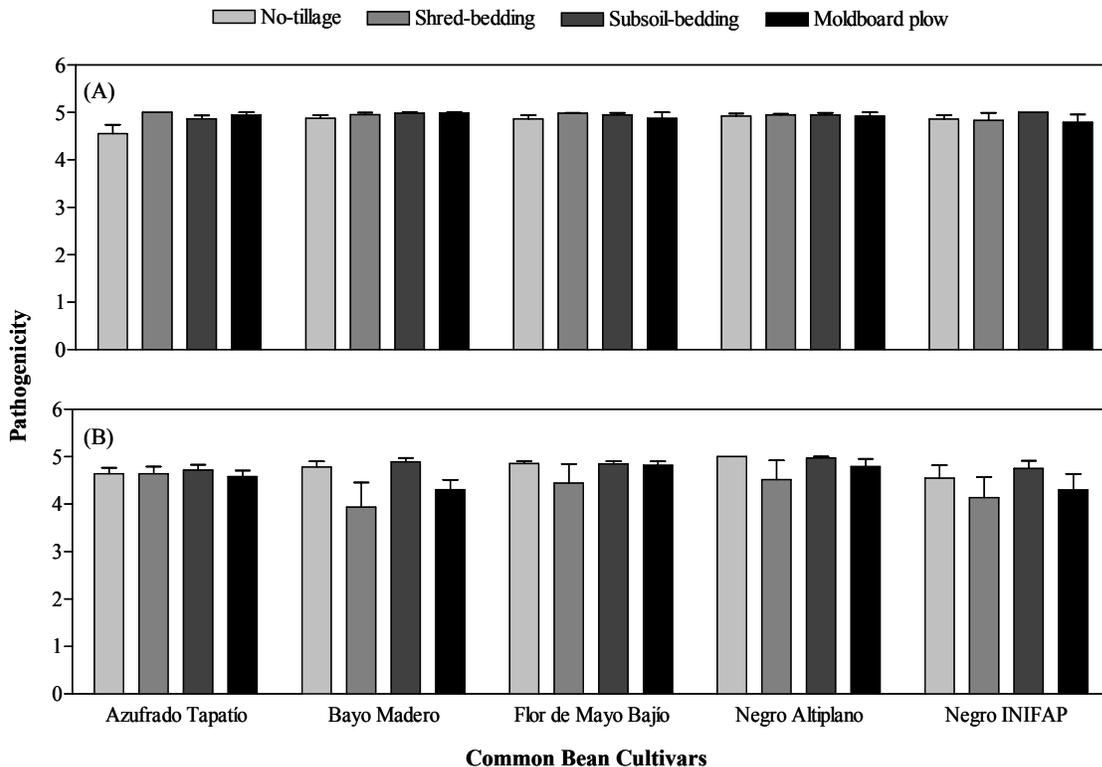


Fig. 1. Tillage effects in *M. phaseolina* pathogenicity of isolates obtained in 2005 (A) and 2006 (B) from Rio Bravo, Tamaulipas, México. Vertical lines indicate \pm standard error.

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NATURAL INCIDENCE OF *PHYTOPHTHORA PHASEOLI* IN DRY BEAN GROWN IN THE HIGHLANDS OF MEXICO

R. Navarrete-Maya¹, J.A. Acosta-Gallegos², E.I. Cuéllar-Robles³, J.A. Ocampo H.⁴ and J. Navarrete-Maya¹.

¹UNIGRAS, FES-C, UNAM, km. 2.5 carr. Cuautitlán-Teoloyucan, México, 54700, Bean Program of INIFAP, Campo Experimental Bajío, Km 6.5 carretera Celaya-San Miguel de Allende, CP 34110, Celaya, Guanajuato, México ³Campo Experimental Valle del Guadiana INIFAP, Durango, Dgo., CP 38000, Mexico, ⁴Comite Estatal de Sanidad Vegetal (CESAVEG), V. Rodriguez S/N, Frac. La paz, Irapuato, Gto., Mexico. rosa_navarrete@hotmail.com

Downy mildew is induced by *Phytophthora phaseoli* or *Phytophthora parasitica* var. *nicotianae*; a disease that occurs in Central and South America and the West Indies, infecting lima bean (*Phaseolus lunatus*) (3) and has been classified as sporadic. The first notice of the disease in Mexico was in 1968, with posterior reports in 1973, 1975, 1978 and 1980, mainly at the central-western states of Jalisco, Michoacán and State of Mexico (the Neovolcanic axes region), a region of sub-humid temperate climate. In this region it has been observed infecting different cultivars of *P. vulgaris* and *P. coccineus* (1). In 1980 the damage rated on the cv. Bayomex (a type I) in the State of Mexico was 50% (1).

The disease is characterized by the white growth of the fungi at the tip of branches and main stem of the plants, turning down or bending the growing points including leaflets and petioles. After those plant parts wilt and bend, the lesion is then covered by white mycelia and sporangia until death. The fungi can grow on the pods and flowers, stops their development and causes its death; the death organs remain attached to the plant. The fungi can penetrate into the pods and seeds tissues (3). With low temperature (13-25°C) and high humidity the entire pod may be infected, shrivel and dried up (2).

In spite of the sporadic classification given to downy mildew, many of the cultivars in our nurseries grown at the Valle de Mexico Experimental Station (CEVAMEX) had shown damage by this disease, mainly when the weather has been cold and humid. CEVAMEX is located near Texcoco, State of Mexico at 2250 m.a.s.l. and has a total yearly rainfall of 640 mm, most of it occurring from June to September. In this site the disease was observed in 1998, 1999, 2000, 2001 and 2002 (after 2002 the authors have not work at this location). For example, in 1998 its occurrence was recorded on the CIAT's bush core collection that included 685 accessions; 19% of the accessions displayed some damage; sixty susceptible accessions were of the type III growth habit, 28 of the type II and only three from the type I. Most susceptible type III accessions originated in Mexico (38) and in South American countries (20). In 1999, 2000, 2001 and 2002 the disease damaged experimental genotypes of all sorts, particularly of indeterminate growth habit, types III and II, among those: San Cristobal 83, BAT 477, Bayo Mecentral, Bayo INIFAP, Flor de Junio criollo, ICA Palmar, Negro 8025, A193, Flor de Junio Marcela, Azufrado Pimono 78, FEB 190, Negro Cotaxtla 91, VAX 2 and Pinto Sierra.

In 2002 downy mildew was registered at the Bajío Experimental station near Celaya, Guanajuato a site located at 1765 m.a.s.l. causing damage on cultivars Bayo Madero, Negro Veracruz, 97RS326, Azufrado Namiquipa, B98311, TLP 19, MC6, 97RS110, G 1977, G 2846, G 6849, Flor de Mayo M38, Negro Jamapa and Azufrado Peruano 87. In this site the disease was observed again in 2006, as well as in Salvatierra, Guanajuato a site located at 1650 m.a.s.l. Yearly rainfall pattern at these locations is above 500 mm, concentrated from July to September. In both sites where the disease has been observed, the climatic conditions were suitable for its development (4). At those sites most years the same type of cultivars are grown season after season and the disease does not occur every year, on the contrary in these sites it does in a sporadic way. At the CEBAJ downy mildew was observed in 2006 attacking cultivars from different origin and type, including cultivars from the three races in the Mesoamerican pool. A nursery introduced from Michigan, USA, consisting of Pinto, Great Northern and Red Kidney cultivars was badly damaged. Also four *P. acutifolius* accessions showed a strong attack. Since we could not find references of downy mildew attacking this species (3, 4), this is the first time that this disease has been observed attacking tepary bean. In this location the disease was also scored on 100 plants of a segregating F₂ population derived from Pinto Saltillo X PS 99 and in a Bc₂ population of Pinto Zapata/(Pinto Zapata/BAT 477). The severity range was from 3.0 to 6.0 (1.0 to 9.0 scale, where 1.0 is free of disease), with an average of 3.7 and >20% of disease incidence. In the area of Salvatierra Guanajuato, where the disease has been occurring with more frequency than at the CEBAJ, the dominant cultivar during the last decade under irrigated and rainfed conditions has been Flor de Junio Marcela, which has shown susceptibility to downy mildew.

Also in 2006 the disease was observed in commercial fields at Pánuco de Coronado, Durango (> 2000 m.a.s.l.) in plots of cultivars Flor de Junio Marcela, Flor de Mayo Anita, Pinto Saltillo, Pinto Zapata and Pinto Villa showing symptoms of the disease with a high level of severity, particularly on the Pinto seeded cultivars. Same cultivars showed similar severity level at the CEBAJ. The damage of the disease was not quantified but the incidence was detected in cultivars from the R6 to R8 phenological stages; therefore is highly probable that losses due to downy mildew might have been considerable, mainly due to the death of blossoms, pods, petioles and young stems. This is the first time that downy mildew has been observed in the semiarid region in the state of Durango.

Due to the potential damage of the disease, it must be observed and studied in order to prevent it, while resistant cultivars in preferred commercial classes are being developed.

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ROOT ROT RESISTANT SNAP BEANS ADAPTED TO ORGANIC PRODUCTION

James Nienhuis and Michell Sass
Dept. of Horticulture, University of Wisconsin – Madison

Regardless of whether the production system is conventional or organic two of the most important constraints to snap bean production in the upper Midwest, are root rot disease and nitrogen availability, both of which may be physiologically and genetically related (Pfender and Hagedorn, 1982a; 1982b; Bliss and Miller, 1988). Snap bean is a legume that develops a symbiotic relationship with N₂-fixing bacteria; nevertheless snap beans do not obtain adequate N from N₂-fixation alone and supplemental fertilization is required for commercial production (LaRue and Patterson, 1981). The ability of snap bean roots to absorb nutrients from the rhizosphere is limited by rotting of lateral roots and infection of the vascular system of the root by several soil-borne pathogens, including *Fusarium solani* (Mart.) Appel & Wr. f. sp. *phaseoli* (Burk.) Snyd. & Hans, *Rhizoctonia solani* Kuenn, *Pythium* spp. and *Aphanomyces euteiches* f. sp. *phaseoli* (Yang and Hagedorn, 1966, Hoch et al., 1975; Pfender and Hagedorn, 1982a; 1982b; Kobriger and Hagedorn, 1984). In Wisconsin, the two most severe root rot pathogens are *P. ultimum* and *A. euteiches* f. sp. *phaseoli* (Pfender and Hagedorn, 1982a; 1982b).

‘Puebla 152’, a black seeded Mexican bean landrace was identified as a potential source of root rot resistance in field trials (Rosas et al., 1984; Nienhuis and Kmiecik, 1992) and for high N₂-fixation (Graham and Rosas, 1977). Our snap bean breeding program developed a series of inbred-backcross populations derived using snap bean cultivars Eagle and Hystyle as recurrent parents and Puebla 152 as the donor parent.

In fields with low and high nitrogen, a strong positive correlation was observed between shoot nitrogen concentration and dry weight in an inbred-backcross population derived from Puebla 152 (Mera, 1993). The results indicated that selection for increased shoot weight is associated with higher N₂-fixation in low nitrogen soils. This result is relevant to the results obtained from selection for root rot resistance in the same inbred-backcross populations derived from Puebla 152 in which a large positive correlation was observed between plant biomass and vigor in root rot infested soils (Navarro, 2006).

Beginning in 1994 we began selection within the populations derived from Puebla 152 for high germination and plant vigor in low-nitrogen high-root rot fields. Selected lines were later backcrossed for several generations to commercial snap bean cultivars to improve pod quality. We identified four inbred snap bean lines that are root rot resistant and possess the plant and pod characteristics necessary for commercial production and processing. These lines were released for licensing through the Wisconsin Alumni Research Foundation (WARF).

In the summer of 2006, the four Wisconsin root-rot resistant cultivars and checks were evaluated in a root rot infested field in which low amounts of supplemental nitrogen (60 lbs/A) was provided in a simulated organic production system using composted chicken guano compared to 60 lbs/A nitrogen supplied by sidedressed ammonium nitrate.

Comparison of yield (T/A) of six snap bean cultivars grown in synthetic and organic fertilizer (60 lbs N/A). The UW lines are root rot resistance.		
Cultivar	Root rot + Synthetic fertilizer	Root rot + Organic fertilizer
Hercules treated seed	0.41	0.53
Hystyle treated seed	0.41	0.85
Hystyle untreated seed	0.00	0.00
UW353-2-0-0	1.74	2.90
UW353-1-0-0	2.00	2.43
UW317-1-0-0	1.56	1.99
UW18-227-0-0	2.33	2.37

The field test indicates that the UW lines are root rot resistant. The UW lines also had uniform stands and vigorous plants, suggesting that the healthy roots associated with the resistant UW lines resulted in greater shoot nitrogen either from uptake from the rhizosphere or from enhanced N-fixation.

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SUSCEPTIBILITY OF GREEN MANURE SPECIES TO TWO SOILBORNE PATHOGENS OF COMMON BEAN

Paula Jr., T.J.^{1,2}; Teixeira, H.^{1,3}; Vieira, R.F.^{1,2}; Lima, R.C.^{1,2}; Silveira, T.B.^{1,2}; Lehner, M.S.¹

¹EPAMIG, Vila Giannetti 47, 36570-000 Viçosa (MG), Brazil (trazilbo@epamig.ufv.br);

²Supported by CNPq, Brasília, Brazil; ³Supported by FAPEMIG, Belo Horizonte, Brazil

Introduction

Green manure crops are grown to be turned under to increase soil fertility. Leguminous green manure crops, i.e. those which can make nitrogen (N) fertilizers from atmospheric N, can provide large quantities of N for the soil. They also add many tons of organic matter to the soil, thereby improving topsoil depth, water-holding capacity, nutrient content, friability, and texture of soil. Furthermore, some leguminous green manure are used to control plant parasite nematodes, but the role of these plants as hosts of some root rot pathogens of dry beans (*Phaseolus vulgaris*) is not clear yet. Among the most important soilborne pathogens in Brazil are *Rhizoctonia solani* and *Sclerotium rolfsii*. The purpose of this research was to know the susceptibility of some leguminous green manure species to root rots caused by these pathogens.

Materials and Methods

Crotalaria breviflora, *Canavalia ensiformis*, *Cajanus cajan*, *Dolichos lablab*, *Stizolobium cinerium*, *S. aterrimum*, and three cultivars of dry beans (Pérola, Valente, and Carnaval) were tested for their susceptibility to *R. solani* (AG-4) and *S. rolfsii*. Pérola is a carioca type, Valente is a black type, and Carnaval is a cranberry type. The latter belongs to the Andean gene pool and the former ones to the Mesoamerican gene pool. The fungi were isolated from dry bean infected plants and cultivated in PDA medium for 10 days. Two 5 mm diameter mycelial-agar disks were transferred from the margin of growing colonies to 200 mL-Erlenmeyer flasks with 50 g of autoclaved rice grains. After six days of incubation in darkness at 25 °C, rice grains were totally colonized by the fungi. Then, the rice grains were dried on trays for 24 hours before being used. Inoculum of each fungus was mixed with solarized soil (2 g/ kg of soil) and put in pots with of 1 kg of capacity. A control with no pathogen mixed with soil was also used. Each pot was sown with three seeds of the leguminous at 3 cm depth. Pots were maintained in greenhouse and irrigated once a day. A randomized complete block design with five replications was used. The number of emerged seedlings was counted 10 days after planting (DAP). The plants were removed from the pots 30 DAP and hypocotyls were evaluated to determine the disease severity according to a 1-9 scale proposed by Abawi and Pastor-Corrales (1990). With these data, the index of McKinney (IM) was calculated according this equation:

$$IM(\%) = \frac{\sum (\text{score} \times \text{number of plants with this score})}{(\text{total number of plants} \times \text{greater score})} \times 100$$

Results and Discussion

The emergence of leguminous seedlings in pots with non-infested soil was 100 %. Sclerotium root rot was more detrimental to the species than root rot caused by *R. solani* (Table 1). When *R. solani* was mixed with soil, seedling emergence of three green manure species and of the cultivar Carnaval dropped to between 67 % (*D. lablab*) and 93 %. Some of the green manures plants, mainly *D. lablab* and *Stizolobium* spp., were more severely infected by this fungus than the bean plants. *S. rolfsii* inhibited the emergence of *C. cajan* and *S. cinereum*. Just *C. ensiformis* had higher emergence than the dry bean cultivars. All species were severely infected by *S. rolfsii*.

Table 1 – Percentage of emergence of leguminous species and severity (index of McKinney) of root rots caused by *Rhizoctonia solani* and *Sclerotium rolfsii*

Crops	<i>R. solani</i>		<i>S. rolfsii</i>	
	Emergence (%)	Index of McKinney ⁽¹⁾	Emergence (%)	Index of McKinney
<i>Crotalaria breviflora</i>	100	14.07	27	76.30
<i>Canavalia ensiformis</i>	100	20.74	53	82.22
<i>Cajanus cajan</i>	87	33.30	0	100.00
<i>Dolichos lablab</i>	67	52.60	13	89.63
<i>Stizolobium cinereum</i>	93	47.40	0	100.00
<i>Stizolobium aterrimum</i>	100	35.55	20	94.07
Common bean cv. Pérola	100	28.90	13	97.04
Common bean cv. Valente	100	18.52	33	91.11
Common bean cv. Carnaval	93	26.70	20	95.55

$$(1) \frac{\sum(\text{score} \times \text{number of plants with this score})}{(\text{total number of plants} \times \text{greater score})} \times 100$$

It can be concluded that the use of *C. breviflora*, *C. ensiformis*, *C. cajan*, *D. lablab*, *S. cinereum* and *S. aterrimum* as green manure crops in areas of dry bean cultivation can contribute to increase or at least to maintain the inoculum of *R. solani* and *S. rolfsii*. Therefore, these species are not suitable as green manure for areas of dry beans with high population of these pathogens.

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ROOT GRAVITROPISM OF GENOTYPES OF COMMON BEANS USED FOR BREEDING IN BRAZIL

Rogério F. Vieira¹; Jonathan P. Lynch²

¹Epamig, CP 216, Viçosa, MG 36571-000 Brazil; ²Dep. of Horticulture, Penn State, 102 Tyson Building, State College, PA, 16803 USA

Introduction – Plagiogravitropic growth of roots strongly affects root architecture and the layers of soil explored, which is important for the acquisition of water and nutrients. Water is ephemeral, mobile and usually deep, whereas phosphorus (P) is stable, immobile and usually shallow. In common bean, drought tolerance has been associated with depth of rooting, while greater P acquisition has been associated with increased soil exploration by roots in surface layers. Basal roots developed from 2-4 definable whorls at the root-shoot interface. In conjunction with the lateral roots that emerge from them, basal roots usually comprise the majority of total root length. Basal root gravitropism is a key determinant of the overall shallowness of the root system. The objective of this research is to attain information about the root gravitropism of genotypes used in breeding programs in Brazil.

Material and Methods - Eighteen common bean genotypes from Brazil were used. Eight of them are high yielding cultivars or lines (Ouro Negro, Diamante Negro, Valente, Talismã, Jalo MG-65, Carnaval-MG, Vi-4899, and Vi-10-2-1), and ten are genotypes used for disease resistance in breeding programs (AB 136, Cornell 49-242, G2333, Kaboon, México 54, México 309, Pi 207262, TO, TU, VC-4). The genotypes DOR 364, G19833, and Carioca were obtained from the International Center for Tropical Agriculture. DOR 364 has a deep root system and G19833 a shallow one. Carioca is the Brazilian landrace most widely grown in the tropics, perhaps because of its tolerance of infertile soils. Number of whorls and basal roots were counted in seedlings with 5-days after germination has begun using the cigar roll method, without P addition, as described by Vieira et al. in other report at this BIC volume. For gravitropism evaluation, the genotypes were grown in a pouch system as described by Liao et al. (2001), without P addition. The intact root system on the germination blue paper was scanned into a computer as a digital image. The root growth angle was measured (using the program GIMP) as the average of growth angles of the half basal roots that grew stuck to the blue paper. The program Image J was used to measure length.

Results – Four genotypes have 3 whorls (with between 10.6 and 11.5 basal roots), the genotype TO has 1 whorl (5.6 basal roots), and the others, 2 whorls (see Table). G19833 had the shallower root system, followed by Talismã, Carioca, Vi 4899 (carioca types), and Diamante Negro (black seeds). The genotype Vi-10-2-1 (black) had the deepest root system, followed by TU (black), DOR 364, AB 136 (red), México 54 (black), VC-4 (yellow), and G2333 (red).

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Table – Characteristics of 21 genotypes of common beans and their root gravitropism response to P deficiency

Genotype	Seed color ¹	Plant type ²	Seed weight (g)	# of whorls	# of basal roots ³	Basal root growth angle (degrees from vertical) ⁴					Basal root length (cm) ⁵	Basal root length at the top 2 cm ⁴	% of basal root at the top 2 cm
						Day 1	Day 2	Day 3	Day 4	Total			
AB 136	red	IV	0.29	2	8.0	62,2	38,0 D	24,2 D	29,0 C	153,5 C	1,83 (72,3)	1,34 (22,3) C	33,5 B
Carnaval MG	multic.	I	0.43	3	10.9	67,5	46,7 C	34,0 C	26,2 C	174,5 C	1,92 (84,1)	1,54 (35,5) A	42,0 B
Carioca	carioca	III	0.27	2	8.3	72,2	58,7 A	47,7 A	37,2 B	216,0 A	1,82 (66,7)	1,51 (32,4) B	49,6 A
Cornell	black	III	0.24	2	7.8	79,2	54,7 B	38,2 B	27,2 C	199,5 B	1,79 (64,8)	1,42 (26,3) C	43,8 A
Diam. Negro	black	II	0.27	2	8.8	76,7	59,7 A	43,2 B	36,2 B	216,0 A	1,82 (71,3)	1,51 (32,4) B	50,1 A
DOR 364	red	II	0.24	2	8.2	66,7	42,2 C	23,2 D	14,7 D	147,0 C	1,78 (63,0)	1,34 (22,1) C	38,4 B
G19833	multic.	IV	0.48	3	10.6	72,0	62,2 A	53,7 A	52,5 A	240,5 A	1,81 (65,7)	1,60 (40,0) A	61,0 A
G2333	red	IV	0.31	2	8.5	66,7	44,5 C	32,0 C	25,0 C	168,2 C	1,72 (54,4)	1,32 (21,2) C	41,3 B
Jalo MG 65	yellow	III	0.44	3	11.5	69,5	51,7 B	38,5 B	26,7 C	186,5 B	1,98 (96,3)	1,64 (43,6) A	45,6 A
Kaboon	white	I	0.51	3	10.7	65,2	50,7 B	39,7 B	30,7 C	186,5 B	1,71 (53,5)	1,44 (28,2) B	54,0 A
México 54	pink	IV	0.48	2	8.0	64,0	42,2 C	26,7 C	15,0 D	148,0 C	1,92 (83,6)	1,42 (26,2) C	31,6 B
México 309	black	III	0.28	2	7.6	75,5	51,7 B	38,2 B	35,2 B	200,7 B	1,74 (57,5)	1,39 (24,6) C	44,9 A
Ouro Negro	black	III	0.26	2	8.7	72,0	49,0 B	32,0 C	26,7 C	179,7 B	1,84 (71,1)	1,48 (30,9) B	43,6 A
Pi 207262	carioca	III	0.30	2	8.1	69,5	51,2 B	38,7 B	29,7 C	189,2 B	1,82 (68,6)	1,41 (26,4) C	41,6 B
Talismã	carioca	II	0.24	2	7.8	72,2	59,7 A	49,0 A	41,0 B	222,0 A	1,77 (60,7)	1,48 (31,0) B	51,1 A
TO	carioca	I	0.34	1	5.6	67,2	53,2 B	44,0 B	43,5 B	208,0 A	1,64 (44,3)	1,21 (16,8) C	37,6 B
TU	black	III	0.23	2	9.1	58,5	36,2 D	22,2 D	15,2 D	132,2 D	1,86 (73,8)	1,44 (28,5) B	38,7 B
Valente	black	II	0.22	2	9.3	73,7	51,2 B	34,0 C	25,2 C	184,2 B	1,78 (61,9)	1,46 (29,4) B	48,3 A
VC-4	yellow	III	0.22	2	8.1	65,5	44,5 C	31,0 C	21,0 C	162,0 C	1,85 (71,8)	1,36 (23,3) C	33,1 B
Vi-10-2-1	black	II	0.26	2	8.7	55,7	29,2 D	13,2 E	9,7 D	108,0 D	1,89 (79,1)	1,38 (24,4) C	31,3 B
Vi 4899	carioca	I	0.22	2	8.2	68,5	60,2 A	50,5 A	37,2 B	216,5 A	1,80 (64,8)	1,46 (29,2) B	46,1 A
Mean						68,6	49,4	35,9	28,8	182,8	1,81 (68,1)	1,44 (28,3)	43,2
CV (%)						11,9	14,3	16,9	24,2	11,1	5,5	6,1	17,5

¹ multic. = multicolored, carioca = cream color of the background and the brown color of the stripes.

² I = determinate habits, II = indeterminate bush types, III = indeterminate semiclimber, IV = indeterminate climber.

³ Means of 20 seedlings.

⁴ Daily angles of basal roots one day after seedlings with emerging radicles 2-3 cm length have been transferred to growth pouches. Total = sum of the angles at each day.

⁵ Before analysis of variance, means were transformed by logarithmic (number in parentheses refers to original data).

SIMPLE METHOD TO EVALUATE ROOT HAIRS OF A LARGE NUMBER OF COMMON BEAN GENOTYPES

Rogério F. Vieira¹; Celestina N. Jochua²; Jonathan P. Lynch²

¹ Epamig, CP 216, Viçosa, MG 36571-000 Brazil; ²Dep. of Horticulture, Penn State, 102 Tyson Building, State College, PA, 16803 USA

Introduction - The capacity of plants to absorb both water and mineral nutrients from the soil is related to the plant's ability to develop an extensive and well-located root system. Of the total root surface area, root hairs can contribute up to 67 %. (Nielson et al., 2001). For determination of root hair length and density, Miguel (2004) stained roots of 14-days old plants that were observed under microscopy at 40x magnification. Using a comparative picture taken at the same magnification with micrometer scale, actual root dimensions were determined. Pictures were taken using a digital camera attached to dissecting scope and saved using Adobe Photoshop 7.0 software. Then the pictures were transferred to the Scion Image 1.63 software for root length determination at that magnification. For root hair density, a known surface area at that magnification was selected and the number of root hairs in the selected area was determined. After having root hair measurements (length and density) further calculations were made to convert to real root hair length in millimeters, and root hair density in number of root hairs per square millimeter. These calculations were made using as inputs, the picture magnification, micrometer scale and conversion of the selected root surface area to the unit of root surface area in square millimeters. Thus, root hair length (mm) and root hair density (number of root hair per mm² of root surface area) was determined for basal and lateral roots. This method is very laborious in a breeding program where a large number of genotypes are evaluated. The objective of this research was to test a simple method of root hair evaluation.

Material and Methods - Twenty-one genotypes were evaluated in relation to root hair density/length. Seeds of 18 common bean genotypes from Brazil were used. Eight of them are high yielding cultivars or lines (Ouro Negro, Diamante Negro, Valente, Talismã, Jalo MG-65, Carnaval-MG, Vi-4899, and Vi-10-2-1), and ten are genotypes used for disease resistance in breeding programs (AB 136, Cornell 49-242, G 2333, Kaboon, México 54, México 309, Pi 207262, TO, TU, VC-4). The genotypes DOR 364, G 19833, and Carioca were obtained from the International Center for Tropical Agriculture (CIAT). DOR 364 is known as inefficient under P-deficient conditions. Carioca is the Brazilian landrace most widely grown in the tropics, perhaps because of its tolerance of infertile soils. G19833 is a landrace from Peru that is relatively well adapted to P-limited conditions. Seeds were surface sterilized with 0.5% NaOCl for one minute, rinsed thoroughly with distilled water and scarified with a razor blade. They were placed 2 cm from the top of brown germination paper soaked in 0.5 mM CaSO₄ and with radicles pointed toward the bottom of the paper. The paper was then rolled into a moderately tight cigar roll configuration and placed in a 1 L beaker with 100 mL of 0.5 mM CaSO₄ at the bottom. Beakers were wrapped with cellophane plastic punctured with holes before being placed in a germination chamber at 28 °C. Five days later, shoots of seedlings were eliminated and roots of each genotype were separate in basal and taproots. They were conserved in 25% v/v ethanol immediately after harvest. Root hairs were visually evaluated after been stained with 0.05% trypan blue using a rating scale of 1-9 to rank the density/length as follow: 1 = no root hairs; 3 = between 1 and 5 rating scale; 5 = intermediate root hair density/length, as RILs 28 and 32, 7 =

between 5 and 9 rating scale; 9 = abundant root hairs, as RILs 13 and 53. These RILs were selected from the thesis of Miguel (2004). Data were analyzed as a completely randomized design (CRD), with each treatment replicated four times.

Results and Discussion – As seen in Fig. 1 and 2, the method worked well with relatively low SE of mean for each genotype. In general SE was lower for basal roots than for primary roots.

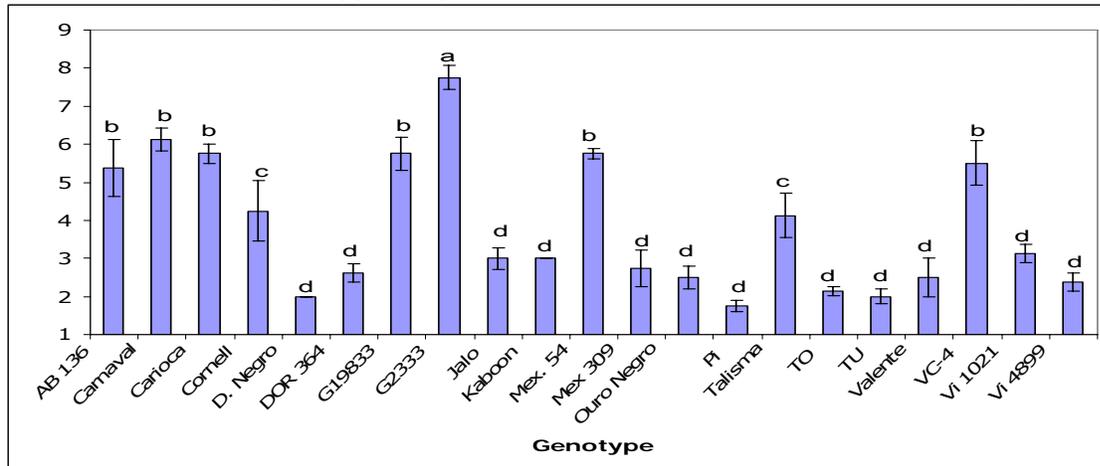


Fig. 1. Root hairs on basal roots 5 d after germination of 21 genotypes of common bean. Data shown are means \pm SE (n = 4). Columns with different letters belong to homogenous groups by Scott-Knott test.

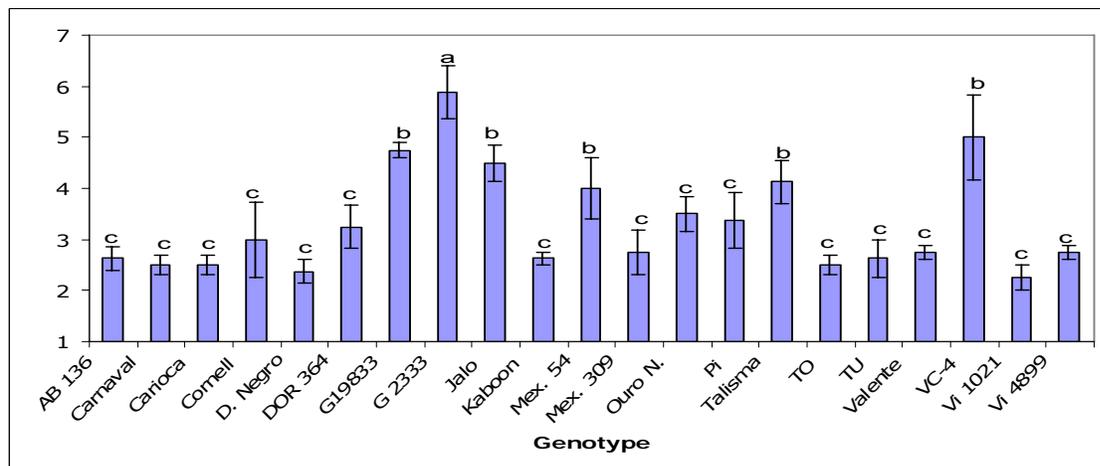


Fig. 2. Root hairs on primary roots 5 d after germination of 21 genotypes of common beans.

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BREEDING COMMON BEANS FOR DROUGHT TOLERANCE IN SOUTHERN ETHIOPIA

Asrat Asfaw, Fistum Alemayehu and Fekadu Gurum

South Agricultural Research Institute, Awassa Research Centre, P.O.Box 6, Awassa, Ethiopia

Introduction

Beans are the most important food legume for direct human consumption in the world, and are a traditional staple in many parts of East Africa (Wortman et al., 1998). In eastern and southern Africa, beans are the second most important protein sources and the third most important caloric source after cassava and maize (Pachico, 1993). In Ethiopia, beans are among the main export crops and evolving as an important source of foreign currency and cash income for small-holder farmers. Drought is one of the serious problems for bean production in Ethiopia. Climate models predict that several regions where drought is already a problem such as the drought stressed areas of Africa will become successively drier over the next decades (Jones and Thornton, 2003). Ameliorating the effects of drought in common beans production in Ethiopia calls to have a genetic tolerance incorporated into cultivars such that they are prepared to produce under sub-optimal conditions.

Material and Methods

Five contrasting populations were created at Awassa in 2002. The F_{1-2} generations were first evaluated at Awassa under early and late sown conditions in 2003 mehere season (Main season). The best performing F_{1-2} 's were advanced to F_3 . At F_3 seeds of each family were divided into three equal parts and planted at three contrasting environments to test the families for different constraints under natural condition in 2004 belg (short rain season). The locations at which the 169 F_{1-3} families planted were Awassa (1750 m altitude for CBB and rust screening), Kokate (2161 m altitude for low soil P and N screening) and Amaro (1426 m altitude for drought screening). The best families were then selected and advanced to F_4 using single pod bulk method. The selected 71 F_{1-4} families were planted at Amaro in 2004 mehere season and advanced to F_5 using plant bulk. At F_5 five best plants from each best performing families planted at Amaro in 2005 belg were selected to raise plant-to-progeny rows (lines selection). A total of 265 $F_{(1-5)6}$ lines generated and planted in plant-to-row along with three checks (Omo-95, Red wolayta and DOR-554) at Awassa on-station in the year 2005 main season. The lines were planted in single row of 2 meter long spaced 80cm. From the 265 lines, the best performing 95 lines were advanced to F_7 and planted in advanced yield trials at six contrasting locations in 2006 mehere season along with five checks.

Results

Among 169 F_{1-3} families planted at Awassa, Amaro and Kokate in *Belg* season 2004, 71 families that combined high yield with biotic and abiotic stress resistance/tolerance under natural conditions were advanced to F_4 . Among the advanced, 12 families expressed remarkable adaptation to drought. The 12 families gave grain yield of more than 1000 kg/ha with prevalent severe moisture stress in 2004. Among the 12 drought tolerant families, CAW-02-03-10-7, CAW-02-04-7-7 and CAW-02-01-2-1 gave the top yield 1549.1, 1520.3 and 1441.9 kg/ha where as among the checks DOR-554 and Omo-95 gave 678.4 and 660.9 kg/ha, respectively. These families also expressed good yielding potential and resistance to disease both at Awassa and kokate test locations. The $F_{(1-5)6}$ lines expressed large variability for grain yield in 2005. The

grain yield (g/2 meter long row) of the $F_{(1-5)6}$ lines ranged from 251.3 (CAW-02-04-7-7-2) to 966.7 (CAW-02-03-8-1-1). The frequency of lines out yielded the best check included in the trial is presented in Table 1. From the 265 lines evaluated in preliminary yield trial, the best performing 95 lines were advanced to F_7 and planted in advanced yield trials at six contrasting locations in 2006 mehere season along with five checks. The mean grain yield data (kg/ha) of two locations of some promising lines is presented in Table 2. The mean grain yield ranged from 3954.8 to 1593.5 kg/ha. Remarkable number of lines out-yielded the checks in advanced yield trials.

Table 1. Frequency and mean grain yield (g/2m row) of lines out-yielded the best check at Awassa in 2005

Families	Pedigree	frequency	Mean yield of best yielded lines
CAW-02-01	R.W.///DOR-716/ICTAJU-95-2//G-6/DICTA-106	1	796.6
CAW-02-02	R.W.///XAN-314/EMP-375//MOC-106/DOR-554	2	754.0
CAW-02-03	RWR-719///R.W./ICTAJU-95-4//XAN-317/DOR-794	30	711.42
CAW-02-04	ROBA///EMP-445/DFA-64//R.W./RAB-589	17	601.8
CAW-02-05	J.local///DOR-716/ICTAJU-95-2//G-6/DICTA-106	1	698.8
Omo-95 (RWR-719) (check)			622.4
DOR-554 (check)			499.2
Red wolyata (check)			557.2

Table 2. Mean grain yield (kg/ha) of some $F_{(1-5)7}$ lines in 2006 main season at four locations.

Lines	Pedigree	Mean grain yield
CAW-02-05-2-7-5	J.local///DOR-716/ICTAJU-95-2//G-6/DICTA-106	2965.2
CAW-02-03-6-4-2	RWR-719///R.W./ICTAJU-95-4//XAN-317/DOR-794	2898.5
CAW-02-05-2-7-2	J.local///DOR-716/ICTAJU-95-2//G-6/DICTA-106	2858.5
CAW-02-05-2-7-1	J.local///DOR-716/ICTAJU-95-2//G-6/DICTA-106	2898.5
CAW-02-04-8-3-1**	ROBA///EMP-445/DFA-64//R.W./RAB-589	2720.5
CAW-02-01-1-1-3 **	R.W.///DOR-716/ICTAJU-95-2//G-6/DICTA-106	2557.2
CAW-02-01-1-1-1 **	R.W.///DOR-716/ICTAJU-95-2//G-6/DICTA-106	2578.1
CAW-02-01-1-1-4 **	R.W.///DOR-716/ICTAJU-95-2//G-6/DICTA-106	2532.1
CAW-02-02-5-1-2 **	R.W.///XAN-314/EMP-375//MOC-106/DOR-554	2344.7
Omo-95 (check)		2548.8
DOR-554 (check)		2181.5
Red wolyata (check)		2330.9
Roba (check)		2249.5
Local check		2087.1

**lines developed from drought tolerant families

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COMMON BEAN IMPROVEMENT FOR HIGHLAND ADAPTATION IN SOUTHERN ETHIOPIA

Asrat Asfaw, Fistum Alemayehu, Fekadu Gurum and Yasin Goa
Awassa Agricultural Research Center, P.O.Box 6, Awassa, Ethiopia

Introduction

Common bean is the main grain legume grown as source of protein and cash by smallholder farmers in south regional state of Ethiopia. Its production in the region is concentrated at low land areas. In high altitude areas faba bean and field pea are widely grown. The faba bean and field pea production in the high lands of southern Ethiopia is constrained by disease and pest problems, and theft of raw-eaten green pods. Common bean is free from theft of row-eaten green pods. Hence beans can be potential crop for diversifying crop chooses as protein and income source for high land inhabitants in the cereal and 'enset'(*Ensete ventricosum*) based farming system.

Materials and Methods

Diverse bean genotypes obtained from regional bean net work were evaluated from 2000 to 2004 at different stages of trial in the high lands of SNNPR state of Ethiopia. In the PYT (Nursery-I & II) the genotypes were evaluated at Hosiana (7°34'12.1" N & 37°50'04.6" E at altitude of 2265m a.s.l.) in 2000 and 2001. At MLT (initial and advanced multi-location performance trial), the genotypes were evaluated in two independent sets (small-medium seed size set and large seed size set). At initial MLT the materials were evaluated at three locations namely Hossina, Angecha (7°19'38.6" N & 37°50'50.4"E at altitude of 2377 m a.s.l) and Bulle (6°18'03.9"N and 38°24'18.0"E at altitude of 2837m a.s.l.) in 2002. In advanced MLT (2003 & 2004), the selected genotypes were evaluated at three locations: Hosaina, Kokate (6°52'43.9"N & 37°48'22.1"E at altitude of 2161m a.s.l.) and Waka (2300m a.s.l.) using RCBD replicated three times. 'Redwolyta with small seed size, and 'Ibbado' & 'Brown speckled' with large seed sizes were used as checks in respective seed class trials. The checks were varieties released for lowland adaptation. In the trials grain yield, days to flowering and maturity was recorded for analysis. The best adapted entries were identified using the probability of outperforming a best check as described by Eskridge (1996). According to Eskride (1996) the reliability or the probability was calculated for each test entries as $P(X_i - X_c > 0)$, where X_i and X_c are the responses of the i^{th} entry and that of the best check in the j^{th} environment.

Results and Discussion

The genotypes numbering 334 at nursery-I expressed remarkable variation for some economically important traits. About 43.2 and 75.4% of the test genotypes flowered and matured earlier than the mean number of days required the checks to flower and mature, respectively. Majority of the lines expressed field level resistance to major diseases (CBB, ALS, halo blight). Grain yield ranged from 93.2 (CIFAC 91126) to 1040.8 (A-195) g/ 4m long single row plot. Among the test genotypes, 16.1 and 0.95% gave grain yield greater than mean yield of checks and best check yield, respectively. In nursery-II in 2001 at Hosaina the genotypes numbering 64 also expressed remarkable variation for economically important traits. The grain yield ranged from 330.6 to 3288.8 kg/ha and it required 46 to 67 days for the 50% the genotypes to flower in a plot.

At initial MLT in 2002 the genotypes were not adapted at Bulle in extreme highland. In the rest two locations mean grain yield of small-medium seed size set ranged from 755.8 (NEP-2) to 1508.5(MAM-38) kg/ha. Three genotypes MAM-38 (1508.5 kg/ha), A-797 (1481.4 kg/ha) and A-774 (1340.0 kg/ha) significantly out-yielded the check Red wolyta (1053.2 kg/ha). In large seed size set, the mean grain yield of the genotypes ranged from 659.2(Brown specked) to 1707.7(POA-13) kg/ha. The top three yielders, POA-13 (1707.7 kg/ha), ICA-CAFETERO (1516.0 kg/ha) and SEQ-1041(1508.9 kg/ha) significantly (P=0.05) out-yielded the check Brown speckled (659.2 kg/ha). Generally the genotypes expressed relatively good yielding potential at Hossaina and performed poor at Angecha. The poor performance at Angencha was due to late sowing that resulted in the plants to experience moisture stress at pod filling stage.

The mean grain yield and yield reliability estimates of genotypes at advanced MLT are presented at Table 1. The mean grain yield of small-medium seed size set ranged from 954.4 (EMP-291) to 1544.1 (MAM-38) kg/ha. Three genotypes MAM-38 (1544.1 kg/ha), A-797 (1382.7 kg/ha) and OBO-A-064 (1438.0 kg/ha) were most reliable having about 86% chance of out performing the check 'Redwolyata'. On the other hand EMP-291, G-3929 and VAX-3 have less than 50 % chance of out performing the check 'Redwolayta'. In large seed size set, the mean grain yield ranged from 1162.4 (Brown speckled) to 2005.5 (POA-13) kg/ha. POA-13, SEQ-1041 and SEQ-1020 with mean grain yield 2005.5, 1949.4, and 1963.6 kg/ha, respectively, were most reliable genotypes having about 86% chance of out performing the best yielding check either 'Ibbado' or 'Brown speckled'. In general MAM-38, A-797 and OBO-A-064 from small and medium seed class and POA-13, SEQ-1041 and SEQ-1020 from large seed class were best adapted genotypes to southern high lands of Ethiopia.

Table 1. Mean grain yield (kg/ha) and reliability estimates of genotypes evaluated at 7 environments in southern Ethiopia

S.No	Small and medium seed size set			Large seed size set		
	Genotypes	Grain yield	R*	Genotypes	Grain yield	R*
1	ICJASYUA	1186.4	0.57	POA-13	2005.5	0.86
2	IAN-309	1201.3	0.57	ICA-CAFETRIO	1422.7	0.29
3	A-774	1456.5	0.71	SEQ-1041	1949.4	0.86
4	A-686	1396.2	0.71	OBO-A-075	1862.0	0.57
5	FEB-190	1332.7	0.57	SEQ-1020	1963.6	0.86
6	MAM-38	1544.1	0.86	G-19833	1527.1	0.43
7	A-797	1382.7	0.86	G-10-BRB-191	1499.5	0.43
8	MX-9065-9T	1428.1	0.71	OBO-A-088	1585.0	0.43
9	EMP-291	954.4	0.14	DFA-64	1588.2	0.43
10	EMP-161	1228.8	0.57	FOT-61	1567.8	0.43
11	OBO-A-064	1438.0	0.86	AND-1083	1626.2	0.29
12	EMP-289	1364.0	0.57	CAL-180	1596.1	0.29
13	G-3929	1204.2	0.43	POA-11	1727.4	0.57
14	VAX-3	1253.5	0.43	A-36	1696.2	0.57
15	Red wolyta	1179.0	-	Ibbado	1655.7	-
16				Brown speckled	1162.4	-

*, Yield reliability

References: Eskiridge, K.M. 1996. Analysis of multiple environment trials using the probability of outperforming a check. In: Kang, M.S. and Gauch, H.G. (Eds.), Genotype by Environment Interaction. CRC Press. Pp 273-308.

LEAF AREA OF TWO BEAN VARIETIES UNDER SOIL WATER DEFICIT

Antonio García-Esteva¹; Josué Kohashi-Shibata² and José Luis Cervantes-Flores³

^{1,2,3}*Campus Montecillo, Colegio de Postgraduados. C.P. 56230 Montecillo, Edo. de Méx., México. Fax (595) 95 2 02 47. Autor for correspondence: jkohashi@colpos.mx*

Introduction. Growth is one of the physiological parameters affected by plant water stress. Such stress is most commonly due to soil water deficit. It is well known that this deficit inhibits plant and especially leaf growth.

The objective of the present work was to compare the effect of a progressive soil water deficit on the leaf area of two bean varieties contrasting in their response to drought.

Materials and Methods. Two type III bean (*Phaseolus vulgaris* L.) varieties were employed: Bayo Madero (BM) susceptible to drought and Pinto Villa (PV) tolerant (Acosta 1982; Acosta *et al.*, 1993). The plants were grown in a greenhouse, one plant per plastic pot filled with nine kg of sandy loam soil. The soil water constants were: field capacity 13.80% and permanent wilting percentage 7.10%. Normal watering was provided until the first compound leaf unfolded, at the beginning of V3 stage (CIAT, 1982), when the plants were 16 days (d) of age. At this stage the water treatments started: Normal watering continued for the control (NW), while watering was withheld (WW) in the soil water deficit treatment. Plant and soil were sampled every three days. Four plants were taken for each variety and treatment. The leaves were cut and the laminar leaf area was determined in an electronic area meter. The experiment was suspended at the eight sampling, when the first compound leaf exhibited the permanent wilting condition (the leaf borders were folded and did not regain water the next morning). Soil moisture samples were taken from a layer located in the middle of the pot, oven-dried and the moisture content calculated. The average values and standard deviation were calculated for the soil and plant data.

Results. Leaf area was inhibited in WW in both varieties. The leaf area declined as the soil water depletion progressed. The higher values of leaf area reduction occurred from the 12th d after the suspension of watering (Tables 1 and 2). The reduction of leaf area was smaller in BM than PV. At 21st and 24th d after the suspension of watering (ages 37 and 40 d), the degree of wilting in PV was less than that in BM.

As expected in WW the water loss was faster and the beginning of the experiment and was declined subsequently. The soil reached the percentage wilting condition at the end of the experiment in both varieties. In PV when the experiment ended, flowers and small pods occurred while only flowers in BM.

The high reduction of leaf area with the concomitant reduction of water loss by transpiration enabled PV plants to maintain a better water condition for a longer time as compared to BM. This behavior in PV might be a strategy to withstand drought.

Table 1. Leaf area (dm² per plant) of the compound leaves from the normal watering (NW) and those of the withheld watering (WW) treatment. The number in parenthesis represents the percentage of reduction of the leaf area of WW plants with respect to NW.

DASW ¹	BM ²	BM	PV ³	PV
	NW	WW	NW	WW
3	0.98	1.19 (----)	0.88	0.67 (23.9%)
	§±0.35	±0.69	§±0.27	±0.15
6	2.99	2.39 (20.1%)	1.97	1.81 (8.1%)
	±0.33	±0.52	±0.77	±1.08
9	5.27	3.37 (36.0%)	4.52	2.74 (39.4%)
	±0.71	±0.67	±0.55	±1.01
12	9.04	3.55 (60.7%)	8.34	2.96 (64.5%)
	±1.91	±0.58	±2.03	±0.46
15	13.07	4.08 (68.8%)	12.79	3.13 (75.5%)
	±1.17	±1.21	±5.13	±0.65
18	16.46	4.07 (75.3%)	18.83	3.46 (81.6%)
	±3.47	±0.64	±4.07	±0.80
21	24.67	3.94 (84.0%)	19.92	3.45 (82.7%)
	±2.83	±0.72	±3.53	±0.86
24	28.77	4.23 (85.3%)	27.31	3.43 (87.4%)
	±4.84	±0.60	±2.34	±1.06

¹DASW=Days after the suspension of watering, ²BM=Bayo Madero, ³PV=Pinto Villa, §±standard deviation.

Table 2. Soil water percentage of soil where bean plants of the normal watering (NW) and plants of the withheld watering treatments. The number in parenthesis indicates the reduction of WW soil with respect to the NW treatment.

DASW ¹	BM ²	BM	PV ³	PV
	NW	WW	NW	WW
3	21.1	17.2 (18.5%)	21.3	18.0 (15.5%)
	§±0.60	±0.80	§±1.44	±0.45
6	22.1	13.4 (39.4%)	24.6	14.8 (39.8%)
	±0.33	±0.99	±2.27	±0.66
9	19	9.5 (50.0%)	22.2	12.4 (44.1%)
	±1.23	±1.46	±0.74	±0.58
12	20.4	10.2 (50.0%)	25.7	11.4 (55.6%)
	±1.41	±1.11	±0.88	±0.31
15	15.3	7.8 (49.0%)	18.2	10.1 (44.5%)
	±1.90	±0.63	±3.75	±0.06
18	11.7	7.0 (40.2%)	14.2	8.3 (41.5%)
	±3.43	±0.50	±3.15	±0.39
21	15.9	5.9 (62.9%)	19.8	6.4 (67.7%)
	±3.77	±0.42	±1.72	±0.60
24	18.5	6.0 (67.6%)	18.8	5.8 (69.1%)
	±1.80	±0.40	±3.13	±0.42

¹DASW=Days after the suspension of watering, ²BM=Bayo Madero, ³PV=Pinto Villa, §±standard deviation.

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INHERITANCE OF DROUGHT TOLERANCE TRAITS IN ANDEAN X ANDEAN AND ANDEAN X MESOAMERICAN F₂ POPULATIONS.

Godwill S. Makunde¹, Stephen Beebe², Matthew W. Blair², Rowland Chirwa³, Davies Lungu⁴

¹Crop Breeding Institute, P. O Box CY550, Harare, Zimbabwe, ²CIAT – International Center for Tropical Agriculture - A. A. 6713, Cali, Colombia, ³CIAT – Malawi, Chitedze Station, ⁴University of Zambia, School of Agricultural Sciences

Introduction

Common bean is an important food and cash crop in Zimbabwe and other parts of Eastern and Southern Africa that is mainly grown by small-scale farmers for whom it is a major protein source for the human diet. Of late, common bean is also recognized to be a good source of essential micronutrients. The average per capita bean consumption across Africa in 2001 was 2.7 kg per year, with high-end consumption at 27.7 kg per year in Rwanda (FAO, 2003). Despite its importance in nutrition and income generation, common bean production is often threatened by drought episodes. In addition, for many countries such as Zimbabwe, the area under common bean is expanding into semi-arid areas suppressing average national production, which for Zimbabwe remains low at 500kg/ha even as total production increases due to greater total hectareage devoted to the crop. The chief cause of this low productivity is drought. Drought management through supplementary irrigation and good agronomic practices (mulching, tie ridging etc) has been of little use with small-scale farmers due to a number of factors, especially the high cost of irrigation equipment and monthly water charges. Genetic improvement of commercially grown cultivars for drought tolerance is the only cheap and viable option for small-scale farmers of Zimbabwe. This study is aimed at understanding the mode of inheritance of drought tolerance from Andean and Mesoamerican sources crossed with common bean cultivars important to Zimbabwe and will form the basis for developing drought tolerant bean varieties for the farming community.

Materials and Methods

A North Carolina design II was used to generate 49 experimental F₂ populations from 5 large seeded varieties popular in Zimbabwe (Red Canadian Wonder, CAL143, SUG131, PAN147, Natal Sugar) and drought tolerant sources including 5 males of Andean origin (RAA21, SEQ1003, SAB259, ICA Quimbaya, ICA Palmar) and 5 males of Mesoamerican origin (SER8, SER16, SER22, SEC16, SEQ11). One cross between Red Canadian Wonder and SER22 failed due to dwarf lethality but all other F₁s produced F₂ seed which was produced in Cali, Colombia in September 2005. F₂ seed was planted at the Save Valley Experiment Station, Zimbabwe during the 2006 winter season in two experiments, one under irrigation and the other one under drought stress, both with incomplete lattice designs and three replicates. In the drought stress trial, sprinkler irrigation was withdrawn three weeks after emergence of the plants and resumed at mid podding stage. Plots under irrigation were irrigated every two weeks throughout the season. Each irrigation cycle consisted of applying 40 mm water. The drought stressed plots received a total of 160 mm and irrigated plots received a total of 380 mm water throughout the whole growing season.

Results and Discussion

Specific combining ability (dominance) effects were higher than the general combining ability (additive) effects for grain yield, days to maturity and days to 95% flowering under both irrigated and water stressed conditions for both the Andean x Andean and Andean x Mesoamerican F₂ populations. Similarly, SCA effects were also higher for 100-seed weight under both irrigated and water stressed conditions for the Andean x Mesoamerican F₂ populations. However, SCA effects were smaller than GCA effects for 100-seed weight under water stressed conditions in the Andean x Andean F₂ populations but not under irrigated conditions.

Table 1. Contribution of GCA and SCA effects (%) to the variability of grain yield, 100-seed weight, days to maturity (DTM) and days to 50% flowering (DTF) among the Andean x Andean and Andean x Mesoamerican F₂ populations under irrigated and water stressed environments.

Andean x Andean F ₂ populations									
Irrigated environment					Water stressed environment				
Source	Yield	DTF	DTM	100-seed weight	Yield	DTF	DTM	100-seed weight	
GCA effects	12.97	10.57	16.61	22.05	28.41	26.28	13.66	36.46	
SCA effects	74.06	78.86	66.79	55.91	43.65	47.43	72.68	27.08	
Andean x Mesoamerican F ₂ populations									
Source	Yield	DTF	DTM	100-seed weight	Yield	DTF	DTM	100-seed weight	
GCA effects	13.33	20.31	13.93	21.84	25.89	21.63	15.22	26.32	
SCA effects	73.34	59.38	72.14	56.33	48.22	56.73	69.67	47.37	

Sprague and Tatum (1942) and Allard (1971) found that by choosing parents for crosses on merit alone, genetic variance for additive effects (GCA) can be reduced, increasing the relative importance of dominance gene effects (SCA). This could also hold for our crosses since the male parents were selected because of their history of drought tolerance and female parents due to their high productivity in Zimbabwe without previous studies of combining ability.

Consequently, narrow sense heritability is likely to be low from this scenario where GCA effects are lower than SCA effects for the yield and phenology traits measured but higher for 100 seed weight under drought stress where the opposite was true. Since dominance gene effects are non-fixable, it may be difficult to start selections based on yield under drought in the early breeding cycles. Selection methods such as single seed descent and bulk method could be of practical benefit in populations exhibiting low heritability for individual traits with selection beginning at F₅ upwards.

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INDICATORS OF DRY BEAN PERFORMANCE UNDER RAINFED CONDITIONS AT THE HIGHLANDS OF MEXICO

J. S. Padilla-Ramírez¹, E. S. Osuna-Ceja¹, E. Acosta-Díaz² and J. A. Acosta-Gallegos³

¹ Bean Program. INIFAP. Campo Experimental Pabellón. Apdo. Postal 20, Pabellón de Arteaga, Ags. México. C. P. 20660. ² Bean Program. INIFAP. Campo Experimental General Terán. ³ Bean Program. INIFAP. Campo Experimental Bajío. Email: jsaulpr@yahoo.com.

Introduction. Common bean is the second most important crop in Mexico with near 2 million hectares cultivated each year. However, almost 65% of the total area planted with dry beans is cultivated under rainfed conditions, where precipitation is limited (300 to 350 mm) during crop cycle. Thus, this crop is generally subjected to drought stress, which in turn affect grain yield. The evaluation of dry bean genotypes under soil water limited conditions is essential to select those materials showing good adaptation. There are different plant indicators of the crop performance under drought, although some of them need specialized laboratory equipment. In the present study, total biomass, harvest index, the assimilation rate to grain yield and their relationships were used as indicators of the dry bean performance under rainfed conditions in the semiarid highlands of Mexico.

Materials and Methods. The study was conducted at the Experimental Station of Sandoval (21° 54' N; 102° 04' W and 2000 masl) located in Aguascalientes state, during the summer of 2005 and 2006. Experiments were planted at the beginning of the rainfall season on July 5th and July 14th in 2005 and 2006, respectively. Six dry bean cultivars were evaluated in the first year, while at the second year 14 cultivars were included. Dry bean cultivars have different growth cycle including early, intermediate and late. The experimental design was a randomized complete block with four replications. The experimental unit consisted of four rows of 8.0 m long and 0.76 m apart. Meteorological data (rainfall and temperature) were registered at daily bases from a near climatological station in each growing season. Plant traits recorded in each plot were days to maturity, total aerial biomass (leaves excluded) and grain yield. Harvest index (grain yield/total aerial biomass) and a daily assimilation rate to grain yield ($\text{kg ha}^{-1} \text{d}^{-1}$) were estimated (1) and used to establish some relationships among them.

Results and Discussion. Early cultivars showed the lowest grain yields in both years with an average of 822 kg ha^{-1} , while intermediate and late cultivars had an average of 960 kg ha^{-1} and 1012 kg ha^{-1} , respectively (Table 1). These results are opposite to those reported previously (2) in which early genotypes had higher grain yield than late genotypes mainly due to the scarcity and untimely rainfall distribution. In contrast, during 2005 and 2006 total rainfall during the growth cycle was 316 and 498 mm, respectively, which had a better distribution especially at the reproductive period. However, these conditions are more uncommon and rainfed crops are usually exposed to terminal drought. Thus, earliness in dry beans has been considered as an escape mechanism to drought. In the other hand, harvest index was higher (60%) in the early cultivars than in the late cultivars (54%). Outstanding cultivars regarding to grain yield were: Azufrado Tapatío and Pinto Villa (early); Pinto Zapata Sel-CEPAB (intermediate) and Tlaxcala-62 (late), which showed either high harvest index or high biomass accumulation, similar results were reported (3). The relationship days to maturity vs assimilation rate to grain yield clearly separated those cultivars that can be more efficient to produce higher grain yields for each growth cycle. Pinto Villa, Pinto Zapata Sel-CEPAB and Tlaxcala-62 were among the cultivars showing the highest values of the assimilation rate to grain yield with an average of $12.0 \text{ kg ha}^{-1} \text{d}^{-1}$ (Figure 1). These results suggest that total biomass production, harvest index and the

assimilation rate to grain yield are good indicators to select dry bean cultivars having better adaptation to semiarid conditions.

Table 1. Grain yield (kg ha^{-1}) and growth cycle of dry bean cultivars evaluated under rainfed conditions at the Experimental Station of Sandoval in Aguascalientes, Mexico.

Cultivar	2005	2006	Mean	Growth Cycle
Pinto Villa (PV)	781	1002	891.5	Early
Pinto Zapata (PZ)	877	582	729.5	Early
Azufrado Tapatío (Az Tap)	956	916	936.0	Early
Pinto Bayacora (P Bay)	NI ¹	772	772.0	Early
Pinto Saltillo (P Sal)	NI	781	781.0	Early
Bayo Madero (BM)	NI	1008	1008.0	Intermediate
Bayo Victoria (BV)	NI	904	904.0	Intermediate
Blanco Español (B Esp)	NI	874	874.0	Intermediate
Flor de Junio Victoria (FJV)	NI	791	791.0	Intermediate
PZ Selección CEPAB (Sel PZ)	NI	1178	1178.0	Intermediate
Negro Altiplano (Ng Alt)	NI	1007	1007.0	Intermediate
Flor de Mayo M-38 (FM M38)	996	742	869.0	Late
Bayo Criollo del Llano (BCrLI)	1109	1039	1074.0	Late
Tlaxcala-62 (Tlax-62)	1063	1127	1095.0	Late

¹NI = Not Included

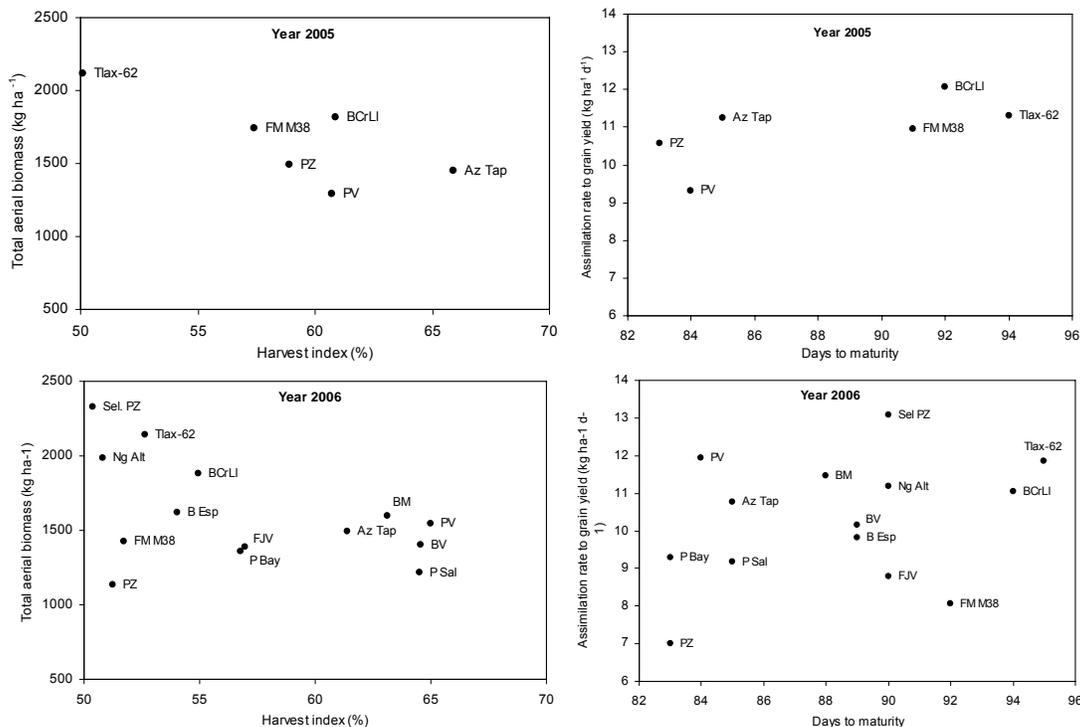


Figure 1. Relationships between harvest index vs total aerial biomass (left) and days to maturity vs assimilation rate to grain yield (right) in dry bean cultivars cultivated under rainfed conditions at Aguascalientes, Mexico.

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SOURCES OF VARIATION OF COMMON BEAN FOR DROUGHT TOLERANCE

A. P. Rodiño, M. Riveiro, M. Santalla and A. M. De Ron

Plant Genetic Resources Department, Misión Biológica de Galicia - CSIC, P.O. Box 28, 36080 Pontevedra, Spain

Introduction

Drought is considered the major factor limiting crop production worldwide and is responsible for heavy production losses in food legumes. Some losses are due to intermittent drought during the vegetative phase while other is due to terminal drought during reproductive development (Serraj et al. 2004). The severity of drought stress is unpredictable as it depends on many factors such as occurrence and distribution of rainfall, evaporative demands of the atmosphere and moisture storing capacity of the soils. Moderate to high drought stress can reduce biomass, number of seeds and pods, days to maturity, harvest index, seed yield and seed weight in common bean. Management practices can contribute to a decrease in yield loss in water-deficient environments, but major progress can also be achieved through genetic improvement. Plant responses to water stress include morphological and biochemical changes that lead first to acclimation and later, as water stress become more severe, to functional damage and the loss of plant parts (Chaves et al. 2002). In common bean, the main selection criteria for drought resistance are parameters related to plant growth and grain production. The objective of this work was to identify common bean sources of variation for breeding for drought tolerance.

Materials and Methods

Thirty nine common bean accessions (31 landraces and 8 resistant and susceptible checks) were evaluated in two locations in the northwest of Spain, 1) Location 1 (Pontevedra) (42° 24' N, 8° 38' W, 40 masl) and 2) Location 2 (Lalin) (42° 39' N, 8° 06' W, 745 masl) for drought tolerance under non stressed (NS) and drought stressed (DS) conditions according to a randomized complete block design with two replications. Each experimental plot consisted of four 25-plant rows with a crop density of 200000 plant ha⁻¹. The NS plot received additional irrigation and the DS plot not received irrigation. The Drought Intensity Index (DII) for each location was calculated as $DII=1-X_{ds}/X_{ns}$, where X_{ds} and X_{ns} are the mean of all genotypes under DS and NS environments, respectively. Drought Susceptibility Index (DSI) for each genotype was calculated as follows: $DSI=(1-Y_{ds}/Y_{ns})/DII$, where Y_{ds} and Y_{ns} are the average yields of a given genotype under DS and NS conditions, respectively. The following traits were determined: days to flowering, days to maturity, 100 seed weight (g), seed yield (g plant⁻¹) and Percent of Reduction (PR), measured in the two central rows of each plot. For data analysis, locations and replications were considered as random effects and DS versus NS treatments and common bean genotypes as fixed effects. All data were analyzed using as SAS statistical package (SAS Institute, 2000).

Results and Discussion

The effect of treatments was significant for days to maturity, 100 seed weight and seed yield in Pontevedra and for all characters except seed yield in Lalin. The effect of accessions in location 1 was significant for days to flowering and maturity, and 100 seed weight and in location 2 for all characters except seed yield. The interactions between genotypes and DS *versus* NS conditions were also significant for days to flowering and 100 seed weight in Pontevedra and they not were significant in Lalin. The DII based on seed yield of all genotypes was higher (0.77) in Pontevedra than in Lalin (0.031). Among the 39 accessions used in this study, SEA 5 (31.4 g plant⁻¹) and L88-18 (13.25 g plant⁻¹) have the highest yield in NS conditions in location 1 and 2, respectively (Table 1). Under the DS conditions, PHA-0471 (3.02 g plant⁻¹) and PHA-0683 (19.0 g plant⁻¹) had the highest yield in the two locations, respectively. The lowest yield in NS conditions was for PHA-0493 and PHA-2076 in Pontevedra and Lalin and for DS conditions were Ica Palmar and Pinto Sierra. In general, seed yield for 39 accessions in DS was significantly lower than in NS conditions. The drought resistant controls did not adapt well to the conditions from the Northwest of Spain. Drought stress, on the average, reduced bean yield by 40% and reduction in seed weight due to drought stress was ranged from 0-27%. Teran and Singh (2002) also reported yield reduction between 41 and 95 % due to DS. It is possible identify different accessions as drought resistant from those environments with higher DII values. PHA-0122, PHA-0483, PHA-0493,

PHA-0543, PHA-0595 had low DSI and Pinto Sierra, SEA 5, L88-18, PHA-0006 and PHA-0118 had high DSI. PHA-0432, PHA-0471, PHA-0683, PHA-2074, Linex, and Alavesa, possessed moderate levels of drought resistance; they yielded well in DS and moderate in NS conditions. PHA-0483, PHA-0543, PHA-0595, LEF2RB, UI239, and Othello had high level of drought resistance, with DSI<1.0 indicating below-average susceptibility to drought. Variation for drought tolerance was found in other studies (Muñoz et al. 2006). Breeding crops for drought resistance is often considered to be a slow and difficult process. Moreover, drought may not be representative of that occurring in the major drought endemic regions of the world. Thus, while breeding for resistance to drought stress, either PR or DSI could be used in combination with yield in DS and NS or GM yield to identify superior genotypes. Effects of DS versus NS environments for seed yield and differences among genotypes for all characters except PR and DSI were significant. Under such conditions common bean genotypes with high yield in NS and DS environments and low DSI value are desirable.

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Table 1. Seed yield for common-bean selected genotypes evaluated in DS and NS conditions in two locations

Accessions	Seed yield (g plant-1) (Pontevedra)				Seed yield (g plant-1) (Lalin)			
	NS ^a	DS ^b	PR(%)	DSI	NS ^a	DS ^b	PR(%)	DSI
ALAVESA	9.21	2.53	72.53	0.94	11.59	5.30	54.27	17.51
L88-18	8.63	1.43	83.43	1.08	13.25	8.02	39.47	12.73
LEF2RB	3.20	2.31	27.81	0.36	2.36	7.88		
LINEX	8.70	2.65	69.54	0.90	11.34	6.54	42.33	13.65
OTHELLO	8.83	1.97	77.69	1.01	7.38	5.85	20.73	6.69
ICA PALMAR	4.94	0.66	86.64	1.13	4.22	2.38	43.60	14.07
PHA-0006	5.32	1.14	78.57	1.02	4.64	5.16		
PHA-0118	7.66	0.92	87.99	1.14	4.63	2.80	39.52	12.75
PHA-0122	3.54	1.16	67.23	0.87	2.21	3.73		
PHA-0432	7.38	2.36	68.02	0.88	5.97	10.09		
PHA-0471	8.22	3.02	63.26	0.82	9.15	10.82		
PHA-0483	4.12	1.45	64.81	0.84	5.56	5.14	7.55	2.44
PHA-0493	1.83	0.77	57.92	0.75	6.57	7.87		
PHA-0543	8.01	2.80	65.04	0.84	7.26	5.30	27.00	8.71
PHA-0595	6.85	2.43	64.53	0.84	10.47	7.76	25.88	8.35
PHA-0683	7.26	0.96	86.78	1.13	9.58	19.00		
PHA-2074	4.51	1.79	60.31	0.78	2.51	10.03		
PHA-2076	6.24	1.29	79.33	1.03	1.33	3.39		
PINTO SIERRA	7.69	0.75	90.25	1.17	4.59	1.77	61.44	19.82
SEA 5	31.40	2.16	93.12	1.21	12.25	6.35	48.16	15.54
UI 239	6.54	2.07	68.35	0.89	7.97	8.85		
Overall average	6.62	1.56	73.23	0.95	6.12	6.23	37.22	12.01

^aLSD ($p \leq 0.05$)=6.0 ^bLSD ($p \leq 0.05$)=4.3

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PATTERN OF MACRONUTRIENTS ABSORPTION BY COMMON BEAN CVS. BRS MG TALISMÃ AND OURO NEGRO AT CONVENTIONAL AND NO TILL SYSTEMS

M. J. B. Andrade¹, N.M.B.Vieira¹, J. G. Carvalho², J. Alves Junior¹, A.R.Moraes³, and P. M. Rezende²

Departamentos de Agricultura¹, Ciências do Solo² and Ciências Exatas³ of the Universidade Federal de Lavras, Caixa Postal 37, 37200-000 - Lavras, MG, Brazil.

Introduction: The growth curves and nutrients patterns of absorption in function of the plant phenologic status allow us to know the amounts of nutrients absorbed and the relative intensity of absorption of each culture. For your exam, the periods of larger absorption of essential nutrients are evidenced, giving basic information about the most appropriate times for the fertilizer application. In order to characterize the pattern of macronutrients absorption by the BRS MG Talismã (recently recommended in Brazil) and Ouro Negro cultivars, at conventional and no tilling systems, were carried out one field experiment atn winter-spring sowing season, in the typical dark red latossol at experimental area of the Departamento de Agricultura, Universidade Federal de Lavras (UFLA), and three field experiments at summer-autumn sowing season, in a acric red yellow soil at commercial area, Madre de Deus de Minas, both places at Minas Gerais State, Brazil.

Material and Methods: The experimental design was randomized blocks with three replications and twelve or eleven treatments (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 15 or 16 seeds per meter. The experiment was conducted under conventional irrigation by aspersion at Lavras and without irrigation at Madre de Deus de Minas. The bean cultivar BRS MG Talismã, a carioca commercial type, originating from the UFLA Bean Genetic Improvement Program, showed growth habit III (semi prostrated), normal cycle and resistance to the 89 race of *Colletotrichum lindemuthianum* and the gold mosaic virus (EMBRAPA, 2002). The bean cultivar Ouro Negro, a black commercial type, showed growth habit III, normal cycle and resistance to the anthracnose, high capacity nitrogen biologic fixation and cold tolerance (Informativo..., 1997). Each time, ten or twenty plants were sampling for macronutrients levels determinations. The collected material was dry with circulation of air to 65-70 °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:

The maximum accumulation of N and P it happened between 75-89 DAE (days after emergency) and K, at 70-78 DAE (Figures 1, 3, 5 e 7). Ca presented larger absorption between 46-66 DAE, Mg showed your higher absorption levels between 57-84 DAE and S, at 72-84 DAE (Figures 2, 4, 6 e 8). The N, P, K e Mg showed larger extractions at no tilling system and the absorption sequence was N>K>Ca>Mg>P>S. The cv. Talismã absorbed more Ca in relation to cv. Ouro Negro.

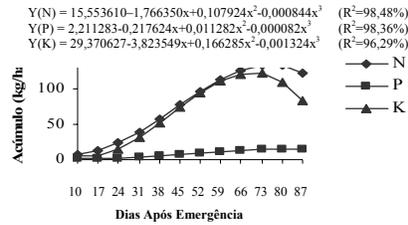


FIGURE 1 – Pattern of primary macronutrients absorption by the bean plant cv. Ouro Negro at no tilling system, during the crop cycle.

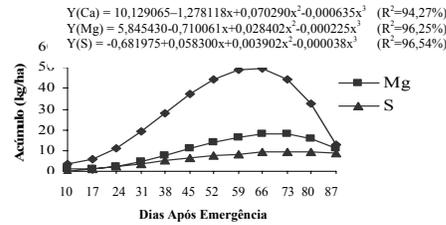


FIGURE 2 – Pattern of secondary macronutrients absorption by the bean plant cv. Ouro Negro at no tilling system, during the crop cycle.

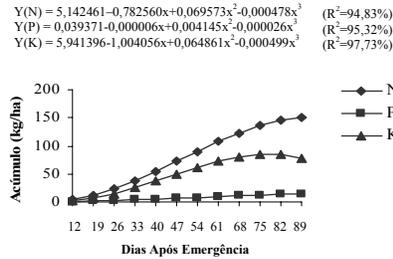


FIGURE 3 – Pattern of primary macronutrients absorption by the bean plant cv. BRS-MG Talismã at no tilling system during the crop cycle.

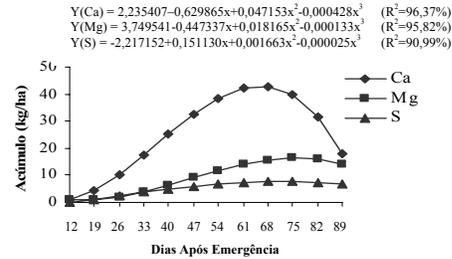


FIGURE 4 – Pattern of secondary macronutrients absorption by the bean plant cv. BRS-MG Talismã at no tilling system, during the crop cycle.

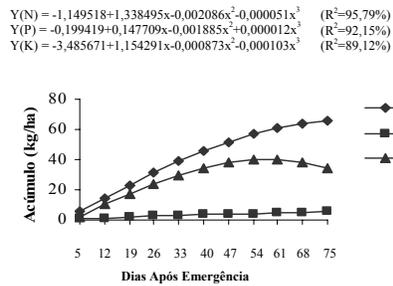


FIGURE 5 – Pattern of primary macronutrients absorption by the bean plant cv. Ouro Negro at conventional system during the crop cycle.

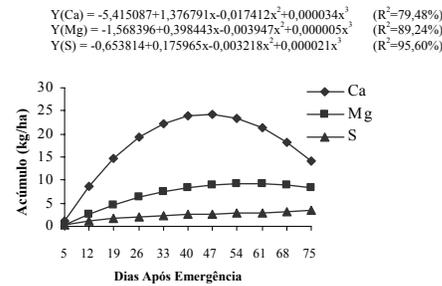


FIGURE 6 – Pattern of secondary macronutrients absorption by the bean plant cv. Ouro Negro at conventional system during the crop cycle.

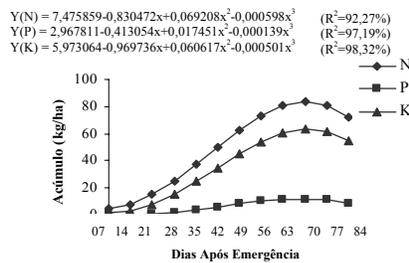


FIGURE 7 – Pattern of primary macronutrients absorption by the bean plant cv. BRS-MG Talismã at conventional system during the crop cycle.

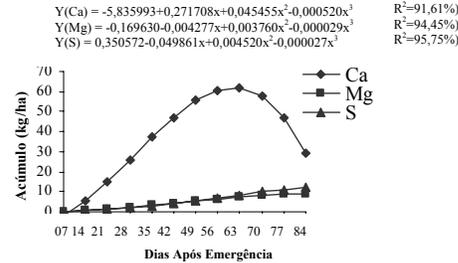


FIGURE 8 – Pattern of secondary macronutrients absorption by the bean plant cv. BRS-MG Talismã at conventional system during the crop cycle.

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PATTERN OF MICRONUTRIENTS ABSORPTION BY COMMON BEAN CVS. BRS MG TALISMÃ AND OURO NEGRO AT CONVENTIONAL AND NO TILLING SYSTEMS

N.M.B.Vieira¹, M. J. B. Andrade¹, J. G. Carvalho², J. Alves Junior¹, A.R.Moraes³. P. M. Rezende²

Departamentos de Agricultura¹, Ciências do Solo² and Ciências Exatas³ of the Universidade Federal de Lavras, Caixa Postal 37, 37200-000 - Lavras, MG, Brazil.

Introduction: In the current agriculture, where the profitability and the sustainability are important aspects, the micronutrients became used in more routine way in the fertilization. In spite of having recognized as essential elements for the development of the plants, studies related were, in your majority, recent and relatively scarce. In general, your absorption for the vegetable depends, among other factors, of the stadium of plant development, of the micronutrient metabolic activity, besides a series of factors related with the conditions of the environment. In order to characterize the pattern of micronutrients absorption by the BRS MG Talismã (recently recommended in Brazil) and Ouro Negro cultivars, at conventional and no tilling systems, were 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), and three field experiments at summer-autumn sowing season, in a acric red yellow soil at commercial area, Madre de Deus de Minas, both places at Minas Gerais State, Brazil.

Material and Methods: The experimental design was randomized blocks with three replications and twelve or eleven treatments (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 15 or 16 seeds per meter. The experiment was conducted under conventional irrigation by aspersion at Lavras and without irrigation at Madre de Deus de Minas. The cv. BRS MG Talismã, a carioca commercial type, originating from the UFLA Bean Genetic Improvement Program, showed growth habit III (semi prostrated), normal cycle and resistance to the 89 race of *Colletotrichum lindemuthianum* and the gold mosaic virus (EMBRAPA, 2002). The cv. Ouro Negro, a black commercial type, showed growth habit III, normal cycle and resistance to the anthracnose, high capacity nitrogen biologic fixation and cold tolerance (Informativo..., 1997). Each time, ten or twenty plants were sampling for macronutrients levels determinations. The collected material was dry with circulation of air to 65-70 °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:

The maximum accumulation of B, Fe and Mn it happened between 54-73 DAE (days after emergency), whereas the Cu and Zn showed your higher absorption levels between 72-87 DAE (Figures 1-4). The absorption sequence was Fe>Zn>Mn>B>Cu. The no tilling system showed larger Cu absorption and the Ouro Negro black cultivar absorbed more Fe in relation to the Talismã carioca cultivar.

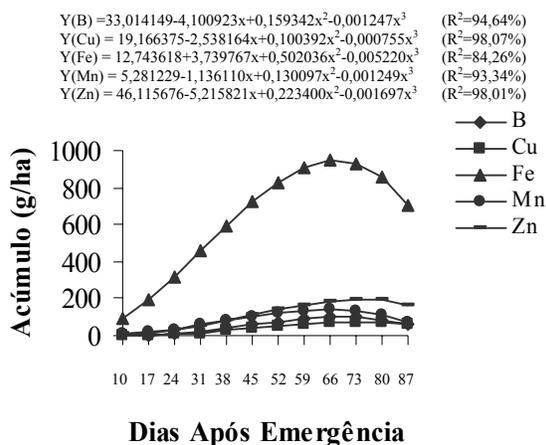


FIGURE 1 – Pattern of micronutrients absorption by the bean plant cv. Ouro Negro at no tilling system, during the crop cycle.

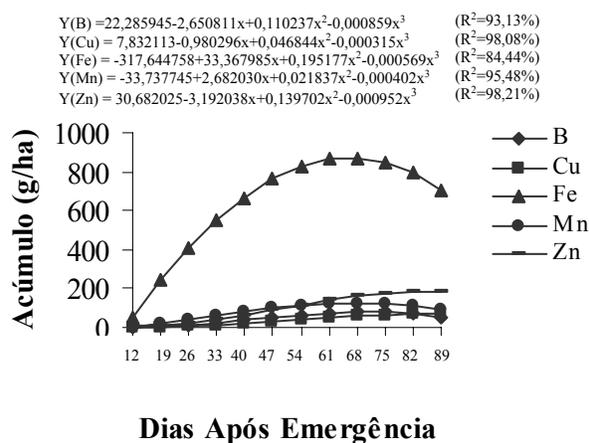


FIGURE 2 – Pattern of micronutrients absorption by the bean plant cv. BRS-MG Talismã at no tilling system, during the crop cycle.

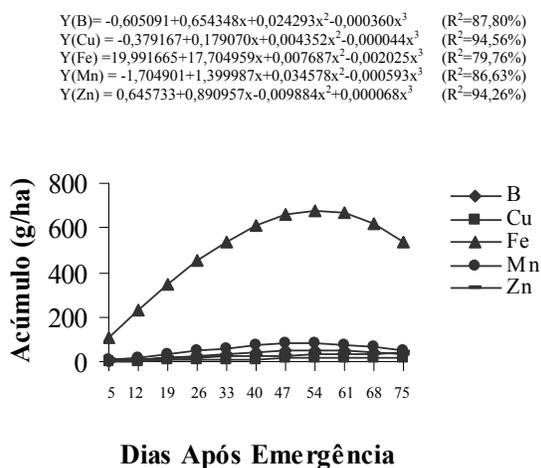


FIGURE 3 – Pattern of micronutrients absorption by the bean plant cv. Ouro Negro at conventional system, during the crop cycle.

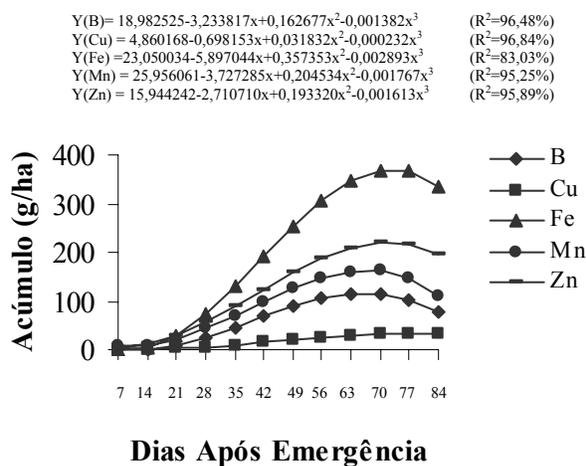


FIGURE 4 – Pattern of micronutrients absorption by the bean plant cv. BRS-MG Talismã at conventional system, during the crop cycle.

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BEAN (*PHASEOLUS VULGARIS* L.) YIELD REDUCTION BASED ON WEED INTENSITY COMPETITION

J. Alberto Escalante-Estrada and Ma. Teresa Rodríguez-González.

Colegio de Postgraduados. Campus Montecillo. Montecillo, Edo. Méx Méx. México. 56230. E-mail: jasee@colpos.mx ; mate@colpos.mx.

INTRODUCTION

The presence of weeds has been shown to lead to reductions in crop yield (Serrano *et al.*, 2001). The size of this reduction will depend on the intensity of the interspecific competition generated by the weeds population. In other crops mathematical models have been generated to evaluate this relation (Cousens, 1985). Nevertheless, since that the crop sensitivity to the weed competition will depend on the crop species, crop variety and weed species that occurred in the crop should be looking a model for each growth conditions and crop. Therefore, to determine the model that explains the degree of reduction of the bean yield Flor de Durazno (recommended by Campos *et al.*, 1998 for highlands and rainfall conditions), based on the weeds population was the objective of the present study.

MATERIALS AND METHOD

Sowing Time of bean (*Phaseolus vulgaris* L.) Flor de Durazno of determinate growth habit, type I, pink with beige color of seed, seed size of 410-530 mg and seed protein of 26 % was on May 26, 2006, under rainy conditions in Montecillo, Mex.(19°29 ' N, 98° 45'W, 2.250 ms of altitude, tempered to semi-arid climate).The density was 33 plants by m² in rows of 40 cm apart in a Fluvisol type. All the plots were fertilized with 100-100-00 of NPK. Once the species of weeds had the appropriate size to be identified (30 days after sowing time, das) was come to give the following treatments of weed elimination (E): 1) without elimination (0%E), complete competition; 2) 100% E, without competition; 3) 75% E, 25% of competition; 4) 50%E, 50% of competition; and 5) 25%E, 75% of competition. The design was a randomized blocks with four replicates. The phenology and the grain bean yield (dry matter, g) were evaluated.

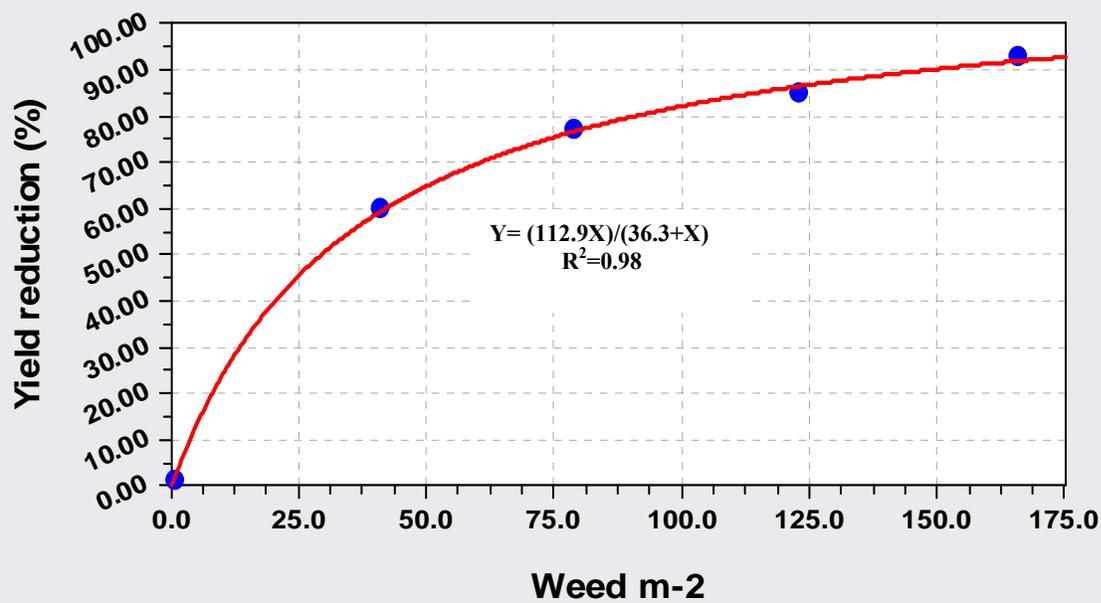
RESULTS AND DISCUSSION

The bean phenology was not affected by the weed competition with weeds. The beginning of flowering occurred 28 das and physiological maturity 100 das. The weeds population for complete competition (comp) (0% E) was of 166 plants by m²; 75% comp, 123 plants by m²; 50% comp, 79 plants by m²; 25% comp, 41 plants by m²; and without competition (0% comp).The bean yield reduction was of 93, 85, 77, 60 and 0%, respectively, The bean yield was of 20, 41, 63, 110 and 277 g m⁻², respectively. The saturation growth rate model ($Y = (ax) / (b+x)$) was the one that showed to a determination coefficient ($R^2=0.98$) highest to explain this relation (Figure 1), which differs to the $Y=(ID) / (1+ (ID/a))$ reported previously for other crops, growth conditions and different weed species in competition (Cousens, 1985) . The competition beans-weeds was by 70 days. Eleven weeds species in the experimental area were registered. The highest important by abundance order were: *Simsia amplexicaulis* (cav) Pers, *Chenopodium* sp, *Amaranthus hybridus* L., and some species of gramines.

CONCLUSION

The saturation growth rate model was the one that better predicts the losses of yield bean Flor de Durazno with base to weed density.

**Figure 1.- Bean yield reduction and weed population.
Montecillo Méx. México. Summer 2006.**



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EFFECTS OF INDENTATION, TIDE RIDGING AND TILLAGE SYSTEMS ON SEED YIELD OF DRY BEAN IN THE SEMIARID HIGHLAND OF MEXICO

E. S. Osuna-Ceja², J.S. Padilla-Ramírez², M.A. Martínez-Gamiño², E. Martínez Meza and J.A. Acosta-Gallegos²

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²INIFAP Bean Research Group. Apartado Postal 20, Pabellón de Arteaga, Aguascalientes, México. C.P. 20660. esosuna@yahoo.com.mx

Introduction. Limited and erratic rainfall in the semiarid highlands of Mexico often results in low dry bean yield and sometimes in total crop failures. Soil water conservation practices can greatly improve the potential success in many dry land farming systems. Conservation tillage systems that contribute to minimize soil erosion and retain crop residues on the soil surface have generally increased water conservation. Seed yield of dry beans under rainfed conditions may be enhanced by utilizing improved dry bean cultivars in conjunction with cultural practices such as in situ rainwater catchments techniques, combined with reduced tillage (1). Also, furrow ridges, which are small dikes or micro-catchments along the rows at 3-m intervals throughout the length of the field and the “Aqueel”, which is the trade name for a unique means of creating indentations in a loose soil surface. These ridges along the rows and the indentations, act as water reservoirs to store rainwater. Both dry land conservation technologies have increased dry bean yield, reduced runoff and control soil erosion (2). In a semiarid environment, the efficient use of precipitation is necessary to reduce production risks. The purpose of this study was to validate an integral dry bean production strategy by including several technological components in combination with improved cultivars.

Material and Methods. The study was conducted at different sites located in the region known as “El Llano” in the northeast of the state in Aguascalientes (21° 54' N; 102° 04' W) during summer 2005 and 2006. This area is characterized as semiarid with an annual rainfall average < 450 mm at an altitude of 2000 m.a.s.l. The soil is sandy clay loam with a pH value of 7.9 and < 1% organic matter; it is superficial with 1% slope. Trials were conducted in four sites in 2005 and five in 2006. Plots of one ha each were planted after the onset of the rainy season on July 5th in 2005 and on July 15 and 27th in 2006. Three technological components were validated: 1) Improved bean cultivars (Flor de Mayo Bajío and Flor de Mayo Sol); 2) Tillage methods (disc plowing (DP) and “Multiarado” (Mult), chisels that break the ground without turning the soil) and 3) In situ water catchments and conservation (Aqueel “Aq” and Ridges “R”). The Aqueel wheel makes continuous rows of indentations; it was attached to the rear of the planter at the time of sowing. Tide ridges were 0.15 to 0.20 m high and were form in each furrow at spacing of 3-m with hoe like devices attached to the cultivator (35 days after planting). Treatments were established on strips of six to eight rows 0.76 m wide and 100 to 150 m long. Seed yield was determined on four samples of 6.08 m² (2 x 0.76 x 4) per treatment.

Results and Discussion. In 2005 total rainfall from June to October was similar to the long-term average and above it in 2006 (Table 1). Within each growing season there were dry and wet spills; in 2005 early in the season (June) rainfall was below average, considerably above average during July and August, and below average in September and October. Rainfall in 2006 was above average from July to September and below average in October. Rainfall distribution is important when water catchments are built during the crop season since they will have a positive impact only if it rains after they are form.

TABLE 1. Average long-term rainfall (30 yr) and rainfall occurred in 2005 and 2006 at “El Llano”, Aguascalientes, Mexico.

MONTH	Rainfall (mm)		
	2005	2006	30-yr avg
June	25	34	96
July [†]	105	156.4	100
August	167	195.2	84
September	14	67.4	61
October	3.8	4.0	30
Total	314.8	457	371

[†] Total growing season rainfall was 273 mm in 2005 and 343 mm in 2006.

TABLE 2. Average seed yield (ton ha⁻¹) of two dry bean cultivars grown under four technological components in five sites at “El Llano”, Aguascalientes, Mexico during 2005 and 2006

Technological Component	Experimental Sites					Average
	Tildio I	Tildio II	Copetillo	Sandoval	Sta. Rosa	
Cultivar:	2005					
Flor de Mayo Bajio	1.035	0.416	0.527	0.782	-	0.690
Flor de Mayo Sol	0.957	0.365	0.581	0.980	-	0.721
Tillage methods:						
Disc plowing	1.028	0.333	0.560	0.893	-	0.703
Multiarado	0.964	0.447	0.547	0.868	-	0.707
Water catchment:						
Ridges	1.061	0.428	0.554	0.942	-	0.746
Without Ridges	0.931	0.353	0.554	0.789	-	0.657
Cultivar:	2006					
Flor de Mayo Bajio	626.8	156.2	0.476	1.014	0.567	0.568
Flor de Mayo Sol	866.6	115.3	0.627	1.012	0.717	0.668
Tillage methods:						
Disc plowing	690.7	142.5	0.457	1.123	0.597	0.602
Multiarado	802.7	129.0	0.646	1.170	0.679	0.685
Water catchment:						
Aqueel	-	-	0.612	1.203	0.639	0.819
Without aqueel	-	-	0.491	0.902	0.634	0.676
Ridges	757.6	153.4	0.558	1.163	0.745	0.675
Without Ridges	735.8	118.1	0.545	1.023	0.546	0.594

In 2005 and 2006 cv Flor de Mayo Sol outperformed Flor de Mayo Bajio by 4% and 15%, respectively. Both tillage methods had a similar yield in 2005 and the ‘Multiarado’ had 12% higher yield than disc plowing; this is important since the ‘Multiarado’ uses have the time and energy to prepare one ha than the traditional disc plowing. As for the Aqueel, an implement that was tested in 2006, this was the component with the higher yield, 17% higher than the control. The use of ridges also improved yield by 12%. The implement for building ridges costs less than the aqueel, although the aqueel can further increase seed yields. The introduction of technological components in the semiarid highlands of Mexico can greatly reduce the risks of producing rainfed dry beans.

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OXADIARGYL: A PRE EMERGENCE HERBICIDE TO CONTROL BLACK NIGHTSHADE (*SOLANUM NIGRUM*) IN DRY BEAN

Alberto Pedreros and Juan Tay
INIA Quilamapu
Casilla 426, Chillán, Chile
e-mail : alberto.pedreros@inia.cl

Introduction

Dry bean production is affected by several weed species during crop development, and yield can decrease more than 60% under central valley of Chile. Use of herbicides is the most important weed control system, however some species, such as black nightshade (*Solanum nigrum*) and lambsquarters (*Chenopodium album*), are not well controlled. Thus, these weeds can reach high populations during bean flowering (Pedreros, 1993; Tay et al., 2005). On the other side, besides weed interference, there are high amount of black nightshade fruits still wet at harvest time. The objective of this experiment was to evaluate the effect of oxadiargyl herbicide on black nightshades density and dry bean yield.

Materials and Methods

A field trial was carried out during 2005-2006 season. Dry bean cv. Torcaza-INIA was planted during November 2, 2005. The herbicide treatments were applied with a CO₂ sprayer, delivering 200 l/ha with a pressure of 241 kPa. Oxadiargyl (Topstar) and S-metolachlor (Dual Gold) were pre-emergence applied, while Trifluraline (Treflan) was pre plant incorporated and Bentazon (Basagran) was post emergence sprayed. The experiment was arranged in a randomized complete blocks with four replications; plots size was 5 m long x 2 m wide in rows spaced at 0.4 m. Dry bean yield was determined by weighing 3 m long of the two central rows, while yield components were taken from two 0.5 m x 0.5 m quadrants of each plot.

Results and Discussion

No visual phytotoxicity symptoms were detected during growing of bean cv. Torcaza-INIA (data no shown). None treatments affected bean density of plants evaluated at harvest time. On the other side the treatments weedy check, S-metolachlor, trifluraline and bentazon significantly decreased pod per plant, while affected grain per pod. Only weedy check decreased weight of grain in comparison with oxadiargyl at rates between 0.6 and 1.0 kg a.i. ha⁻¹ while the other treatments were similar to untreated (Table 1).

Table 1. Effect of herbicide treatments on yield and yield components of Torcaza-INIA bean cv. Chillán, Chile 2005-2006.

Treatments	Rates a.i l or kg ha ⁻¹	Density plant/ m ²	Pod per plant	Grain per pod	Weight of 100 grains	Yield t ha ⁻¹
1. Weedy check	-	27 a	9 b	3 c	34.8 b	0.99 f
2. Oxadiargyl (PRE)	0,6	27 a	13 a	4 abc	37.6 a	2.20 ab
3. Oxadiargyl (PRE)	0.8	27 a	14 a	4 ab	37.6 a	2.21 ab
4. Oxadiargyl (PRE)	1.0	27 a	14 a	4 a	38.0 a	2.41 a
5. Oxadiargyl (PRE)	1.2	28 a	13 a	4 abc	35.5 ab	2.01 bc
6. S-Metolachlor (PRE)	1.152	29 a	11 b	4 abc	37.1 ab	1.73 d
7. Trifluraline (PPI)	0.72	27 a	10 b	4 bc	34.5 ab	1.26 e
8. Bentazon (POST)	0.96	27 a	13 a	4 bc	37.1 ab	1.91 cd
Coefficient of variation		10.6	16.5	12.7	4.8	9.2

1 Means within a column followed by the same letter are not different, LSD (0.05).

2 Similar values with different letters are due to make up to a round numbers.

The main weed in the untreated control, at 45 days after treatments, was black nightshade (*Solanum nigrum*), with a population over 80 plants/m². The herbicide oxadiargyl, at any rate, controlled a 100% of this weed at 45 days after planting, and only S-metolachlor was similar, although it had 4 plants/m² at evaluation time (Table 2). This herbicide could be evaluated in mixture or sequential applications with others that do not control this weed.

Table 2. Effect of herbicide treatments on density and dry matter of black nightshade 45 days after treatments. Chillán, Chile 2005-2006.

Tratamientos	Rates a.i l ó kg ha ⁻¹	Black nightshade	
		Population (plants/m ²)	Dry matter (g/m ²)
1. Weedy check	-	84 a	38.2 a
2. Oxadiargyl (PRE)	0,6	0 c	0.0 c
3. Oxadiargyl (PRE)	0.8	0 c	0.0 c
4. Oxadiargyl (PRE)	1.0	0 c	0.0 c
5. Oxadiargyl (PRE)	1.2	0 c	0.0 c
6. S-Metolachlor (PRE)	1.152	4 c	1.1 c
7. Trifluraline (PPI)	0.72	44 ab	13.2 b
8. Bentazon (POST)	0.96	32 b	26.6 ab
Coefficient of variation		42.5	51.0

1 Means within a column followed by the same letter are not different, LSD (0.05).

2 Data were transformed to (log x + 1) to stabilize variances, they are reported as original values.

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SNAP BEAN (*PHASEOLUS VULGARIS* L.) YIELD AND WEED CONTROL WITH SUNFLOWER (*HELIANTHUS ANNUUS* L.) RESIDUE

Nicolás Salinas-Ramírez¹, J. Alberto Escalante-Estrada¹, Ma. Teresa Rodríguez-González¹ and Eliseo Sosa-Montes². ¹Colegio de Postgraduados. Montecillo, México, 56230.

²Univesidad Autónoma Chapingo, Chapingo, México, 56230

INTRODUCTION In the last years the per capita consumption of snap bean has been increased, which leads to the search for higher yields, than is possible to be obtained by means of the control of the abiotic and biotic factors that limit yield. The weed occurrence can cause reductions in a 100% of the crop production by the competition of the ecological substrate (space, water, nutrients and light), in addition to being damaged by plagues and diseases (Carvalo and Torres, 1994). The weed control in snap bean generally is made by manual, mechanical and chemical form, being this last, fast and efficient. Nevertheless, the excessive use of agrochemical (Metobromuron) for its control can cause resistance of the weed to these products, environmental contamination and in some cases reduction in the growth and the yield pods (Park and Hamill, 1993; McNaughton *et al.*, 2004). The weed control with products of vegetable origin has been set out, particularly with sunflower residues, that when degrading, they release allelopathic substances that inhibit the germination and weed growth (Rodríguez, 1994); in addition can be a source of organic matter to the soil. Thus, the aim of this study was to determine the effect of the soil incorporation of sunflower residues on weed control and yield in snap bean.

MATERIALS AND METHODS The study was made in the experimental field of the Colegio de Postgraduados, Montecillo, México (19° 29 ' N, 98°53 ' O, to 2250 m of altitude), and climate BS1, the least dry of the arid climates, with rains in summer, annual average temperature of 14,6 °C and 558,5 mm of rainfall (García, 2005). The soil is of clay texture (molic Fluvisol, Flm), with 2 to 3% of organic matter and pH of 8 in the first 30 cm of profile. The sowing of snap bean Hav-14 habit of climbing growth indeterminate was made on May 2, 2005, under rainy conditions with trellises to the density of 5.2 plants by m², with application of 1.5 kg m⁻² of sunflower residue 15 days before crop sowing, and without application (control). The design was a randomized block whit four replicates. The phenology of snap bean was determined on the criteria of Escalante and Kohashi (1993). The fresh pod (fresh weight) yield (ton), and the pod number, was the sum of the harvest. The weeds were collected to the 30 days after sowing. Metallic marks of 50X50 cm were used, (quadrants) that were located in the central part of each parcel and the plants were collected for their identification, counting and biomass (g) by specie (Cox, 1978).

RESULTS AND DISCUSSION The phenology, snap bean yield (g) and the number of pods by m⁻² were not affected by the application of the sunflower remainder. Thus, the flowering occurred to the 56 days after sowing (das) and the date of the last harvest of pod to the 104 (das). The pod yield and number of pod were of 1170 g m⁻² and 258 pods m⁻², respectively. That eleven species of weeds that occurred in the control (without application of remainder), *Chenopodium album* L. and grass species showed greater number of plants by species (figure 1). With the application of sunflower remainders, the population and production of biomass (g) of the weeds was reduced (figure 2). This result suggest that application of sunflower residues in addition to being a source of organic matter for the improvement of the soil, is an alternative for the weed control in the crops that does not affect the snap bean yield.

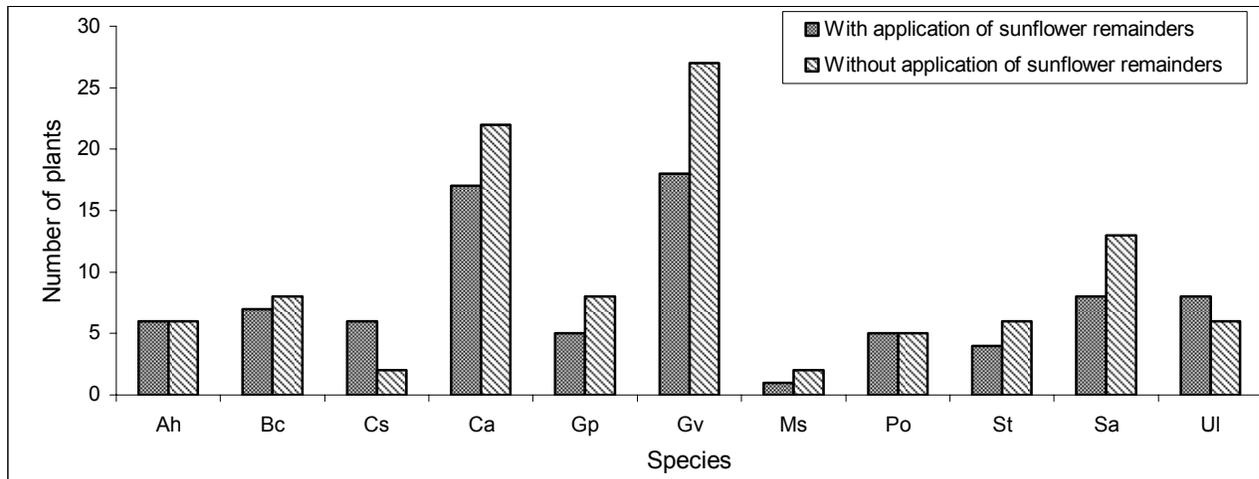


Figure 1. Weed species and number in the crop of snap bean. Means of five replicates; with or without sunflower treatment. Ah: *Amaranthus hybridus* L.; Bc: *Brassica campestris* L.; Cs: *Cyprus* sp.; Ca: *Chenopodium album* L.; Gp: *Galinsoga parviflora* Ca.; Gv: Gramines; Ms: *Malva* spp.; Po: *Portulaca oleracea* L.; St: *Salvia tiliifolia* Vahl.; Sa: *Simsia amplexicaulis* (Cav.)Pers.; Ul: *Urocarpidium limense* (L.) Kaprov.

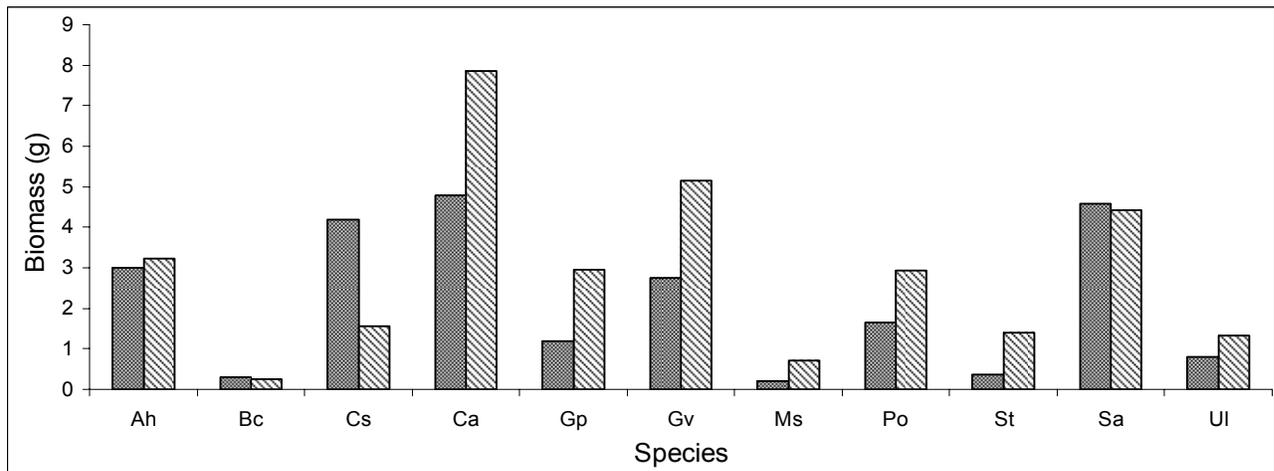


Figure 2. Biomass weeds in the crop of snap bean. Mean of five replicates. Colegio de Postgraduados, Campus Montecillo, Mexico. May 2005.

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EFFECTS OF WASTE CASSAVA PLANT ON EMERGENCE OF SNAP BEAN (UEL-2) SEEDLINGS

Brito¹, O. R.; Assunção², F. Q., Miglioranza¹, E., Brito², R. M.

¹ State University of Londrina, Agronomy Department. Email: osmar@uel.br. ² Graduate Agronomy Students. UEL, Brazil

INTRODUCTION

The waste from cassava plant is a liquid produced during the cassava processing for flour production. It is a pollutant residue with high biochemistry oxygen demand (HESS, 1962). The agriculture utilization is an alternative for disposition these residue. This experiment was carried out with the objective of evaluating the effects of waste cassava plant application on emergence of snap bean seedlings.

MATERIALS AND METHODS

The experiment was carried out under greenhouse conditions. The pots were filled up with a Typic Haplorthox soil with the following chemical properties: pH (CaCl₂)=4.7; Organic matter=17 g kg⁻¹; P=7.6 mg dm⁻³; K, Ca, Mg and Al equal to 0.18; 5.72; 0.76; 0.0 and 5.80 cmolc dm⁻³, respectively. The pots were irrigated with deionized water until they reached 70% of the total retention capacity. The waste cassava plant had the following characteristics: pH=5.5, and K= 4230, P= 270, Ca= 470, Mg= 270, Fe=30.60, Cu=1.40, Zn= 4.82 and Mn= 8,60 mg L⁻¹, respectively. Mineral fertilization was not used. The experimental design was entirely randomized. The treatments resulted of an arrangement of 4 residue levels (0, 20, 40 and 80 m³. ha⁻¹) and 5 sowing times (0, 2, 4, 8 and 10 days after the residue application (daa)), with 4 replications.

RESULTS AND DISCUSSION

The emergence of the snap bean seedlings (cv UEL-2) was affected by residue level and sowing time. For the sowing performed in the day of the application, the effects of waste cassava plant was higher than other sowing days (Figures 1 e 2). In this case, when the waste cassava plant dose was higher than 40 m³ha⁻¹, no seedling emergency was observed. In other sowing time, reduction of the effects of waste cassava plant doses on seedling emergence could be observed, probably because there were toxic degradation components. Then, the values of seedling emergence were higher than 10% and 49 %, for the 2daa and other sowing time days, respectively (Figure1). The reduction of deleterious effects of the waste cassava plant doses on the snap bean seedling emergence was evident according results presented in Figure 2. These results are according to Barana (2000) who worked with rice, sesame and mustard and also with Fioretto (1987), who worked with corn and cotton seeds. These authors observed that waste cassava plant decreased the seed germination and seedling emergence of these plants. For Fioretto (1987) the reduction of the seedling emergence can be attributed to the combined action of cyanide contend in the waste of cassava plant and to the increase of soil solution saline concentration. On the other hand, Barana (2000) does not recommend this waste application in previous or simultaneous irrigation of the sowing of these cultures.

Figure 1. Emergence percentage of the seedlings of snap bean (cv. UEL2) in function of doses of the waste cassava plant application and sowing times.

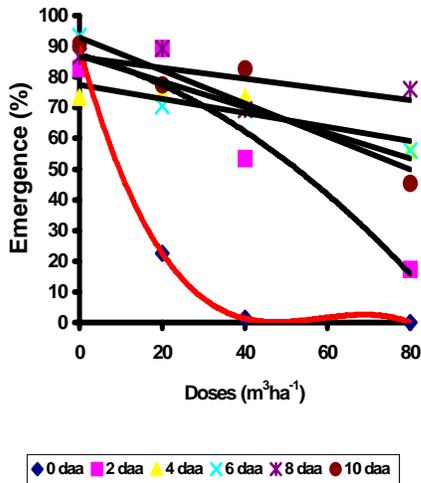
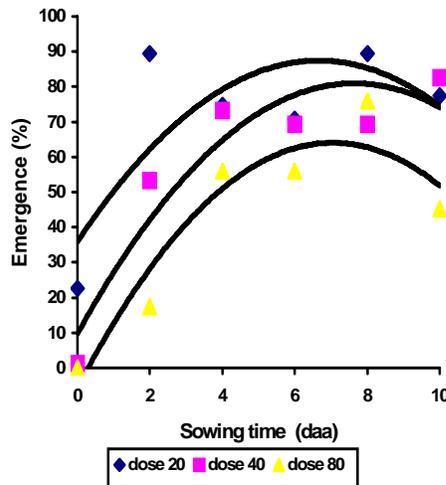


Figure 2. Emergence percentage of the seedlings of snap bean (cv. UEL2) in function of sowing times and doses of the waste cassava plant application.



CONCLUSIONS

The waste of cassava plant dose and sowing time influenced the snap bean seedlings emergence.

There was no seedling emergency of the snap bean (cv UEL-2) when the sowing was performed in the application day with doses higher than $40 \text{ m}^3\text{ha}^{-1}$.

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COMMON BEAN CULTURE NITROGEN FERTILIZATION UNDER NO-TILLAGE SYSTEM

¹ Brito, O. R., Kuhn², O. J., Richart¹, A. Miglioranza¹, E.

¹ Agronomy Department. UEL, Londrina-PR, Brazil. ² Graduate Student, ESALQ-USP, Piracicaba-SP, Brazil.

INTRODUCTION

The common bean is a crop of great economic importance for Brazil. The bean is a basic component of the Brazilian daily meal, contributing with the supplementation of proteins, carbohydrates and iron (YOKOYAMA et al. 1996). Besides being the greatest world-wide producing and consuming, Brazil is also a bean importer. The State of Paraná is the largest Brazilian producer with about 20% of the national production. The average productivity of the dry bean culture in Brazil is very low (550 to 700 kg ha⁻¹) being lower than the productive potential obtained in field assays that can vary of 3859 kg ha⁻¹ (IAPAR, 2007) to 6000 kg ha⁻¹ (WHITE & IZQUIERDO, 1991), mainly due low soil natural fertility and inadequate practices of culture techniques management.

MATERIAL AND METHODS

The field experiment was carried out in the period of autumn/winter of 1999, under no tillage system with mulching (8000 kg ha⁻¹ of dry matter of millet plant) in the experimental farm at Universidade do Oeste do Paraná (UNIOESTE) (54° 01' 9.09" W, 24° 31' 42.17" S and 400 altitude m) in Marechal Cândido Rondon. The soil is a typical oxisoil, with the following chemical characteristics: P = 8.25 mg dm⁻³; C = 20.8 g dm⁻³; pH CaCl₂ = 5.3; Al = 0.0, Ca = 4.84 cmol_cdm⁻³, Mg = 2.37 cmol_cdm⁻³, K = 0.26 cmol_cdm⁻³ and CEC = 13.68 cmol_cdm⁻³. A randomized blocks design with five treatments and four replications was used. The treatments were: T1 = Control (0+0+0); T2 = 30 kg ha⁻¹ of N in the sowing (30+0+0); T3 = 30 kg ha⁻¹ of N to the 25 DAE (days after the emergency) (0+30+0); T4 = 30 kg ha⁻¹ of N in the sowing + 30 kg ha⁻¹ to the 25 DAE (30+30+0); T5 = 30 kg ha⁻¹ of N in the sowing + 30 kg ha⁻¹ of N to the 25 DAE and + 30 kg ha⁻¹ of N to the 45 DAE (30+30+30). The number of pod per plant, the number of grains per pod and yield was evaluated. The obtained data were submitted to the variance analyses and the averages were compared by Tukey test to 5%.

RESULTS AND DISCUSSION

The different forms and amounts of N applied in the common bean culture influenced significantly the grains productivity, however no significant effect was observed for the number of pod per plant and the number of grains per pod (Table 1).

The highest productivities were observed for the treatments T5 and T4, which differed significantly from the control treatment, indicating the need of one or two complementary cover nitrogen fertilizations. Although KRANZ et al. (1975) consider unnecessary the cover nitrogen fertilization, this practice is already recommended by different authors as AMBROSANO et al., (1997) and PARRA (2000)

Table 1. Average productivity values of common beans culture in function of the types of nitrogen fertilization. Paraná State, Brazil.

Nitrogen Dose (kg.ha ⁻¹)	Productivity (kg.ha ⁻¹)
T5 = 30+30+30*	1796 a**
T4 = 30+30+00	1780 a
T2 = 30+00+00	1721 a b
T3 = 00+30+00	1628 a b
T1 = 00+00+00	1406 b
MDS	357

* (at sowing time + 25DAE +45 DAE)

** Averages followed by different letters, differ significantly by Tukey test to 5%.

CONCLUSIONS

The nitrogen fertilizations applied in the sowing and applied in covering increased the productivity of the beans culture.

The productivity of the common bean culture was not influenced by nitrogen fertilization applied only in sowing or only covering.

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NODULATION AND YIELD OF BUSH AND CLIMBING BEANS INOCULATED WITH RHIZOBIA STRAINS

Paul Kimani¹, G.K Gicharu², N. Mburugu², H. Boga³ and R. Cheruiyot²

¹Regional Program on Beans in Eastern Africa, University of Nairobi; ²Dept of Biological Sciences, Kenyatta University ³Dept of Botany, Jomo Kenyatta University of Agriculture and Technology, P.O Box 29053, Nairobi, Kenya

Introduction

Bean crops depend on symbiotic fixation to meet part of their nitrogen requirements. The balance is obtained from the soil. Nitrogen deficiency is the most important constraint to bean production in sub-Saharan Africa causing annual losses of more than 389,000 t per year. N is deficient in bean producing areas of Madagascar, South Africa, Zimbabwe, Malawi, Tanzania, Rwanda, Burundi, DR Congo, Uganda, Angola, Kenya and Ethiopia (Wortmann et al, 1998). Most smallholder farmers in eastern Africa do not apply fertilizers to their bean crops. This implies that productivity of bean cultivars developed for smallholder farmers in Africa depends on their ability to produce in soil low in N, and on symbiotic fixation. Although several bush and climbing bean varieties have been developed for smallholder production in eastern Africa, the effectiveness of their symbiosis with available inoculants and native rhizobia species is not well known. Moreover, the effect of inoculation on grain yield of the new varieties and advanced lines has not been determined. Our objectives were: i) to determine variation for nodulation and nitrogen fixation in bush and climbing bean lines, and ii) study the effect of inoculating bean genotypes with rhizobia strains on grain yield.

Materials and Methods

Three trials were conducted. In the first trial, 60 bush and 60 climbing bean lines were evaluated for nodulation in separate trials at Jomo Kenyatta University of Agriculture and Technology Research Farm, Juja during short rain and long rain seasons. Four weeks after germination plants were uprooted and nodules carefully detached from the roots and counted. In the second trial, three bush and three climbing bean lines with low (Ayenew and NG 224-4), medium (ECAB 0807 and Cargamanto), and high (GLP 24 and G59/1-2) nodulation potential were grown in polythene sleeves containing sterile vermiculite in the greenhouse and inoculated separately with three rhizobia strains (CIAT 899, USDA 2674, USDA 2676) recommended for bean inoculation in Kenya, and a mixture of the three strains. Rhizobia strains were obtained from Microbial Resource Centre (MIRCEN), University of Nairobi. Plants were irrigated with sterilized nitrogen free nutrient solution (Somasegaram et al, 1985) to ensure that the only available nitrogen for the bean plants was from nitrogen fixation. Plants were harvested at flowering (28 days for bush and 50 days for climbers), and shoot and root dry weight determined, and nodules counted. In third trial, the six bean lines were grown with and without inoculation in the field at Juja, Kenya. Data was recorded on nodule number, shoot and root dry weight, and grain yield.

Results and Discussion

Results showed that there was considerable variation in nodulation among the 120 bean lines. Climbing beans had more nodules per plant compared to bush beans. Average nodule number per plant among the bush bean lines varied from 16 (Awash-1) to 58 (ECAB 0097) with a mean of 33. Among the climbing bean lines, the range was 14 (G50330, Decelaya and MLV 6-90E) to 100 nodules per plant (G59/1-2) with a mean of 37. The nodules in all bean cultivars occurred

mainly on the lateral and finer roots with very few on the tap root. Majority of the nodules were medium in size with a diameter of 1 to 2 mm and were pink in color. Results of the greenhouse trial showed that there were significant differences in nodulation among the bush bean lines. Uninoculated plants produced the smallest number of nodules. Among the inoculated plants, lines inoculated with USDA 2676 produced the smallest number of nodules (50 nodules plant⁻¹). Plants inoculated with USDA 2674 had the highest number of nodules (70 nodules plant⁻¹). Ayenew nodulated best when inoculated with USDA 2674, GLP 24 with CIAT 899, and ECAB 0807 with the three strains. This suggested specificity among genotypes and rhizobia strains. Among the climbers, G 59/1-2 had the highest nodule number with an average of 109.7 nodules plant⁻¹. Inoculated plants produced significantly more nodules than control plots (Table 1). Cargamanto and NG 224-4 nodulated best when inoculated with USDA 2676. In contrast, G59/1-2 nodulated best when inoculated with CIAT 899. Biomass production was highest in inoculated plots in all cases. In the field trial, results showed significant differences among the cultivars and effectiveness of rhizobia strains. Ayenew had the lowest number of nodules (42.8 nodules plant⁻¹), while GLP 24 and ECAB 0807 had 59 and 73.9 nodules plant⁻¹, respectively. Among the bush lines, plants inoculated with CIAT 899 had the largest number of nodules (76.9 nodules plant⁻¹). ECAB 0807 inoculated with CIAT 899 had the highest number of nodules (128 nodules plant⁻¹). Among the climbers, inoculated plants had better nodulation compared with the control. However, nodulation varied with strains and genotypes. Cargamanto nodulated best when inoculated with USDA 2676 (72 nodules plant⁻¹), NG 224-4 (147 nodules plant⁻¹) with USDA 2674 and G59/1-2 (167 nodules plant⁻¹). Results showed that mean grain yield of inoculated bush bean lines was higher compared with the control plots. The multistrain inoculation gave better yield performance compared with single strain and control plots. However, responses varied with genotypes. Ayenew (GLP X92) showed good response to inoculation. This genotype performed best when inoculated with combined strains showing more than 100% yield increase over the uninoculated plots. GLP 24 and ECAB 0807 gave best yields with multistrain inoculation. However, GLP 24 seems to have good performance with native rhizobia and hence showed modest increases with inoculation. ECAB 0807 had a 27.6 % yield increase with multistrain inoculation. Among the climbers, Cargamanto showed highest yield increases when inoculated with USDA 2676, and NG 224-4 and G59/1-2 with combined strains. Yields of climbers were below expected levels probably because of infection by fusarium wilt and bean common mosaic virus.

Table 1. Effect of rhizobia inoculation on nodulation in bush and climbing bean lines.

Inoculum	Variety and growth habit					
	Bush nodules plant ⁻¹			Climbing nodules plant ⁻¹		
	Ayenew	GLP 24	ECAB 0807	Cargamanto	NG 224-4	G 59/1-2
Control	6.8	1.8	3.8	5.5	8.0	8.5
CIAT 899	54.8	68.5	52.2	81.0	93.5	155.0
USDA 2674	89.5	41.8	80.0	84.0	99.0	131.5
USDA 2676	54.2	35.2	60.5	127.5	82.5	122.5
Combined	63.0	54.0	92.8	84.0	86.5	131.0

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THE EFFECT OF NITROGEN USE ON GROWTH AND DEVELOPMENT OF COMMON BEAN AND SUNFLOWER INTERCROP

Edgar J. Morales-Rosales¹, J. Alberto Escalante-Estrada²

¹Universidad Autónoma del Edo. de México. ² Colegio de Postgraduados. Montecillo, Méx.

CP 56230. E-mail: ejmorales@colpos.mx jasee@colpos.mx

INTRODUCTION

The efficiency in the use of the resources has been the main reason by which the multiple crops continue being used at the present time. Nevertheless, the response to the nitrogen fertilizer in these systems has not been well detailed. The supply of nitrogen in the combined crops where a legume is included must be considered, since evidence exist of, with a minimum or excessive application of nitrogen could modify in significant way the response of these agrosystems to the addition of this element (Fukai and Trenbath, 1993). By such reason, a study was made whose aim was to determine the effect of different nitrogen levels on the growth, production of biomass and seed yield in combined sowing of common bean and sunflower.

MATERIALS AND METHODS

The investigation was made during summer of 2003 under rainy season conditions in Montecillo, México (19° 29' North, 98° 53' West and 2250 m of altitude) . The treatments consisted of the supply of four nitrogen levels (40, 80, 120 and 160 kg ha⁻¹) and a control treatment without fertilizer, as source of nitrogen was used urea (46% of N) applying half of nitrogen at time of sowing and to the rest to the first weeding. The sowing was made on May 22nd of 2003 to the density of population of 8.3 plants m⁻² (15x80 cm) having alternated a plant of common bean and one of sunflower. The variables evaluated were: number of leaves m⁻², leaf area index, intercepted radiation, total biomass, harvest index and seed yield m⁻². This evaluation was done under an experimental design of randomized complete blocks with four replications.

RESULTS AND DISCUSSION

All used nitrogen levels, with exception of 160 kg N kg ha⁻¹ increased the production of dry matter and seed yield (Table 1). With the supply of 80 and 120 kg N ha⁻¹ the number of leaves m⁻², leaf area index and intercepted radiation were increased, which resulted in a bigger seed yield (504 and 501 g m⁻², respectively) (Figure 1), which is an indicator of that, with those amounts of nitrogen fertilizer, the advantage of the components crops of the system was greater, meaning that, the common bean and sunflower were complemented themselves mutually in the use of this resource (Morales *et al.*, 2006). The harvest index was not modified by the application of the treatments.

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Table 1. Analysis of variance and comparison of means for total biomass, seed yield and harvest index, in the common bean-sunflower intercropping system, with four levels of nitrogen and control treatment, Montecillo, Méx. 2003.

Nitrogen kg ha ⁻¹	Total Biomass (g m ⁻²)	Seed Yield (g m ⁻²)	Harvest Index (%)
0	1375 c	321.4 c (57% + 43%)	24
40	1745 abc	434 b (55% + 45%)	25
80	2148 a	504 a (51% + 49%)	24
120	2092 ab	501 a (56% + 44%)	24
160	1567 bc	369 c (59% + 41%)	24
Prob F	**	**	ns
HSD _{0.05}	384	65	12

** = P ≤ 0.01; in the parenthesis the first amount refers the seed yield percent of common bean and the second to the sunflower; HSD_{0.05} = Honestly significant difference test 0.05; In a column, means followed by common letter are not significantly different.

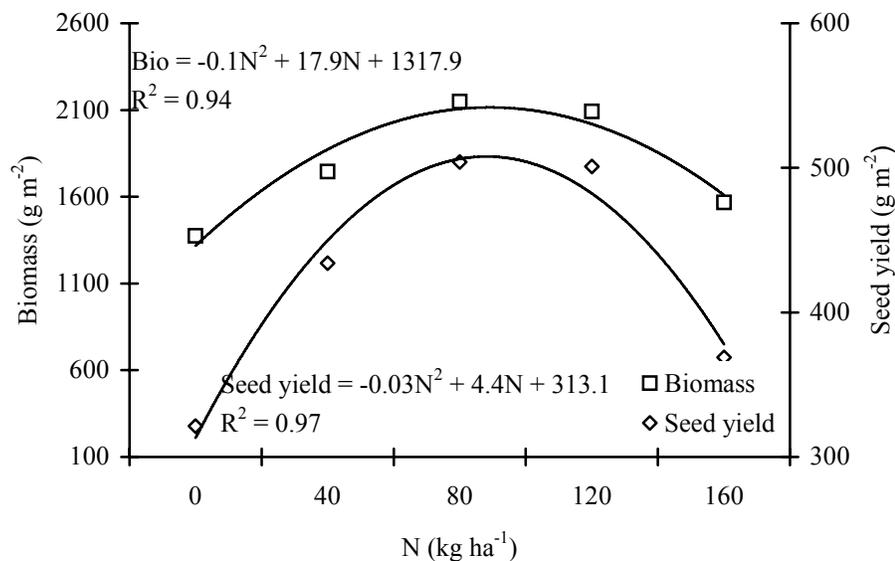


Figure 1. Total biomass and seed yield in the common bean-sunflower intercropping system, with four levels of nitrogen and control treatment, Montecillo, Méx. 2003.

LEGUME PIPE – A TIME SENSITIVE RESOURCE FOR THE AMERICAN BEAN INDUSTRY

H. F. Schwartz, Dept. of Bioagricultural Sciences & Pest Management,
Colorado State University, Fort Collins, CO 80523-1177

Proceedings from the 2005 Biennial Meeting of the Bean Improvement Cooperative emphasized the importance of the common bean community in establishing dialogue with and accessing resources available from the soybean community as they face imminent threats from soybean rust in the Americas (Schwartz et al., 2006). The following article provides a summary of a successful linkage made to a national project that has provided a great deal of timely benefit to the soybean industry, and one that has opened the door for mutual benefit to the common bean industry by expanding our knowledge about the soybean rust threat to common beans.

The goal of the Sentinel Plot component of the National Legume Risk Management Tool Development Project which is also known as the Pest Information Platform for Extension and Education (PIPE) is to provide useful information for legume pest management through a national network of plots that will be monitored by state specialists for legume pests with an emphasis upon soybean rust (SBR, caused by *Phakopsora pachyrhizi*). All states in the network monitored soybean plots during the 2004-2006 growing seasons, and some southern states also monitored “early sentinels” to determine overwintering success of SBR on soybean and kudzu. Non-soybean hosts including other legumes (e.g., common bean, lima bean, lentil, chickpea, field pea) and kudzu were planted in sentinel plots during 2005-2006. Each sentinel plot was monitored over the course of the growing season for approximately 12 weeks. Other pests that were monitored include the soybean aphid (SBA, *Aphis glycines*), in addition to other prevalent problems such as white mold, common bacterial blight, and even some viruses.

PIPE integrates people and computers, distributed throughout the nation, who are networked and facilitated by "state-of-the-art" Information Technology. It supports observation networks, diagnostic laboratories, data management, modeling, interpretation, and the dissemination of timely information on a well-integrated platform to help farmers combat plant diseases, insect pests, and weeds. An important philosophical underpinning of PIPE is that extension and education activities for both integrated pest management and risk management associated with crop insurance should proceed hand-in-hand. PIPE is built on the existing USDA, university, and state departments of agriculture infrastructures and benefits from an informal partnership with industry (Isard et al., 2006).

Colorado State University coordinated the 2006 network of legume sentinel plots (8 to 9 in each state, and fewer in each province) located in the western U.S. (Colorado – H. Schwartz, Idaho – S. Singh, Oregon – C. Ocamb, Washington - P. Miklas) and Canada (Alberta - R. Howard, Manitoba - B. Conner, Saskatchewan - B. Gossen). The State/Provincial Coordinator: (1) confirmed involvement of local cooperators and provided diagnostic training; (2) established linkage with the State Diagnostician (NPDN = National Plant Diagnostic Network contact) to share primary pest information on Soybean Rust and other pests generated by the Sentinel Plot and/or other activities during the season; and (3) established linkage with the USDA/CSREES Soybean Rust Web Site and protocol to access resources and upload weekly survey data that was then made available to the public via <http://sbrusa.net>. During 2006, the western network of

more than 25 Sentinel Plot specialists and observers monitored more than 40 legume (primarily common bean or *Phaseolus vulgaris*) plots in 4 states and 3 provinces from May to September for SBR, SBA and other prevalent diseases and pests. Plans are underway to expand SBR (and other pest) monitoring on legume crops such as common bean during 2007 with the addition of other western states, such as Arizona, California, Montana, New Mexico, Utah, and Wyoming to the 2006 network members. In addition, other states in the Midwest and eastern U.S. will also contribute to the network as they monitor other legume crops which may include common bean, especially snap beans. These legume plots will also be used to monitor for and determine the distribution of virus diseases that can be seed-borne or insect-vectored to common bean and/or soybean. Plant samples will be collected during the late vegetative and early pod fill stages of growth by field specialists for NPDN diagnostic testing following advanced protocols being developed in cooperation with Agdia and a national committee of legume virus experts.

In 2005, USDA-APHIS funded the coordinated framework for soybean rust surveillance and monitoring, predictive modeling, web-based dissemination of information to stakeholders, development of management (fungicide) guidelines, and communication and outreach. The USDA Risk Management Agency (RMA), Cooperative State Research, Education, and Extension Service (CSREES), and USDA-APHIS reached an accord to maintain and expand the system for 2006 and 2007. In September 2005, RMA provided \$2.4 million to CSREES through an interagency agreement to track the spread of soybean rust in 2006 and to create the Pest Information Platform for Extension and Education to provide producers with information about additional legume pests and diseases. The challenge now is to establish a basis for sustaining the PIPE beyond 2007. The system couples extension and education activities for both integrated pest management and risk management associated with crop insurance. PIPE enhances the role of extension specialists in IPM by providing near real-time access to observations, model output, pest management information and guidelines from other states, as well as communication tools for dissemination of this information to support pest management decision making by growers during that growing season.

Monitoring of Sentinel Plots in western states and provinces of North America did not detect any suspicious outbreak of soybean rust on legume crops that included soybean and common bean during 2006. This contributed valuable information to the national program involved with monitoring the outbreak and movement of the fungus in southeastern and now Midwestern states. Timely reporting in the west also allowed pest management specialists to advise crop consultants and growers regarding disease status and threat. As a result, 240,000 acres of common bean grown in Colorado (100,000 acres), Idaho (100,000 acres), Oregon (10,000 acres) and Washington (30,000 acres) were not sprayed needlessly with a preventive fungicide which provided economic benefits to growers and reduced chemical exposure to the environment and food supply. Additional benefits to the common bean and crop insurance industries could include more accurate diagnoses of pest problems that are responsible for crop loss assessments.

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COMMON BEAN FARMER'S CRITERIA IN CULTIVAR CHOICE

Camila Bonemann Chollet¹, Irajá Ferreira Antunes²

UFPEL, CEP 96001-970, Pelotas, RS, Brazil¹; EMBRAPA-Clima Temperado, Caixa Postal 403, CEP 96001-970, Pelotas, RS, Brazil² e-mail: cbchollet@yahoo.com.br

Introduction

Brazil is the principal common bean world producer - three million tons (FAO, 2005) - and consumer - imports additional 100.000 tons - in annual basis. At Rio Grande do Sul State the annual bean crop reaches about 160.000 ha (395,382.9 acres), being the farmer a smallholder, with a cropping area of about two hectares. The role that plant breeding plays in feeding the world population is widely recognized. The evolution of cultivar development is the main reason why the predictions formulated by Malthus were not transformed in reality. The hungry found nowadays, it is believed, is due to the lack of global policies of distribution rather than to the lack of enough food production. Many breeding programs look for farmer's opinion about the performance of breeding lines before releasing them as new cultivars. This work was designed in order to identify the criteria farmers take into consideration when choosing a given cultivar.

Material and Methods

In 2004, it was elaborated a questionnaire aiming to determine which are the criteria farmers consider most important in cultivar choice. A number of 127 questionnaires were submitted to farmers engaged in the Common Bean Demonstration Unity System -SUDF-, which started in 1990, having as main goal to spread knowledge on new cultivars released by research programs. The Demonstrative Unity consists of about fifteen cultivars that are sown in four 4m rows plots, and carried out according to each farmer's cultural practices. Sixty-one questionnaires were answered, coming from the main common bean production regions of the State. An analysis based on the software SPSS was performed.

Results and Discussion

The main characteristics involved in cultivar choice by the common bean farmer in Rio Grande do Sul State are shown in Table 1. It can be observed that seed yield is the most important characteristic for the common bean farmer. Summing up the percentage of farmers that mentioned seed yield as an important characteristic, the total reaches 76.0%. The second trait of importance for the farmers, is cooking quality, appointed, alone or together with other characteristics, by 45.4% of them. The third cultivar characteristic in importance is disease resistance; 35.6% of the farmers have mentioned it as such. The fourth and last one referred was plant architecture, by 18.4% of farmers. Most of the farmers that were involved in this query - (54.9%)- considered, when choosing a common bean cultivar, more than a single characteristic.

The simultaneous consideration of seed yield, cooking quality and disease resistance was subject of concern for 39.5% of them. The results have shown that in Rio Grande do Sul State, the common bean breeder should take as main parameters to integrate into cultivar development, seed yield and cooking quality. Disease resistance has a lower level of importance probably to the easy of control through the use of chemicals. The knowledge on which are the cultivar most important qualitative features from the point of view of the farmers should be a periodical recurrent strategy for breeding programs.

Table 1. Cultivar characteristics (in percentage) of importance in farmers' choice

Trait	Percentage
Seed yield	28,8
Seed yield and cooking quality	13,3
Seed yield, cooking quality and disease resistance	13,3
Seed yield and disease resistance	9,9
Seed yield and disease resistance and plant architecture	4,7
Seed yield and plant architecture	3,0
Seed yield, plant architecture and cooking quality	3,0
Cooking quality	8,1
Cooking quality and disease resistance	3,0
Cooking quality and plant architecture	4,7
Disease resistance	4,7
Plant architecture	3,0
TOTAL	100

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Acknowledgements

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LOCAL AGRICULTURAL RESEARCH COMMITTEE (LARC): A PARTICIPATORY STRATEGY TO IMPROVE DRY BEAN PRODUCTION IN ECUADOR

Mazón, N¹., E. Peralta¹, D. Ruonavaara²

¹Técnicos del Programa Nacional de Leguminosas y Granos Andinos, Estación Experimental Santa Catalina, INIAP. Panamericana Sur km 14, telefax 59322693360, Quito – Ecuador.

²Michigan State University, MSU Extension, 240 Agriculture Hall, East Lansing, Michigan 48824

The communities in the Chota and Mira river valleys, in Northern Ecuador (Imbabura and Carchi provinces), are two of the most important bean producers areas; however, these communities have been widely neglected by the government attention, especially in infrastructure and basic services. The main food and financial sources of these families are originated in the agriculture, where common bean is the most important crop. Farming production in those areas is less profitable each time as consequence that landholding size of the farm is small (80% of producers have less than 2 ha), the low fertility of soils (mainly by the low content of Phosphorus, Zinc and Organic Matter), lack of technical assistance, the organizational culture is minimum, the prices of the bean production is fluctuating and the access to the markets is uncertain (Mazón and Peralta, 2005).

In order to face some of these problems, it has been developed participatory methodologies in the last years. INIAP through the Legume Program has been working with LARC methodology, which is a research service directed by farmers and they are responsible for their action to the community (Ashby, *et al.*, 2001). LARC methodology is a cyclical process conformed by eight stages (Ashby, *et al.*, 2001; Mazón, *et al.*, 2005): motivation, election of the committee, participatory diagnostic, participatory planning, experiment execution, experiment evaluation, analysis of the results and information to the community.

The work is been conducting in La Concepción and Santa Lucia communities (Mira river valley) and El Tambo and San Clemente (Chota river valley). Participatory diagnostics (PD) were accomplished in each community with the objective of identifying the strengths and weaknesses related to the agricultural production and especially with bean crop; the institutions that support the communities in different areas (development, health, education, religion, credit, etc.) were identified; the crops that form their production systems and some other social and economical aspects. The topics of research were delineated in the four communities by farmers of the LARC. (e.g.: Evaluation of advanced bean lines in Santa Lucia, parish La Concepción) and the objectives were constructed (e.g.: To select new improved cultivars with high yield, possessing disease resistance to the main pathogens, with good acceptance by producers and consumers).

A total of 44 advanced lines and varieties of bush bean from three different commercial seed classes such as red mottled, yellow (canario) and white were evaluated by the farmers members of the LARCs. In the first experiments, participatory evaluations were performed in flowering, pod filling stage and dry seed after harvest time though the formats of “absolute evaluation”. The main selection criteria identified by farmers in the four communities were: the amount of pods, foliar and seed health, plant vigor, earliness, size of pods, and size, color, uniformity and brightness of the seed. In La Concepción, in the first cycle of evaluation two new varieties of red

mottled commercial classes were selected: 'Mil Uno' (released as INIAP-424 'La Concepción') and INIAP-414 'Yunguilla'. The line 'Yunguilla' x 'Mil Uno' s23 was selected in La Concepción in a second evaluation cycle as well as in El Tambo and San Clemente. The lines 'Yunguilla' x 'Mil Uno', s35 and 'Yunguilla' x 'Mil Uno', s6 were selected in Santa Lucía.

Santa Lucía, El Tambo and San Clemente selected the variety INIAP 420 'Canario del Chota' and line ACE1 x ('Cocacho' x 'San Antonio') s26p1, a yellow commercial seed classes. The variety of white seed commercial class, INIAP 422 'Blanco Belén', was selected in Santa Lucía, El Tambo and San Clemente.

The LARCs have already initiated the process of seed production and distribution. With the new red mottled varieties, they have reached yields around 1100 kg.ha⁻¹, 1050 kg.ha⁻¹ with yellow seed (canario) and 1150 kg.ha⁻¹ of white seed. Due to the potential of the new varieties and the implementation of new practices in the hands of the farmers, it could be considered a gain compared with the prior situation.

With this new strategy, the farmers are not longer objects used by researchers. At the present, they are an active part of the research process taking decisions and changing experiences with researchers. This new concept allows a certainty of high impact technology adoption and production improvement since the researcher is not the only one taking decisions.

With this new strategy, the farmers became subjects with knowledge and experiences and no more objects used by researchers as in the past. At the present, they are an active part of the research process taking decisions and changing experiences with researchers. The researchers have included farmers' criteria and requests in the process of development of new varieties. This innovation produces varieties with morph agronomic features accepted and spread quickly in a determinate community. On the other hand when farmers take place in the participatory research process they get self estimation and they develop abilities to be better in the community and in relationships with others communities and institutions.

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GENOTYPE EVALUATION OF BLACK BEAN GROUP IN MARINGÁ, PARANÁ STATE, BRAZIL

Carlos Alberto de Bastos Andrade¹, Pedro Soares Vidigal Filho¹ and Tanea Aparecida Crispim Splendor²

⁽¹⁾Departamento de Agronomia, Universidade Estadual de Maringá, Av. Colombo, 5790, CEP 87.020-900, Maringá-PR, Brazil. E-mail: cabandrade@uem.br, ⁽²⁾PGM/UEM, Maringá State University.

Introduction

In general, cereal and legume grains are the base of human being's food. In Brazil, agriculture researches has been valuing grain cultivation, especially common bean. Among most commercial group cultivated, the black bean group assumes the second place of highest consumption in Brazil, and it is considered the favorite in South and Southeast regions of the country. Therefore, the objective of this work was to evaluate the grain yield capacity of 18 bean genotypes belonged to the black commercial group.

Material and methods

The experiment was carried out at Fazenda Experimental de Iguatemi (FEI) that belongs to the Universidade Estadual de Maringá (UEM). The black bean genotypes Negro, FT 120, FT Nobre, FT Soberano, IAC Una, Iapar 44, IPR Chopim, IPR Graúna, IPR Uirapuru, LP-98-122, LP-98-123, LP-99-85, LP-99-96, LP-01-51, Rio Tibagi, Xamego, LP-98-158 and BRS Valente were evaluated. This study was carried out in 2005/2006 using traditional soil tillage system, and the experimental design used was the randomized complete blocks with four repetitions. The plots consisted in four lines of 5.0 m of length, spaced with 0.45 m, and the sowing density was 13 plants per meter. The genotypes yield was obtained through the harvest of two central plants lines, despising 0.50 m of each line extremity. Ten plants were used to determinate the number of pods per plant (NPP), number of seeds per pod (NSP) and mass of 100 seeds (MS). The data analysis was made according to the methodology proposed by Campos (1984). The genotypes means were compared utilizing the method of Scott-Knott (1974) at a level of 5% of probability.

Results and discussion

The variance analysis showed no significant differences between genotypes in relation to number of seeds per pod and grain yield. On the other hand, it was observed a significant effect among genotypes in relation to number of pods per plant and mass of 100 seeds (Table 1).

According to Table 2 the genotypes that pointed out for mass of 100 seeds were: IRP Uirapuru (21.00), IPR Chopim (20.50), LP-98-123 (20.25), LP-01-51 (20.00), IAC Una (20.00), LP-98-158 (19.75), Negro (19.75) and IPR Graúna (19.25); whereas for number of pods per plant were: IPR Chopim (21.38), Iapar 44 (19,10), IPR Graúna (17.20), LP-01-51 (17.05), LP-98-123 (16.58), IPR Uirapuru (16.20) e LP-99-85 (15.95). On the other hand, IPR Uirapuru (4.76), FT Soberano (4.59), LP-01-51 (4.58), Rio Tibagi (4.52), Iapar 44 (4.33) and LP-99-96 (4.25) were the ones that pointed out for number of seed per pod.

Table 1 – Mean square of four agronomic traits in 18 genotypes of black bean commercial group. Fazenda Experimental de Iguatemi, Maringá, PR - 2005/2006.

Variation source	GL	Mean square ¹			
		Yield	MS	NPP	NSP
Gen	17	148,347.79	26.05*	25.54*	0.45*
Rep	3	16,582.87	0.68	9.90	0.17
Error	51	96,855.41	1.43	9.06	7.64
CV (%)		24.96	6.89	19.90	9.35
General mean		1,247.08	17.32	15.13	4.14

*Significant at 5% probability.

Table 2 – Mean values of grain yield, mass of 100 seeds, number of pods per plant, and number of seeds per pod of black bean commercial group genotypes. Fazenda Experimental de Iguatemi, Maringá, PR - 2005/2006.

Genotype	Means ¹			
	Grain yield (kg ha ⁻¹)	Mass of 100 seeds (g)	Number of pods per plant	Number of seeds per pod
Negro	1025	19.75 a	12.23 b	3.98 b
FT 120	1165	15.50 b	13.53 b	4.12 b
FT Nobre	1257	15.00 b	13.25 b	4.10 b
FT Soberano	1062	15.50 b	12.78 b	4.59 a
IAC Uma	1120	20.00 a	14.75 b	4.13 b
Iapar 44	1122	15.00 b	19.10 a	4.33 a
IPR Chopim	1412	20.50 a	21.38 a	4.20 b
IPR Graúna	1007	19.25 a	17.20 a	3.40 b
IPR Uirapuru	1775	21.00 a	16.20 a	4.76 a
LP-98-122	1147	15.00 b	12.15 b	4.05 b
LP-98-123	1177	20.25 a	16.58 a	3.92 b
LP-99-85	1160	15.00 b	15.95 a	4.10 b
LP-99-96	1300	15.25 b	12.95 b	4.25 a
LP-01-51	1417	20.00 a	17.05 a	4.58 a
Rio Tibagi	1170	15.00 b	15.08 b	4.52 a
Xamego	1427	15.00 b	12.38 b	3.85 b
LP-98-158	1475	19.75 a	14.50 b	3.74 b
BRS Valente	1225	15.00 b	15.35 b	3.91 b

¹Means followed by the same letter in each column belong to the same group, according to Scott-Knott clustering at 5% of probability.

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GENOTYPE EVALUATION OF COLORED BEAN GROUP IN MARINGÁ, PARANÁ STATE, BRAZIL

Carlos Alberto de Bastos Andrade¹, Tanea Aparecida Crispim Splendor² and Pedro Soares Vidigal Filho¹

⁽¹⁾DAG/UEM, Maringá State University, Av. Colombo, 5790, 87020-900, Maringá, Paraná, Brazil; cabandrade@uem.br ; ⁽²⁾PGM/UEM, Maringá State University.

Introduction

The common bean (*Phaseolus vulgaris* L.) is one of the most essential components of the diet food in the majority of the low income population. Therefore, Brazil seems to be the higher producer and consumer of bean in the world. One of the alternatives to increase bean production is the selection of superior genotypes adapted to distinct environments. Considering the importance of genotype and environment interaction, it was developed this work aiming to evaluate the production of cultivars and lineages of bean of colored commercial group in the experimental site of the State University of Maringá (UEM), located in Iguatemi district, in the municipal area of Maringá, Paraná State, Brazil.

Material and methods

The experiment was conducted in the Experimental Farm of Iguatemi (FEI), which belongs to the State University of Maringá (UEM). It was utilized 18 genotypes among cultivars and lineages, of the following colored commercial group bean: Carioca, FT Bonito, FT Magnífico, IAC Eté, IAC Tibatã, Iapar 31, Iapar 80, Iapar 81, IPR Juriti, IPR Saracura, LP-99-63, LP-99-79, LP-20-108, Claro, LP-01-38, Pérola, Rubi, SEL 37-10 and Rudá. The seeds were provided by IAPAR. The experimental scheme was of random blocks (DBC), four repetitions. The parcels in the field were constituted by four lines of 5 meters length, spacing 0,45 m between them, with planting density of 13 plants by linear meter, being adopted as useful parcel to evaluate the two central rows, disregarding 0,50 m of each tip of the lines. The seeding step was made in 10/14/2005 and the traditional soil preparation was used. The chemical fertilizing proceeding was made according to the chemical analysis of the soil and recommendations to bean culture according to the IAPAR (2000). Before the culture efflorescence begins it was made two manual cleaning of the area, in order to keep the culture without weeds and indeed two applying insecticides to control plagues. The crop was made into the useful area of each parcel, represented by two central lines after field maturation of each genotype. It was made 10 samples to determine the number of green beans by plant, number of grains by green bean and mass of 100 grains in the useful area during the crop. It was cropped indeed the entire useful area in order to calculate the grain production. In the evaluation process of the agronomical features it was used the statistical analysis of Variance by making the "F" test and to complete data, it was compared average of genotypes using the grouping criterion of Scott-Knott (1974) at a probability of 5%.

Results and discussion

In the Analysis of Variance (Table 1), it was observed a significant effect between distinct genotypes studied related to the grains production variable, mass of 100 grains and number of grains by green bean, but did not present a significant effect among the studied genotypes related to the variable number of green bean by plant. The Table 2 presents the studied genotypes behavior related to the average of each variable. By using the grouping criterion of Scott-Knott, it was formed significant average groups to each variable. In that way, the genotypes which

presented better results in each variable were: production of grains (kg ha⁻¹) – higher production: LP-01-38 (1305), Carioca (1302), IPR Juriti (1197), IAC Tibatã (1117) and IPR Saracura (1082); Mass of 100 grains (g) – higher mass: Claro (30,25) and lower mass: Rudá (15,00) and LP-99-63 (15,00); Number of grains by green bean – higher numbers: FT Bonito (4,36), LP-01-38 (4,17), IAC Eté (3,85), Carioca (3,82), IPR Saracura (3,75), Iapar 31 (3,74), Rubi (3,73), Pérola (3,72), IPR Juriti (3,71) and IAC Tibatã (3,63).

Table 1 – Average Square of four characters evaluated in eighteen genotypes of colored commercial group bean. Final essay on 2005/2006.

Variation source	GL	Average Square ¹			
		REND	M100G	NVP	NGV
GEN	17	124762,50*	51,14*	26,81 ^{ns}	0,44*
REP	3	21893,98	1,00	1,83	0,17
error	51	54840,06	0,71	19,97	9,19
CV (%)		23,89	3,86	34,36	11,74
General Average		980,42	21,78	13,00	3,62

*Significant at 5% of probability by the test of Scott-Knott. ^{ns}Not Significant at 5% of probability, by the test of Scott-Knott. ¹REND = production of grains (kg/ha), M100G = mass of 100 grains (g), NVP = number of green bean by plant, NGV = number of grains by green bean.

Table 2 – Average values of colored commercial bean group. Final essay 2005/2006.

Genotype	Average ¹			
	Production of grains (kg ha ⁻¹)	Mass of 100 grains (g)	Number of green bean by plant	Number of grains by green bean
Carioca	1302 a	20,25 c	10,33	3,82 a
FT Bonito	822 b	19,75 c	17,30	4,36 a
FT Magnífico	905 b	20,50 c	11,13	3,45 b
IAC Eté	960 b	20,25 c	17,43	3,85 a
IAC Tibatã	1117 a	20,50 c	13,70	3,63 a
Iapar 31	972 b	20,25 c	11,53	3,74 a
Iapar 80	960 b	20,75 c	9,65	3,54 b
Iapar 81	1000 b	25,00 b	11,85	3,07 b
IPR Juriti	1197 a	24,50 b	12,83	3,71 a
IPR Saracura	1082 a	24,75 b	13,10	3,75 a
LP-99-63	867 b	15,00 d	13,35	3,44 b
LP-99-79	967 b	20,25 c	12,18	3,25 b
Claro	842 b	30,25 a	10,90	3,21 b
LP-01-38	1305 a	25,50 b	10,80	4,17 a
Pérola	785 b	24,50 b	18,08	3,72 a
Rubi	1027 b	20,00 c	14,35	3,73 a
SEL 37-10	922 b	25,00 b	15,28	3,22 b
Rudá	610 b	15,00 d	10,33	3,46 b

¹ Averages followed by same letter, each column, belong to the same group, according to grouping criterion of SCOTT-KNOTT at 5% of probability.

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BEAN VARIETIES FOR THE HUMID TROPICAL LOWLANDS

Paul Kimani¹, Jean-Paul Lodi Lama², Matondo Nsebua² and Lunze Lubanga³

¹Regional Program on Beans in Eastern Africa, Dept of Plant Science and Crop Protection,
University of Nairobi, P.O. Box 29053-0625 Nairobi, Kenya

²INERA-Mvuasi and ³INERA- Mulungu, DR Congo

Introduction

Beans are traditionally cultivated in the medium and highland agro ecological zones (>1000 masl) of East and Central Africa. Most of the varieties developed in the last 20 years are adapted to the cooler altitudes. However, beans are widely consumed in the lowland areas in the region. But lowland production is limited because much of the research effort has concentrated in developing cultivars for the highland zones. Most of the beans consumed in the lowlands are produced in the highlands and exported to markets in lowland zone. For example, bean consumed in western lowland zone in the DR Congo are imported from the highland production zones in eastern parts of the country, especially in North Kivu province. Because of the poor communication system, beans are either airlifted or transported by river for more than 1000 km to west DR Congo. As a result, prices in Kinshasa and other markets are relatively high because of the high transportation costs, and are hardly affordable to the urban poor, contrary to the popular belief that bean is a 'poor man's meat'. Interest for local bean production is growing in Congo (Brazzaville), Cameroon, Central African Republic, Cabinda (Angola) and other countries in the tropical humid lowlands of west and central Africa. For example, Institut National pour l'Etude et la Recherche Agronomiques (INERA), the national agricultural research Institute of the Democratic Republic of Congo, has identified bean as a priority crop for production in lowland zones of DR Congo because of increase in demand and potential for income generation for smallholder farmers. Development of bean varieties adapted to the humid lowlands can enhance productivity of beans in these regions. A collaborative program between the regional bean program and INERA was initiated to identify bean genotypes adapted to the lowland humid tropical zones in western Congo and neighbouring countries in West Africa. This report highlights progress in this program.

Materials and Methods

Bean germplasm was introduced to INERA-M'vuazi from INERA research stations at Mulungu, Gandanjika, FOFIFA (Madagascar) and University of Nairobi (Kenya). The collection comprised of 80 sugar bean lines, 40 lines tolerant to low soil fertility from BILFA V nursery, 8 entries from FOFIFA bean program, and more than 86 F₂ and F₃ segregating populations from the regional multiple constraint nurseries at University of Nairobi and local collections. The collection was evaluated at M'vuazi, Kisantu and several on-farm sites in Bas Congo, Kinshasa and Bandu Provinces. M'vuazi, the main coordinating center for bean research is located at latitude 5°27'S, longitude 14° 54'E and 470 masl. It has mean annual temperature of 23.6°C and receives 1425 mm rainfall per year. All trial sites were below 1000 masl. The evaluations were conducted in collaboration with farmer groups, NGOs and community based organizations (CBOs).

Results and Discussion

Twelve bean varieties adapted to lowland conditions have been released and are being disseminated in association with NGO's and farmers' associations. The varieties are: More 88002, PVO 14 (local landrace), PVO 14/2, T-3, A445, Diniania, Ntendezi (local landrace), Manseki, Nguaku-Nguaku, Tuta (Congolese landrace), G20854 and Lundamba. Ten varieties are in pre-release stages. These are Mbindi (from local germplasm), G22258, L4 (Congolese germplasm), I7 (Congolese landrace), G22501, Lyamungu 90, G16157, BF12 (Congolese landrace), BF10 and G8047. BILFA lines performing well include ZAA 5/2, G22258, Mwamafutala and AFR 593. Two lines KS 65-2 (sugar) and KS 47-1 (medium yellow) selected from regional nurseries have been identified for release.

Dissemination of the varieties is being conducted in collaboration with 14 NGOs and farmer's associations in areas near Mvuazi, and with INERA's Research and Development (extension) section and farmer associations and field schools in Kisantu, and with CADIM in Plateau de Bateke. Beans are grown over three seasons in the lowlands: Season A (November to February) is the main season. Season B (April to May) is used for seed production. In season C (June-October) beans are cultivated in valley bottoms on residual moisture. Cultivation in seasons A and B is on the 'uplands'. Major disease constraints to production in the lowlands include common bacterial blight, web blight, bean common mosaic virus, root rot, and rust. Major pests include bruchids, aphids and foliage/stem beetle (with symptoms similar to bean stem maggot). A visit to Kinshasa markets revealed that several of these released varieties were being traded. Yellows, whites and sugars dominated the markets in Kinshasa. Yellows were the most expensive (CFR 560 per kg) and dark browns, the cheapest (CFR 200 per kg). INERA M'Vuazi has been instrumental in disseminating bean germplasm to other countries in West and Central Africa. Some of the genotypes distributed to Liberia, Central African Republic and Congo-Brazzaville are presented in Table 1.

Table 1. Bean germplasm adapted to humid tropical lowlands distributed from INERA-M'vuazi.

Destination	Type of Material	Number of accessions
Liberia	Advanced lines and released varieties	15
Central African Republic	*MCR lines	15
	White bean	8
	Sugars	11
Congo-Brazzaville	Released varieties	12

*MCR= multiple constraint resistance

These results suggest that bean may be more plastic than expected. Some of the varieties performing well in lowlands (as low as 470 masl) such as Lyamungu 90 were selected for highland zones. Although climbing beans are traditionally grown at high altitudes, new medium altitude climbers were introduced in central and west Africa. Several climbing bean varieties were performing well at Mvuazi (470 masl). It appears that there is considerable potential for expanding bush and climbing bean production to lowland agroecological zones.

INCREASING BEAN YIELD THROUGH SITE-SPECIFIC CULTURAL PRACTICES AND ADAPTED LINES UNDER CENTRAL PIVOT IRRIGATION SYSTEM IN THE TROPICS

M.Thung, D. M. Soares, H. Aidar and J. Kluthcouski.

Embrapa Arroz e Feijão. P. O. 179, 75.375-000 ; Santo Antônio de Goiás/GO, Brazil.

Intensive pasture production for raising beef cattle under central pivot irrigation system has become a common activity in tropical region of Brazil. However, after 4 to 5 years the pasture production starts to decline because of soil fatigue and self-induced incompatibility of the pasture. So, is crop rotation of grass followed by maize/soybean, sorghum and bean in no-till planting has been introduced with moderate success. Bean yield is still too low due to nitrogen deficiency. Traditional side dressing with N several days after planting did not solved the problem in the area with high organic matter accumulation on the surface. In this region with low latitude and altitude and maximum air temperature around 35° C, bean growth cycle is reduced to less than 70 days. Bean can grow, however, in this region because night temperatures drop to below 28° C and soil maximum temperatures never exceed 30° C at 5 to 20 cm depth. Bean is planted during the dry winter period between May and September under irrigation, but average bean yield has never reached 2 t ha⁻¹. No commercial bred cultivar is available for this region. The objectives of these experiments were to increase bean yield through better nitrogen application methods and evaluate the performance of new lines in the tropical environment.

Materials and methods: Three experiments were conducted at the Triangulo farm in Bonópolis/GO (13° 38' 11" S; 49° 48' 37" ; 290 masl) on Oxisols with the following soil characteristics. Ca 2.8, Mg 0.9 and K 0.26 (all in cmol_c dm⁻³) and 10.4 mg dm⁻³ of P. Soil organic matter is about 25.0 g dm⁻³ and on soil surface there was more than 8 Mg ha⁻¹ of dry sorghum mulch. Perpendicular to the fertilizer application heavy-pneumatic bean planter drilled the seed at the rate of 18 seeds per m at the average depth of about 5 cm. The distance between rows was 45 cm. Basal fertilizers were applied at the rate of 90 kg P₂O₅ ha⁻¹ and 50 kg K₂O ha⁻¹ at planting. Before flowering period two chemigation with urea at 15 kg N ha⁻¹ each were applied to promote the microbial activities for decomposing the organic matter. Split plot design with five replications was used, main plots were the N treatments and subplots were bean lines. Net plot size was 10 m² and the seed yield was adjusted to 13 % moisture content.

Experiment 1, Optimum doses of N: Four N doses of 0, 30, 60 and 90 kg ha⁻¹ were incorporated into the soil 2 days before sowing. Satellite treatment with total of 60 kg N ha⁻¹ as urea was applied as chemigation (four times 15 kg N ha⁻¹) between preflowering and flowering period. The ETA 15, ETA 10, Pitoco and BRS Radiante lines were planted as test materials.

Experiment 2, Optimum time for N application: The 60 kg N ha⁻¹ as urea was applied at 10 and 5 days before sowing and at planting date. Cultivars tested were Rudá, Pérola, BRS Valente and Cranberry.

Experiment 3, Method of N applications for reducing soil compaction: Urea was applied parallel and diagonally (45°) to the sowing direction at the doses of 30 and 60 kg N ha⁻¹. The diagonal drilling of fertilizer was to avoid excessive soil compaction by tractor traffic near the bean rows on humid soil under central pivot system. The lines ETA 15, ETA 10, Pitoco and BRS Radiante were used for this experiment.

Results: Experimental data of experiments is presented in Tables 1 to 3. The overall yield was 2300 kg ha⁻¹, which is 300 kg ha⁻¹ higher than the farmer's average yield. Maximum yield was 2775 kg ha⁻¹ with the application of 60 kg N ha⁻¹ and higher doses did not increase yield. The N application through chemigation gave the same yield as control treatment, probably due to high N volatilization losses. The best time for N side dressing was 10 days before planting. This suggests that 10 days was sufficient for releasing the fixed N by soil microorganisms and also reduced the salt concentration of the nitrogen fertilizer around the root zone. Similar results were obtained by Kluthcouski et al., 2006 in other crops. The diagonal alignment of the N application did not yielded as the standard parallel application, because bean roots do not spread laterally as those in the sub irrigation system (Santos et al.2002). Hence, the

plants could not absorb the N on the far side of the bean row. This fertilization recommendation is site specific and should not be applied to other region before similar research is conducted. In high temperature regions new lines such as ETA 15, ETA 10 (both type III) and Pitoco yielded more than 2300 kg ha⁻¹, about 300 kg ha⁻¹ higher than the older cultivars such as Rudá, Pérola or BRS Radiante. This suggests that prostrate type III plants are better adapted to the high temperature regions than erect type II and still can be mechanically harvested through pulling, windrowing and combining.

Table 1. Yield (kg ha⁻¹) of four bean lines as affected by method and doses of N application at Triangulo farm in Bonopolis/GO, 2006.

Treatment (kg ha ⁻¹)	ETA 15	Pitoco	ETA 10	BRS Radiante	Average
60 N Central pivot	2185	2099	1518	1245	1762 c
0 N	2137	2162	1832	1235	1841 c
30 N	2923	2506	2378	1950	2439 b
60 N	3259	2834	2688	2317	2775 a
90 N	3060	2482	3156	2317	2754 a
Average	2713 a	2417 b	2314 b	1813 C	2314

CV (%) = 16

Table 2. Yield (kg ha⁻¹) of four bean lines as affected by the time of N application at Triangulo farm in Bonopolis/GO, 2006.

Treatment	Rudá	Pérola	BRS Valente	Cranberry	Average
At planting	2208	1982	1956	1633	1945 c
5 DBP	2517	2070	1955	1821	2091 b
10 DBP	2719	2433	2336	1964	2363 a
Average	2481 a	2162b b	2082b b	1806c c	2133

DBP = days before planting. CV (%) = 11.

Table 3. Yield (kg ha⁻¹) of five bean lines as affected by alignment and doses of N application at Triangulo farm in Bonopolis/GO, 2006.

Treatment (kg ha ⁻¹)	ETA 15	Pitoco	ETA 10	BRS Radiante	Average
0 N	2137	2162	1832	1235	1841 d
30 N Diagonal	2420	2650	2064	1587	2180 c
30 N Parallel	2923	2506	2378	1950	2439 b
60 N Diagonal	2373	2909	2382	1614	2319 bc
60 N Parallel	3259	2834	2688	2317	2775 a
Average	2622 a	2612 a	2269 b	1741 C	2311

CV (%) = 14

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COST BENEFIT ANALYSIS OF THE INTRODUCTION OF HEAT TOLERANT BEAN VARIETIES IN ATLÁNTIDA, HONDURAS

Timothy G. Porch¹, Richard Bernsten², Juan Carlos Rosas³, and Molly Jahn⁴

¹USDA-ARS-TARS, Mayaguez, PR, ²Michigan State University, East Lansing, MI, ³Escuela Agrícola Panamericana, Tegucigalpa, Honduras, ⁴University of Wisconsin, Madison, WI.

Rising ambient air temperatures, migration, and deforestation threaten the sustainability of hillside agriculture in Atlántida, Honduras. Currently, farmers avoid climatic constraints to common bean (*Phaseolus vulgaris* L.) production by planting at distinct altitudes during different seasons. However, this practice may become less effective due to climatic change. Farmers in ten villages in Atlántida, five at a lower altitude and five at a higher altitude, were interviewed to determine their knowledge and experience with climatic change, bean production and to collect data for a cost benefit analysis. Differences in bean production and yield between the low altitude and high altitude villages were attributed mainly to climatic constraints, due to differences in elevation. In this study, a cost/benefit analysis was conducted to determine if the development and introduction of heat tolerant bean varieties could help to alleviate heat-related constraints on bean production.

The ex ante cost benefit analysis was carried out using data collected from the farmer survey and additional data collected from the Department of Agriculture of Honduras. Migration rates, farm level bean and corn prices, and production area data were obtained from secondary data (e.g., national statistics/census data). Other data, such as expansion of bean production area and adoption rates were estimated from data collected through the farmer survey of 99 farmers in 10 villages. On the assumption that a heat tolerant variety would be released in 2010, the estimated cost of six years of plant breeding research was factored into the six years preceding 2010. Estimates for the projected annual adoption rates for the 10-year period following release are based on logistic adoption curves. The final estimates of the value of incremental production due to increased bean production was discounted for different scenarios which reflect reductions in corn production (i.e., to plant more area in beans, farmers would have to reduce their corn area). Increasing climatic temperatures are expected to decrease yields over the long term, 50 to 100 years. However, because of minimal effects of warming over the 10-year projection period for this study (about 0.2° C), this effect was not included in the cost/benefit analysis (data not shown). It was assumed that adoption rates would be similar to those of recently released varieties, thus a 33% maximum adoption rate was used. It was assumed that yield would increase more in Primera (20%) than in the Postrera (10%), because of the greater yield advantage of the new variety during the warmer Primera season. It was estimated that it takes six years and costs roughly \$60,000 USD to develop a new common bean variety in Honduras. Furthermore, for the purpose of the farmer survey, it was estimated that this investment in breeding for heat tolerance would increase bean yield by approximately 260 kg per hectare in areas subjected to high temperature stress, i.e. seasonal temperatures averaging above 30° C during the day or 20° C at night.

Surveyed farmers were asked if and how they would change their production system if a new bean variety yielded 260 kg per hectare more than their current variety. The farmers reported that they would increase their bean production area during the Primera season by 68% on average. Farmers in lower elevation villages projected that they would increase their bean growing area by 81% versus 61% in high altitude villages. In addition, few farmers (less than

5%) said that they would acquire new properties for planting the improved bean variety. In order to determine access, acceptance, and use of improved varieties, farmers were asked about their use of common bean germplasm. Almost all of the surveyed farmers in Atlántida grew beans of the small red market class, however, approximately 7% also planted black beans. Approximately 48% of the farmers had heard of one or more of the improved varieties that had been released by the Zamorano Bean Program, and 34% had tested one of the improved varieties through association with a non-governmental organization (NGO) or on their own. Of the improved varieties that have been released by Zamorano, farmers regularly planted only two varieties, ‘Tío Canela 75’ (Rosas et al., 1997) and ‘Dorado’. Farmers in four out of the 10 villages had no knowledge of any of the improved varieties, and 67% of the farmers planted only landraces.

Given the assumptions of population growth, yield increases, production area increases, and adoption rates, we estimated the potential increase in bean yield due to the introduction of a heat tolerant variety in Atlántida. The opportunity cost scenarios used net returns per hectare of maize as the opportunity cost, with various assumptions regarding the proportion of maize area reduction for every additional hectare of common bean (i.e., 1.0 ha allocated to beans results in a 1.0, 0.5, or a 0.25 ha reduction in the maize area). With the introduction of a heat-tolerant variety in 2010, 3,244 to 3,338 additional metric tons of beans could be produced between 2011 and 2020 in Atlántida, with an estimated net present value between \$388,000 and \$720,000 USD depending on the opportunity cost assumption (Table 1). The rate of return for the investment in a heat tolerant variety in Atlántida, discounting plant breeding costs, is therefore between 28 and 38%. The greater willingness of farmers at low altitudes to increase their bean production area, indicates a potential to shift bean production from the fragile higher altitude areas to lower zones during the Primera season.

Table 1. Net present value in USD of projected additional bean production, during the years 2011 to 2020, due to the introduction of a heat tolerant variety in Postrera and Primera in Atlántida, Honduras, given a range of opportunity costs to maize production¹. Projected scenarios based on responses from farmers in high & low altitude villages in Atlántida.

<i>Scenario</i>	<i>Incremental production (Metric Tons)</i>	<i>Net present value OC= 0²</i>	<i>Net present value OC= 1/4</i>	<i>Net present value OC= 1/2</i>	<i>Net present value OC= 1/1</i>
High Altitude	3,244	\$697,561	\$627,162	\$556,762	\$415,962
Rate of Return		38%	36%	34%	29%
Low Altitude	3,338	\$719,575	\$636,636	\$553,698	\$387,821
Rate of Return		38%	36%	34%	28%

¹Estimates calculated from historic maize yield averages and farm level prices.

²Opportunity costs (OC) of increased bean production on maize production. Ratio indicates proportion of maize area reduced for every hectare of bean area increase.

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ON-FARM PARTICIPATORY BREEDING OF THE COMMON BEAN, *PHASEOLUS VULGARIS* L.

Shree P. Singh¹, Henry Terán¹, Margarita Lema¹, Marie Dennis¹, Richard Hayes¹, and David Fullmer²

¹Univ. of Idaho, 3793N 3600E, Kimberly, ID 83341-5076; D. ²Fullmer (Producer), 3581E 3600N, Kimberly, ID 83341.

ABSTRACT

Native Americans domesticated common bean landraces under low-input subsistence production systems. However, modern cultivars have been mostly bred at the Agricultural Research and Extension Centers (ARC) under high-input and intensive management production systems with little or no participation of producers and consumers at least during the early phases of breeding. The latter group of cultivars may have limited adoption. The objective of this study was to determine the effects of on-farm participatory (OFP) evaluation and breeding on seed yield and adoption of common bean cultivars. Three experiments were carried out between 1999 and 2006. First, 25 cultivars released between 1932 and 1998 were evaluated at the Kimberly and Parma ARC and OFP at Hazelton, Idaho from 1999 to 2001. Second, a similar group of 16 cultivars was evaluated at the ARC and OFP at Kimberly in 2004 and 2005. Third, two interracial multiple-parent populations were selected from F₄ to F₇ independently at the ARC and OFP at Kimberly between 2003 and 2006. Seed yields were the lowest at OFP. Furthermore, seed yield at Hazelton OFP was not correlated with seed yields at the Kimberly and Parma ARC in the first experiment. In contrast, a positive association was found between the ARC and OFP seed yields at Kimberly in the second experiment. From the partial results available thus far from the third experiment, we found that 11 of 44 families in one population and 16 in the second population selected at the OFP were common to those selected at the ARC. However, mean seed yield of selected families in both populations at the OFP were significantly ($P < 0.01$) higher than those selected at the ARC, thus, justifying the OFP breeding for future common bean cultivars.

INTRODUCTION

Native Americans in Mexico, Central America, and South America domesticated common bean landraces, from wild populations, under low-input subsistence production systems over millennia that are still grown and form the basis of modern cultivars around the world. The inherent genetic variability among wild populations from different regions (Gepts et al., 1986; Koenig et al., 1990), large edaphic and climatic variations along the domestication range, differences in resistance to abiotic and biotic stresses, and differences in preferences for plant type, maturity, and seed characteristics among the inhabitants resulted in thousands and thousands of distinct landraces often with limited adaptation. In contrast, since organized breeding was initiated a century ago modern cultivars in the U.S. and elsewhere have been developed at the ARC often under high-input well-managed production systems with little or no participation of producers and consumers during the breeding phase. The lack of participation of producers and consumers is believed to be associated with limited or lack of adoption of modern cultivars. The objective of this study was to determine the effects of on-farm participatory breeding on seed yield and adoption of common bean cultivars.

MATERIALS AND METHODS

Twenty-five common bean cultivars of great northern, pink, pinto, and red market classes released between 1932 and 1998 were evaluated at the Kimberly and Parma ARC and at OFP Hazelton, Idaho from 1999 to 2001. A similar group of 16 cultivars was evaluated at the ARC and OFP at Kimberly in 2004 and 2005. Also, F₄ to F₇ families from each of two multiple-parent populations were selected at ARC and OFP at Kimberly between 2003 and 2006. A randomized complete block design with four replicates for the first and three replicates for the second experiment were used. Except for sprinkler irrigation at Hazelton (versus gravity irrigation at other locations) all other inputs including use of fertilizer, herbicide, and cultivation were similar in all experiments. Selection from F₄ to F₇ in the two interracial multiple-parent populations, namely POP1= ‘Topaz’/‘Matterhorn’/‘Mesa’/‘Buster’/Common Red Mexican and POP2= ‘LeBaron’/VAX 3/Common Red Mexican/Matterhorn/‘NW 63’ was practiced for the third experiment. An average of 150 F₄ families from each population was evaluated without replication. From F₅ to F₇ a partially balanced lattice design with three replicates was used to eliminate low yielding undesirable families such that in F₇ only 44 families were left in each population. The comparative evaluation of 10 selected breeding lines from each population from each of the ARC and OFP remains to be determined. Therefore, only partial results will be presented for the third experiment.

RESULTS AND DISCUSSION

Mean seed yield of 25 cultivars over the three years was the highest at Parma followed by Kimberly ARC. While Parma and Kimberly yields were positively correlated ($0.60 P \leq 0.01$) neither yield was correlated with the yield at OFP Hazelton. Hazelton is 30 km east of Kimberly and Parma is 290 km west of Kimberly. The mean ARC seed yield (2739 kg ha^{-1}) was significantly ($P \leq 0.01$) higher than the OFP Kimberly yield (1589 kg ha^{-1}) in the second experiment. But, there was a positive correlation ($0.58 P \leq 0.05$) between the mean seed yield of 16 cultivars at the ARC and OFP. Both locations in Kimberly were within 5 km from each other. Of 44 families 11 were common in POP1 and 16 in POP2 between the ARC and OFP selections in the third experiment. Because climatic conditions were similar for the ARC and OFP for the second and third experiments, differences in soil fertility and moisture and agronomic management were largely responsible for seed yield differences for these two experiments. In addition, differences in selection criteria probably contributed to seed yield differences in the third experiment. Early maturity, upright plant type, and lighter-colored pinto (POP1) and deep red (POP2) seed color were emphasized on the OFP while families and breeding lines with much wider range for each of these traits were selected at the ARC. However, more assertive causes would not be known until a comparative study is carried out and subsequent adoption and impact of cultivars developed at the ARC versus OFP are assessed.

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ADOPTION PROGRAM OF PINTO SALTILLO BRED CULTIVAR IN DURANGO, MÉXICO

Arnulfo Pajarito-Ravelero⁵ and Rigoberto Rosales-Serna¹

INTRODUCTION. Pinto Saltillo is a common bean (*Phaseolus vulgaris* L.) cultivar released in 2001 for rainfed conditions in the Mexican Highlands with seed tolerance to coat darkening under storage. Pinto Saltillo averaged 1,139 kg ha⁻¹ in experimental trials established in several environments in Durango, Chihuahua, Zacatecas and Coahuila (Sánchez *et al.*, 2004). After releasing, some attempts were made to introduce Pinto Saltillo in commercial plantings mainly in states of Durango and Chihuahua located in Mexican Northern Highlands. In Durango, traditional bean cultivars are planted because its high yield potential, but this germplasm also presents production risks due to its delayed maturity and disease susceptibility. Problems were also observed for traditional cultivars marketing due to its seed color, which is appreciated mainly in domestic market, and its high susceptibility to seed coat darkening. There are expectations that Pinto Saltillo seed coat clear background improve acceptance in domestic and external markets, receiving a premium price and a longer shelf life. Governmental programs were implemented in 2006 to promote Pinto Saltillo certified seed utilization in order to obtain yield increments and reduce grain marketing risks. The aim of this study was to analyze results obtained in one promotional program implemented in 2006 for Pinto Saltillo adoption in commercial plantings in Durango, México.

MATERIALS AND METHODS. Field evaluation was carried out for Pinto Saltillo adoption program as a part of support program known as PROMAF (Programa de Apoyo al Maíz y Frijol; Maize and Bean Support Program). Evaluations were performed at three rural developing districts (Distritos de Desarrollo Rural) where common bean is traditionally planted in Durango (01= Durango, 03= Guadalupe Victoria and 04= Villa Ocampo). 4,476 planting lots were sampled among a total of 9,250 plots including a total of 42,500 ha. Hundred and three locations distributed in three districts were included in the evaluation program and data were taken for farmer's number whom received Pinto Saltillo seed (for planting ≈5 ha each), planting date, fertilizer dose, "pileteo" application, days to maturity, planting density and grain yield. "Pileteo" is applied to improve rain-water use and soil retention doing little dams in rows during cultural practices by using an implement known as "pileteadora" consisting in a wheel with a lifting piece and a hoe assembled in the rear. In each planting plot ten 5 m row samples were taken, plant number were registered to obtain planting density and then samples were used in seed yield determinations.

RESULTS AND DISCUSSION. In district 01, 3,030 farmers showed interest in using Pinto Saltillo certified seed and 15,000 ha were planted in this district (Table 1). Planting dates varied from July 4th to August 10th due to delayed seed acquisition and problems in crop plantings due to excessive rain water and machinery scarcity. Fluctuation was observed for days to maturity (83 to 87 days after planting); planting density (67,800 to 91,556 plants ha⁻¹) and seed yield (680- 951 kg ha⁻¹). Average seed yield obtained in district 01 was 782 kg ha⁻¹. In district 03, 4,891 farmers sown Pinto Saltillo cultivar in 2006 and 21,500 ha were planted in this district. Planting dates varied from July 1th to July 30th due to delayed soil preparation, seed distribution and problems caused by excessive rain during crop plantings. Fluctuation was observed for days to maturity (85 to 94 days after planting); planting density (55, 155 to 67, 058 plants ha⁻¹) and seed yield (714- 986 kg ha⁻¹). Average seed yield was 844 kg ha⁻¹. In district 04, 1,329 farmers planted Pinto Saltillo in 6,000 ha and planting dates varied from June 2th to July 28th due to fluctuations in rain occurrence, delayed soil preparation and problems in seed distribution. Variation was observed for days to maturity (89 to 100 days after planting); planting density (51,368 to 68,306 plants ha⁻¹) and seed yield (642- 1132 kg ha⁻¹). Average seed yield was 793 kg ha⁻¹.

⁵Bean Program. INIFAP-Campo Experimental Valle del Guadiana. km 4.5 Carretera Durango-Mezquital, C. P. 34170. Durango, Dgo. México. e-mail: arnulfp@yahoo.com.mx.

In despite of production problems, significant yield increments were observed in all the bean production areas in the State of Durango. Average bean yield observed in Durango (1995 to 2005) was 490 kg ha⁻¹, then grain yield increments obtained with Pinto Saltillo (801 kg ha⁻¹) averaged 39 %. Contradictory comments were declared by farmers on basis to Pinto Saltillo seed size and shape and broth consistency after cooking process, although no problem was observed in marketing Pinto Saltillo by farmers and private and governmental enterprises. Farmers declared satisfied using Pinto Saltillo because significant yield increments, marketing facilities and coat tolerance to darkening, in spite of rains observed during harvest. Softness in Pinto Saltillo grain cooking process was also appreciated by consumers. Pinto Saltillo morpho-agronomical and market traits favoured its adoption by farmers and significant increments in planted area should be expected in next growing cycles at Durango, Chihuahua and Zacatecas. Rain occurred in 2006 favoured bean yields and Pinto Saltillo showed yields over 1000 kg ha⁻¹ in some locations. Pinto Saltillo showed better market acceptance and occasionally greater seed yields compared to Pinto Villa, which is another important bean cultivar planted in the State of Durango.

Additional activities need to be done to obtain increments in bean certified seed supply through stable funding mechanisms and promoting improved cultivars by a continue seed distribution program. Bean monoculture and scarcity and expensive prices of certified seed incremented problems in mechanical mixing of grain produced in Durango, Chihuahua and Zacatecas where several Pinto, Canario (Cream), Flor de Mayo (pink) and black seeded cultivars are planted. Additional programs need to be implemented to provide bean farmers training on appropriate plant population, planting dates and practices required to preserve seed quality from season-to-season. Pinto Saltillo morpho-agronomical attributes, market quality and grain cooking traits made the adoption program a success during 2006 growing cycle.

Table 1. Results obtained in Pinto Saltillo adoption program implemented in Durango, México. 2006.

RDD*	Municipality	Sampled Plots	Planting Date	Days to Maturity	Planting Density	Yield kg ha ⁻¹
01	Nuevo Ideal	184	July 4-30	87	85,000	951
	Canatlán	495	June 7-Aug 7	86	76,610	691
	Durango	477	July 4-Aug 10	83	91,556	680
	Nombre de Dios	172	July 11-30	85	74,740	804
	Poanas	438	July 9-30	85	68,958	776
	Súchil	45	July 15-30	85	67,800	775
	Vicente Guerrero	124	July 13 - Aug 7	86	75,883	797
03	Cuencamé	275	July 6-22	91	63,749	986
	Guadalupe Victoria	729	July 3-29	94	58,102	850
	Pánuco de Coronado	746	July 1-30	89	55,155	827
	Peñon Blanco	120	July 4-29	85	67,058	714
04	Coneto de Comonfort	17	June 2 - July 25	90	64,752	642
	Hidalgo	110	July 13-18	100	54,147	708
	Indé	62	July 4-18	100	55,927	857
	Ocampo	132	July 7-16	100	60,311	1132
	San Bernardo	19	July 15-18	100	51,368	703
	Santa Clara	331	July 2-28	89	68,306	715
Total		4, 476	Average	90	67,024	801

*RDD= rural developing district

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BRS EXPEDITO, A NEW BLACK SEEDED COMMON BEAN CULTIVAR

Irajá Ferreira Antunes¹, Expedito Paulo Silveira², Heloisa Torres da Silva³

EMBRAPA-Clima Temperado, Caixa Postal 403, CEP 96001-970, Pelotas, RS, Brazil¹; FAPEG, Postal 403, CEP 96001-970, Pelotas, RS, Brazil² EMBRAPA- Arroz e Feijão, Caixa Postal 179, CEP 75375-000, Santo Antônio de Goiás, GO, Brazil³. e-mail: iraja@cpact.embrapa.br

Plant breeding, as an applied science, has been efficient in offering solutions to world hungry to a point that the problem, nowadays, is thought to exist not due to lack of food, but to a bad distribution. The cultivar, or in other words, a population of plants with phenotypic uniformity, stability and distinct from others, is the concrete form of contribution from plant breeding.

In general, new cultivars result being more resistant or tolerant to a given biotic or abiotic stress, and usually with improved seed yield.

Brazil is a country in which the common bean (*Phaseolus vulgaris*) is a staple food. Besides being rich in proteins and carbohydrates, bean seed has high levels of calcium, iron, and manganese that are important chemical elements in bone physiology, hemoglobin production and anti-oxidation mechanisms, respectively.

Another important feature of the common bean, is its high level of fiber content that acts in cholesterol reduction and diabetes risk reduction.

This paper presents BRS Expedito, a new black bean cultivar for Southern Brazil. It results from the cross CNF 5491 x FT Tarumã, conducted at Embrapa Clima Temperado, Pelotas, Rio Grande do Sul State, in 1990. Both parental cultivars are black seeded, being the line CNF 5491 originated at Embrapa Arroz e Feijão and FT Tarumã at FT Sementes, a private company. At Embrapa Clima Temperado's experimental fields, were performed the F₂ to F₄ generation advances by single pod descent. In 1994, at the F₄ generation, was selected the plant that resulted in the line identified as TB 94-01. During 1995 and 1996 crop years, in preliminary trials, TB 94-01 has shown a favorable behavior and then was included in advanced State trials. From 1997/98 to 2004/05, from 20 experiments scattered through Rio Grande do Sul State, showed a mean seed yield of 2,359.3 kg ha⁻¹, 11.18% higher than check's yield (Table 1).

In comparison to cultivars available at State level, is highlighted by its resistance to anthracnose (whose agent is the fungus *C. lindemuthianum*) revealed in controlled experiments (Cruz and Balardin, 2001; Alzate-Marin et al, 2001; and results obtained at Embrapa), as well as in field experiments. At the Universidade Federal de Santa Maria showed resistance to nine from thirteen pathotypes, being the most resistant (Cruz and Balardin, 2001). At the Universidade Federal de Viçosa, among breeding lines from different common bean breeding programs, presented the highest number of markers linked to resistance genes, being three to anthracnose, three to rust and one to angular leaf spot.

The seeds of BRS Expedito are larger than most black seeded cultivars available in South Brazil market (28.0g per 100 seeds), and have an uniform black color. Its protein content (29%) is the highest among the cultivar released for cultivation in Rio Grande do Sul (12.8% higher than that of BR-Ipagro 35 Macotaço, the second highest). In tests conducted at the Universidade Federal de Santa Maria, was superior for nitrogen, phosphorus, potassium and calcium content among 19 cultivars released for cultivation in Rio Grande do Sul, being considered as promising

as parental cultivar for breeding proposes (Jost et al, 2006). Its total fiber content is similar to those of the best cultivars for this characteristic.

BRS Expedito is a type II plant architecture cultivar, with good lodging and shattering resistance, being suited to direct harvest. Its mean life cycle is 88 days and is protected at the National Service for Cultivar Protection under Cultivar Protection Certificate 00688.

Table 1. BRS Expedito and check cultivars mean seed yield (kg ha^{-1}) from 1997/98-2004/05 trials in Rio Grande do Sul State, Brazil.

Crop year	BRS Expedito (A)	Check mean yield (B)	Relative yield (A/B)100	Number of locations
1997/1998	1761	1586	111.0	3
1999/2000	2834	2582	109.7	6
2000/2001	2179	1879	116.0	4
2001/2002	2188	2278	96.0	1
2002/2003	1524	1318	115.6	2
2003/2004	3385	3186	106.2	1
2004/2005	2644	1944	136.0	3
Mean	2359.3	2110.5	111.8	20 (total)

Check cultivars: 1997/1998: BR-Ipagro 1 Macanudo and BR-Ipagro 3 Minuano; 1999/2000: BR-Ipagro 35 Macotaço and FTS Nobre; 2000/2001: FTS Nobre and Diamante Negro; 2001/2002: BRS Valente, FTS Nobre and Diamante Negro; 2002/2003: BRS Valente and FTS Soberano; 2003/2004: BRS Valente and FTS Soberano; 2004/2005: BR-Fepagro 44 Guapo Brilhante and Carioca.

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BRSMG MAJESTOSO: ANOTHER CARIOCA GRAIN TYPE BEAN CULTIVAR FOR THE STATE OF MINAS GERAIS

Ângela de Fátima Barbosa Abreu^{1,3}, Magno Antonio Patto Ramalho¹, José Eustáquio de Souza Carneiro², Maria José Del Peloso³, Trazilbo José de Paula Júnior⁴, Luís Cláudio de Faria³, Leonardo Cunha Melo³, Everaldo Gonçalves de Barros², Maurílio Alves Moreira², Israel Alexandre Pereira Filho⁵, Maurício Martins⁶, João Bosco dos Santos¹, Carlos Agustín Rava³, Joaquim Geraldo Cáprio da Costa³ and Aloísio Sartorato³

¹Universidade Federal de Lavras (UFLA), Caixa Postal 3037, 37200-000 Lavras, MG. E-mail: afbabreu@ufla.br. ²Universidade Federal de Viçosa, 36570-000 Viçosa, MG. ³Embrapa Arroz e Feijão, Caixa Postal 179, 75375-000 Santo Antônio de Goiás, GO, E-mail: mjpeloso@cnpaf.embrapa.br. ⁴Epamig, Caixa Postal 216, 36570-000 Viçosa, MG. ⁵Embrapa Milho e Sorgo, Caixa Postal 151, 35701-970 Sete Lagoas, MG. ⁶Universidade Federal de Uberlândia, Caixa Postal 593, 38400-902 Uberlândia, MG.

Bean yield in the state of Minas Gerais is increasing and this state, since 2005, became the major dry bean production in Brazil. However, production in this state is still unstable due to a series of factors including the diversity of management systems and the biotic stresses. In relation to the diversity management systems the state of Minas Gerais presents since the small subsistence farmers that use little technology until the great rural business farmers that adopt all the available technology. Among the biotic stresses, bean diseases play an important role. *Colletotrichum lindemuthianum* and *Phaeoisariopsis griseola*, the causal agents of anthracnose and angular leaf spot, respectively, have been one of the most bean diseases in the state of Minas Gerais. The most economic way to control these diseases is the use of resistant cultivars. However, as pointed out in the literature these pathogens present numerous pathotypes, making the useful life of these cultivars very short. In such a situation the bean breeding programs must be dynamic, releasing to the farmers new bean cultivars with different resistant alleles. Before the releasing of such cultivars they have to be tested in a majority of environment. It is with these objectives that the Universidade Federal de Lavras, (UFLA), Universidade Federal de Viçosa (UFV), Embrapa Arroz e Feijão (CNPAF) and Empresa de Pesquisa Agropecuária de Minas Gerais (Epamig), have been evaluating new cultivars. As a result of this effort it has been released the BRSMG Majestoso as a new carioca grain type bean cultivar to the farmers of the state of Minas Gerais.

BRSMG Majestoso is originated from the cross between Ouro Negro, a black bean cultivar and Pérola, a carioca grain type cultivar. Crosses were performed at the UFLA Biology Department where the F₃ seeds were obtained. From the F₃ population 398 plants were selected all with carioca grain type. These plants were evaluated in the F_{3:4} and F_{3:5} progenies in Lavras and Patos de Minas and 10 of them were selected to be evaluated in different trials. Based on the results of these experiments line OP-NS 331 were selected to participate in the Use and Cultivation Values trials from 2002 to 2004. These trials was performed in 43 environments in the state of Minas Gerais (Table 1) using cultivars BRS Talismã and Pérola as control together with other 17 lines in a complete randomized block design with three replications.

Cultivar BRSMG Majestoso presents a type III indeterminate habit growth. Its lodging and plant type are very similar to the control Pérola, the most cultivated cultivar in the state of Minas Gerais (Table 1). Besides, it is resistant to the pathotypes 55, 89, 95 and 453 of

Colletotrichum lindemuthianum and to bean common mosaic virus and, under field conditions, presented an intermediary reaction to *Phaeoisariopsis griseola*. The 43 environments used to test the VCU trials included several regions in the state of Minas Gerais and the three planting dates (wet season, dry season and fall-winter season). In almost all of them the average yield of cultivar BRSMG Majestoso was superior than the average yield of the controls Pérola and BRSMG Talismã (Table 1), showing its wider adaptation. The overall average yield of BRSMG Majestoso was 11,3% higher than the two controls. In the bean quality test, cultivar BRSMG Majestoso besides of the carioca grain type, which is very important for the market, also presented excellent cooking quality, with a cooking time inferior to both BRS Talismã and Pérola (Table 1).

Due to its high average yield, superior grain quality, resistance to anthracnose and a lower susceptibility to angular leaf spot the cultivar BRSMG Majestoso is another carioca common bean type option for the farmers of the state of Minas Gerais.

Institutions involved in the cultivar evaluation:

Embrapa Arroz e Feijão - Santo Antônio de Goiás, GO.

Universidade Federal de Lavras - Lavras, MG.

Universidade Federal de Viçosa - Viçosa, MG.

Empresa de Pesquisa Agropecuária de Minas Gerais - Centro Tecnológico da Zona da Mata - Viçosa, MG.

Embrapa Milho e Sorgo - Sete Lagoas, MG.

Universidade Federal de Uberlândia - Uberlândia, MG.

Table 1. Some biological and physical characteristics of cultivars BRSMG Majestoso and the controls Pérola and BRSMG Talismã.

Characteristic	BRSMG Majestoso	Pérola	BRSMG Talismã
Plant type	5,2	5,3	5,9
Lodging	5,2	5,4	6,0
Flowering	42,0	45,0	42,0
Maturation	87,0	88,0	84,0
Angular leaf spot	3,0	3,6	4,2
Cooking time	27,0	31,0	31,0
Soluble solids	8,9	10,6	11,0
Protein	23,0	21,3	23,8
100 grain weight	30,6	29,0	26,0
Average yield (kg/ha)	2413,0	2138,0	2199,0

BRS COMETA: A CARIOCA GRAIN TYPE COMMON BEAN CULTIVAR WITH ERECT GROWTH HABIT

Luis Cláudio de Faria¹, Maria José Del Peloso¹, Leonardo Cunha Melo¹, Joaquim Geraldo Cáprio da Costa¹, Carlos Agustín Rava¹, José Luiz Cabrera Díaz¹, Josias Correa de Faria¹, Heloísa Torres da Silva¹, Aloísio Sartorato¹, Priscila Zaczuk Bassinello¹ and José Benedito de Freitas Trovo²

¹Embrapa Arroz e Feijão, Caixa Postal 179, Santo Antônio de Goiás, GO, CEP 75375-000. E-mail: lcfaria@cnpaf.embrapa.br. ²Embrapa Gado de Corte, Rodovia BR 262, Km 4, Caixa Postal 154, Campo Grande, MS, CEP 79002-970.

Common bean is considered to be the Brazilian's people basic protein food, who regionally demands, besides the culinary quality, also different grain color and type. Actually, the Brazilian's dry bean consumption is about 79% of carioca, 17% of black and 4% of other grain types. These beans are produced mostly in the Southern, Southeast and Center-west regions. To attend this demand, the 2006 bean production was about 3,0 million of tons cultivated in an area of 2,9 million hectares, meaning a national average productivity of 1034 kg/ha. If on one side the productivity has been growing, the consumption per capita has been decreasing, resulting in an average estimate of annual consumption, in 2006, of 12,7 kg/inhabitant. The bean breeding program, at Embrapa Rice and Beans, is focused on more productive, disease resistant and erect plant type cultivars. The latest characteristic has the objective to favor bean mechanical harvesting providing farmers to offer to consumers a product with superior quality.

BRS Cometa is originated from the cross A 769 /4/ EMP 250 /// A 429 / XAN 252 // C 8025 / G 4449 /// WAF 2 / A 55 // GN 31 / XAN 170, performed at Centro Internacional de Agricultura Tropical (CIAT), Colombia. In 1994, Embrapa Rice and Beans received from CIAT the F_{1:3} families. From F₃ to F₅, using the bulk method, plants were selected based on the carioca grain type, erect plant type and resistance to angular leaf spot, rust and anthracnose. In F₆, individual plants were selected based on yield, adaptation, erect plant type, resistance to common bacterial blight and carioca grain type. In F₇, line LM 98202147 were selected based on yield and plant type. In 1999 and 2001 this line was tested in the Carioca Preliminary Trials and in the National Trial, respectively, in seven different environment in the states of Goiás (1), Minas Gerais (2), Rio de Janeiro (1), Sergipe (1), Paraná (1) and the Federal District (1). Based on the results obtained by the yield joint analyses and other cultural characteristics, line LM 98202147 were evaluated, in 2003 and 2004 with 11 other lines and 4 controls, in the Use and Cultivation Values trials, with the pre-commercial name of CNFC 9435, in 77 different environment in the states of Goiás (24), Tocantins (7), Mato Grosso (4), Mato Grosso do Sul (4), Sergipe (6), Alagoas (1), Bahia (1), São Paulo (6), Paraná (10), Santa Catarina (10) and Federal District (4).

In the 77 VCU Trials accomplished during 2003 and 2004, cultivar BRS Cometa yielded as much as the cultivars Iapar 81 and Carioca Pitoco (Table 1).

BRS Cometa presented a uniform color and grain size, 100 seed weight of 24,6g and a cooking time of 33m (Table 2). Under artificial inoculation BRS Cometa was resistant to bean common mosaic virus and to pathotypes 55, 95 and 453 of *Colletotrichum lindemutianum*. In the field it was susceptible to angular leaf spot and bean golden mosaic virus and showed an intermediary reaction to rust and common bacterial blight.

BRS Cometa presents an erect plant type with good lodging resistance and a crop cycle of 78 days from emergence to physiological maturity. Due to its erect plant type, yield potential, excellent cooking quality, disease and lodging resistance, the cultivar BRS Cometa is another options to farmers that intend to produce a carioca grain type in the wet and dry seasons in the state of Santa Catarina and Paraná, in the wet season in the state of São Paulo, Bahia, Sergipe and Alagoas, in the dry and winter seasons in the state of Mato Grosso and Mato Grosso do Sul, in the winter season in the state of Tocantins and in the wet, dry and winter seasons in the state of Goiás and Federal District.

Institutions involved in the cultivar evaluation:

Embrapa Cerrados-DF; Embrapa Soja-PR; Embrapa Agropecuária Oeste-MS; Empresa de Pesquisa Agropecuária e Extensão Rural de Mato Grosso-MT; Embrapa Tabuleiros Costeiros-SE; Embrapa Negócios Tecnológicos-PR; Agência Goiana de Desenvolvimento Rural e Fundiário-GO; Fundação de Ensino Superior de Rio Verde-GO; Centro Federal de Educação Tecnológica (GO);Avena S/C Ltda. - Major Vieira-SC; Cooperativa Regional Agropecuária de Campos Novos (SC); C. Vale Cooperativa Agroindustrial-SC; Escola Agrotécnica Federal de Concórdia-SC; Cooperativa dos Produtores de Sementes de Laranjeiras do Sul Ltda-PR; Sementes Campo Verde-PR; Universidade Estadual de Londrina-PR; Cooperativa Agrícola Mista de Prudentópolis-PR; Detec Assessoria Técnica S/C Ltda-SP; Anastácio Ceregatti Sanchez Ltda-SP; Cooperativa Regional Agropecuária de Taquarituba-SP; Empresa Baiana de Desenvolvimento Agrícola-BA.

Table 1. Yield of BRS Cometa according to region/state compared to the average control in VCU Trials, during 2003 and 2004.

Region	State	Season	BRS Cometa (kg/ha)	Control ¹ (kg/ha)	Relative yield (%)	Environment
South	SC/PR	“wet”	2219	2442	91,4	12
		“dry”	2213	2174	100,3	8
Southeast	SP	“wet”	2749	3074	89,1	6
		“wet”	2061	2042	107,4	12
Center-west	GO/DF	“dry”	1369	1396	98,4	4
		“winter”	2304	2546	90,2	12
	MT/MS	“dry”	1451	1570	92,8	6
		“winter”	2292	2541	91,6	2
North	TO	“winter”	1717	2118	88,6	6
Northeast	BA/SE/AL	“wet”	2083	2205	95,8	8
Average			2086	2244	95,3	

¹Iapar 81 and Carioca Pitoco.

Table 2. Bean quality test of cultivar BRS Cometa compared to both controls¹.

Cultivar	Cooking time (min.)	Protein (%)	100 seed weight (g)
BRS Cometa	33	22,2	24,6
Carioca Pitoco ¹	36	-	20,4
Iapar 81 ¹	29	22,5	25,1

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
WASHINGTON, D.C. 20250
and
AGRICULTURAL RESEARCH DIVISION
UNIVERSITY OF NEBRASKA
LINCOLN, NE 68503

**RELEASE OF ABC-WEIHING COMMON BACTERIAL BLIGHT, RUST AND
MOSAIC RESISTANT, SEMI-UPRIGHT, HIGH SEED QUALITY GREAT NORTHERN
BEAN GERMPLASM LINE**

N. Mutlu, C.A. Urrea*, P.N. Miklas, J.R. Steadman, M.A. Pastor-Corrales, D.T. Lindgren, J.
Reiser, A.K. Vidaver, and D.P. Coyne
Univ. of Nebraska, USDA-ARS Prosser, and USDA-ARS Beltsville

The Agricultural Research Service, U. S. Department of Agriculture and the Agricultural Research Division, University of Nebraska, announce the release of the common bacterial blight resistant great northern bean germplasm line ABC-Weihing.

Great northern common bean (*Phaseolus vulgaris* L.) germplasm line ABC-Weihing was bred specifically for enhanced resistance to a major seed borne disease of common bean, common bacterial blight, caused by *Xanthomonas campestris* pv. *phaseoli* [Syn. *X. axonopodis* pv. *phaseoli*] (*Xcp*). ABC-Weihing is the first great northern to combine the XAN 159 and great northern Montana No. 5 sources of common bacterial blight resistance. Combined high levels of resistance was confirmed by the presence of previously developed SCAR markers SU91 and SAP6 tightly linked with quantitative trait loci (QTL) from XAN 159 and Montana No. 5, respectively. Inoculation of ABC-Weihing, evaluated as NE1-05-4, with races 41, 44, 47, 49, 53, 67, 73, and 108 of the bean rust pathogen under greenhouse conditions at Beltsville, MD in 2004 indicated the presence of *Ur-3* and *Ur-6* genes for resistance to common bean rust caused by *Uromyces appendiculatus*. In addition, ABC-Weihing carries the single dominant hypersensitive *I* gene that provides resistance to all non-necrotic strains of the bean common mosaic virus (BCMV), but it is sensitive to the temperature-dependent, necrosis-inducing strains of BCMV and to the temperature-independent, necrosis inducing NL3, NL5, and NL8 strains of the bean common mosaic necrosis virus. ABC-Weihing has partial avoidance to white mold (*Sclerotinia sclerotiorum*) due to its semi-upright plant architecture.

ABC-Weihing is a great northern BC₅F_{3,6} line obtained from five backcrosses ('Weihing' * 5//Chase' / XAN 159). Since there was incompatibility between Weihing and XAN 159 due to the lethality alleles *DL1* and *DL2*, advanced backcross resistant lines from the cross Chase/XAN 159 were used as a donor parent to transfer *Xcp* resistance to the recurrent great northern parent Weihing. XAN 159 developed by the International Center for Tropical Agriculture (CIAT) for common bacterial blight resistance XAN 159 was estimated to have up to five QTLs for resistance to common bacterial blight and is susceptible to rust and BCMV. Chase, a pinto cultivar, has the *Ur-3* rust resistance gene and moderate resistance to common bacterial

blight and brown spot (*Pseudomonas syringae*). Weihing is a great northern cultivar that has the *Ur-3* and *Ur-6* rust genes and resistance to halo blight pathogen *Pseudomonas syringae* pv. *phaseolicola* in Nebraska combined with partial avoidance to white mold due to upright plant architecture. Weihing also has excellent seed quality.

The first cross was made in spring 1997. Only BC_nF₁ plants resistant to *Xcp* isolates Dominican Republic DR-7 and Nebraska SC4A as determined by multiple needle leaf inoculation tests in the greenhouse were used for successive backcrossing. In addition to phenotypic selection for common bacterial blight resistance, marker assisted selection for the resistant QTL-linked markers SU91 and SAP6 was conducted in the BC₁F₁ and BC₂F₁.

Reaction of ABC-Weihing to *Xcp* was consistent across two years. At the West Central Research and Extension Center, North Platte, NE, ABC-Weihing inoculated with the Nebraska LB-72 and SC-4A *Xcp* strains, exhibited a field reaction of 3.6 and 2.0 in 2005 and 2006, respectively. The plants were evaluated 14 days after inoculation using a 1-9 scale, where 1 = immune and 9 = very susceptible. Reactions from 1-4 were considered resistant and from 5-9 susceptible. ABC-Weihing had similar reaction to XAN 159 which scored 3.5 and 2.0 in the same trials. Conversely, the susceptible great northern Matterhorn had a reaction of 5.7 and 9.0 in 2005 and 2006, respectively.

ABC-Weihing exhibits a semi-upright Type 2b indeterminate growth habit. Plants averaged 57 cm in height during 2005 with excellent lodging resistance. ABC-Weihing has white flowers and blooms 45 d after planting. ABC-Weihing is a midseason bean maturing 92 d after planting and ranging in maturity from 90-94 days.

Seed size for ABC-Weihing (34.5 g 100 seeds⁻¹) was slightly larger than ‘Matterhorn’ (33.9 g 100 seeds⁻¹) in 2005 in the Mid-West Regional Performance Nursery (MRPN) grown across four locations: Carrington, ND; Saginaw, MI; Mitchell, NE; and Fort Collins, CO. For the same nursery ABC-Weihing had similar yield to Matterhorn, 2,079 kg ha⁻¹ versus 2,112 kg ha⁻¹, respectively.

The ABC-Weihing breeding line will be useful for improving resistance to common blight in great northern and pinto bean market classes while maintaining rust and bean common mosaic resistance, seed quality and yield potential. Limited quantity of seed is available from C.A. Urrea, University of Nebraska, Panhandle Research and Extension Center, 4502 Avenue I, Scottsbluff, NE 69361 (currea2@unl.edu). We ask that appropriate recognition of source be given when this germplasm contributes to the development of a new cultivar.

RELEASE OF GREAT NORTHERN ‘HUNGERFORD’ DRY BEAN

Shree P. Singh, Henry Terán, Marie Dennis, Margarita Lema, Richard Hayes and Craig Robinson

University of Idaho, Kimberly and Parma Research and Extension Centers, Idaho

ABSTRACT

Hungerford is an extra-large-seeded, adapted to the Western U.S., and full-season great northern cultivar that has the *I* gene resistance to BCMV. Hungerford is also resistant to the race 38 and exhibits an intermediate reaction to the race 53 of *Uromyces appendiculatus*, the cause of bean rust. Hungerford has moderate levels of resistance to heat, drought, and soil zinc deficiency and manganese toxicity. Hungerford has an indeterminate semi-prostrate growth habit Type III with medium to large vine. Hungerford was tested in the Idaho Bean Adaptation Nursery (IBAN), Idaho Dry Bean Trial (IDBT), and the Western Regional Bean Trial (WRBT) as UIG4-6P-3P, ABL 2, or 06I6 from 2003 to 2006.

PEDIGREE AND BREEDING HISTORY

Hungerford was derived from the double-cross population UIG4 = ‘Matterhorn’/ ‘Starlight’//‘Beryl’/‘WeiHING’ made in 1999-2000. The Michigan Agricultural Experiment Station released great northern Matterhorn (Kelly et al., 1999). High yielding broadly adapted Matterhorn (Hang, 2006) has an indeterminate upright or erect growth habit Type II (Singh, 1982) with small to medium length vine in southern Idaho. Matterhorn carries the *I* gene resistance to the US-6 and NY-15 strains of the BCMV. However, when inoculated with the *Bean common mosaic necrosis virus* (BCMNV, a potyvirus) strain NL-3K Matterhorn exhibits top or systemic necrosis including black root. Matterhorn is resistant (no disease symptoms) to the race 38 (Andean) of *U. appendiculatus*. However, Matterhorn exhibits an intermediate reaction or small pustules when inoculated with the race 53 (Middle American) of the rust pathogen. The Nebraska Agricultural Experiment Station released high quality great northern Starlight (Coyne et al., 1991) and WeiHING (Coyne et al., 2000). Starlight has an indeterminate semi-prostrate growth habit Type III and is susceptible to BCMV and BCMNV. However, WeiHING has a similar growth habit and resistance to BCMV and rust as Matterhorn. Beryl is a Rogers/Syngenta great northern cultivar with growth habit Type III. In addition to the *I* gene, Beryl carries a recessive resistance gene such that it exhibits local necrosis or pinpoint lesions when inoculated with the NL-3K strain of the BCMNV. Beryl is resistant to the race 38 and susceptible to the race 53 of *U. appendiculatus*.

Bulk seed of the double-cross F₂ was grown in the field at Parma, Idaho. A single plant selection was made that exhibited the extra-large white great northern seed. The F₂-derived F₃ (F_{2:3}) progeny-row was grown in the field at Kimberly, Idaho where all plants selected for extra-large great northern seed were harvested in bulk followed by a single plant selection in the field at Parma. The F_{4.5} plant-to-progeny row was grown in the greenhouse, selection was made for resistance to the US-6 strain of BCMV and extra-large white great northern seed, and all selected plants were harvested in bulk. Subsequently, seed was increased in the field at Kimberly and six F₆ plants were screened each for the NL-3K strain of the BCMNV, the strains NY-15 and US-6 of BCMV, and the races 38 and 53 of *U. appendiculatus* in the separate greenhouse nurseries.

MATURITY

Hungerford is a full-season cultivar, taking 92 to 102 days with a mean of 95 days compared with a range of 90 to 105 days and mean of 97 days for UI 425 in southern Idaho in 2005 and 2006. Hungerford's maturity ranged from 90 to 103 days with a mean of 97 days compared with a range of 86 to 97 days and mean of 91 days for 'Orion' across five environments in Colorado, Idaho, Nebraska, and Washington in the WRBT in 2006.

SEED YIELD

Average seed yield of Hungerford was 1995 lbs A⁻¹ compared with 2140 lbs A⁻¹ for UI 425 across 15 environments in southern Idaho in 2005 and 2006. In the WRBT across five environments, the average yield of Hungerford was 2322 lbs A⁻¹ compared with 2086 lbs A⁻¹ for Orion in 2006.

SEED WEIGHT

Hungerford and UI 425 had a similar mean 100-seed weight of 40 g in the IDBT in Idaho in 2005 and 2006. In the WRBT in 2006, the weight of Hungerford was 44 g compared with 35 g for Orion.

SEED STATUS

Breeder and Foundation seed of Hungerford will be maintained by the Idaho Foundation Seed Program under the direction of the Idaho Agricultural Experiment Station, University of Idaho, Moscow, ID 83844. However, a small quantity of seed of Hungerford for research purposes is available from S. Singh for the first five years. Appropriate acknowledgement of its developers and the University of Idaho for the use of Hungerford as germplasm would be highly appreciated. The PVP for Hungerford is pending.

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RELEASE OF PINTO ‘KIMBERLY’ DRY BEAN

Shree P. Singh, Henry Terán, Marie Dennis, Margarita Lema, Richard Hayes and Craig Robinson

University of Idaho, Kimberly and Parma Research and Extension Centers, Idaho

Abstract. Kimberly is a high yielding, broadly adapted, and full-season pinto cultivar that has a recessive resistance to BCMV and BCMNV. Kimberly is also resistant to the race 38 and exhibits an intermediate reaction to the race 53 of *Uromyces appendiculatus*. Kimberly is moderately tolerant to BCTV, Fusarium root rot, heat and drought. Kimberly has an indeterminate semi-prostrate growth habit Type III with medium to large vine.

Kimberly was tested in the Idaho Bean Adaptation Nursery (IBAN), Idaho Dry Bean Trial (IDBT), Western Regional Bean Trial (WRBT), and National Cooperative Dry Bean Nursery (CDBN) as UIP7-24P-2P, ABL 13, or 06I15 from 2003 to 2006.

Pedigree and Breeding History. Kimberly was selected from the double-cross population UIP7 = ‘Poncho’/G 17341//‘Kodiak’/BelDakMi-RMR-14 made in 1999-2000. Pinto Poncho is a Rogers/Syngenta cultivar with an indeterminate growth habit Type III which is susceptible to BCMV (US-6 strain), BCMNV (NL-3K) and the race 53 (Middle American) of *U.*

appendiculatus. G 17341 was selected at the Centro Internacional de Agricultura Tropical (CIAT), Palmira, Colombia from a population developed at Cornell University by R.E.

Wilkinson (unpublished). G 17341 has an intermediate level of resistance to common bacterial blight [caused by *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye and *X. campestris* pv. *phaseoli* var. *fuscans*] and a growth habit Type III (Lema et al., 2007; Singh and Muñoz, 1999).

The small semi-shiny pinto seed of G 17341 is variable for a slow darkening trait that allows for improved storage and reduced degradation of color. Stavely et al. (1998) at the USDA-ARS, Beltsville, Maryland, and North Dakota and Michigan Agricultural Experiment Stations cooperatively developed pinto germplasm line BelDakMiRMR-14. BelDakMiRMR-14 has growth habit Type III and pyramided resistance to all known strains of BCMV and BCMNV, and all races of *U. appendiculatus* in the United States. The Michigan Agricultural Experiment Station released pinto Kodiak (Kelly et al., 1999). Kodiak has an indeterminate upright or erect growth habit Type II with small to medium length vine in southern Idaho. Kodiak carries the *I* gene resistance to the US-6 and NY-15 strains of the BCMV. In addition, Kodiak exhibits local necrosis or pinpoint lesions when inoculated with the NL-3K strain of the BCMNV. Kodiak is resistant (no disease symptoms) to the race 38 (Andean) of *U. appendiculatus* however exhibits an intermediate reaction or small pustules when inoculated with the race 53 of the pathogen.

The double-cross F₂ was grown in the field at Parma, Idaho. A single plant selection was made for the light-colored slow darkening pinto seed. The F₂-derived F₃ (F_{2:3}) progeny-row was grown in the greenhouse at Kimberly, Idaho where a single-plant selection was made for resistance to the US-6 strain of BCMV and light-colored slow darkening pinto seed. The F_{3:4} progeny-row was grown in the field at Kimberly and plants selected for slow darkening pinto seed were harvested in bulk. A single-plant selection for slow darkening pinto seed was made in F₅ in the field at Parma. The F₆ progeny-row was screened in the greenhouse. Six plants were screened each for BCMV (NY-15, US-6), BCMNV (NL-3K), and the races 38 and 53 of *U.*

appendiculatus. All plants were harvested in bulk followed by seed increase in the field at Kimberly.

Maturity. Kimberly is a full-season cultivar taking 91 to 103 days with mean of 97 days compared with a range of 86 to 101 and mean of 95 days for Bill Z in the IDBT in southern Idaho in 2005 and 2006. Kimberly's maturity ranged from 99 to 103 days with a mean of 101 days compared with a range of 90 to 98 days and mean of 95 days for Bill Z across five locations in Colorado, Idaho, Nebraska, and Washington in the WRBT in 2005 and 2006. In the CDBN, maturity of Kimberly across 10 locations ranged from 83 to 103 days with a mean of 94 days compared with the respective values of 78 to 93 and 86 days for Othello.

Seed Yield. Average yield of Kimberly was 2323 lbs A⁻¹ compared with 2023 lbs A⁻¹ for Bill Z across 14 environments in the IDBT in southern Idaho in 2005 and 2006. In the WRBT across five environments, the average yield of Kimberly was 2299 lbs A⁻¹ compared with 1942 lbs A⁻¹ for Bill Z in 2005 and 2006. At two locations in Colorado in 2006, the mean yield of Kimberly was 3605 lbs A⁻¹ compared with 3689 lbs A⁻¹ for 'Bill Z', 3466 lbs A⁻¹ for 'Montrose', 3033 lbs A⁻¹ for 'Poncho' and 'Othello', and 2944 lbs A⁻¹ for 'Grand Mesa' (Johnson et al. 2006). In the ADM edible bean trial at two locations in North Dakota in 2006, the mean yield of Kimberly was 2628 lbs A⁻¹ compared with 2437 lbs A⁻¹ for 'Buster', 2090 lbs A⁻¹ for 'Maverick', and 1978 lbs A⁻¹ for 'Pintoba' (Spencer 2006). In the CDBN across 10 locations in the U.S. and Canada in 2006, Kimberly yielded 2713 lbs A⁻¹ compared with 2353 lbs A⁻¹ of Othello (Hang 2006).

Seed Weight. Kimberly and Bill Z had a similar 100-seed weight of 34 g in the IDBT in Idaho in 2005 and 2006. In the WRBT in 2005 and 2006, the respective values for Kimberly and Bill Z were 36 g and 35 g. In the CDBN across 10 environments in 2006, 100 seeds of Kimberly weighed 35 g compared with 36 g for Othello.

Seed Status. Breeder and Foundation seed of Kimberly will be maintained by the Idaho Foundation Seed Program under the direction of the Idaho Agricultural Experiment Station, University of Idaho, Moscow, ID 83844. The PVP for Kimberly is pending.

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RELEASE OF GREAT NORTHERN ‘SAWTOOTH’ DRY BEAN

Shree P. Singh, Henry Terán, Marie Dennis, Margarita Lema, Richard Hayes and Craig Robinson

University of Idaho, Kimberly and Parma Research and Extension Centers, Idaho

Abstract. Sawtooth is a large-seeded, broadly adapted, high yielding, and full-season great northern cultivar that has the *I* gene resistance to BCMV. Sawtooth is also resistant to Fusarium root and to the race 38 and exhibits an intermediate reaction to the race 53 of *Uromyces appendiculatus*, the cause of bean rust. Sawtooth has moderate to high levels of resistance to heat, drought, and to soil zinc deficiency and manganese toxicity. Sawtooth has an indeterminate semi-prostrate growth habit Type III with medium to large vine.

Sawtooth was tested in the Idaho Bean Adaptation Nursery (IBAN), Idaho Dry Bean Trial (IDBT), Western Regional Bean Trial (WRBT), and National Cooperative Dry Bean Nursery (CDBN) as UIG4-53P-2P, ABL 6, or 06I1 from 2003 to 2006.

Pedigree and Breeding History. Sawtooth was derived from the double-cross population UIG4 = ‘Matterhorn’/‘Starlight’//‘Beryl’/‘WeiHING’ made in 1999-2000. The Michigan Agricultural Experiment Station released great northern Matterhorn (Kelly et al., 1999). High yielding broadly adapted Matterhorn (Hang, 2006) has an indeterminate upright or erect growth habit Type II (Singh, 1982) with small to medium length vine in southern Idaho. Matterhorn carries the *I* gene resistance to the US-6 and NY-15 strains of the BCMV. However, when inoculated with the *Bean common mosaic necrosis virus* (BCMNV, a potyvirus) strain NL-3K Matterhorn exhibits top or systemic necrosis including black root. Matterhorn is resistant (no disease symptoms) to the race 38 (Andean) of *U. appendiculatus* but exhibits an intermediate reaction or small pustules when inoculated with the race 53 (Middle American) of the pathogen. The Nebraska Agricultural Experiment Station released high quality great northern Starlight (Coyne et al., 1991) and WeiHING (Coyne et al., 2000). Starlight has an indeterminate semi-prostrate growth habit Type III and is susceptible to BCMV (US-6) and BCMNV (NL-3K). However, WeiHING has a similar growth habit and resistance to BCMV and rust as Matterhorn. Beryl is a Rogers/Syngenta great northern cultivar with growth habit Type III. In addition to the *I* gene, Beryl carries a recessive resistance gene such that it exhibits local necrosis or pinpoint lesions when inoculated with the NL-3K strain of the BCMNV. Beryl is resistant to the race 38 and susceptible to the race 53 of *U. appendiculatus*.

Bulk seed of the double-cross F₂ was space-planted in the field at Parma, Idaho. A single plant selection was made that exhibited the large white great northern seed. The F₂-derived F₃ (F_{2:3}) progeny-row was grown in the greenhouse where a single-plant selection was made for resistance to the US-6 strain of BCMV and large white great northern seed. The F_{3:4} plant-to-progeny-row was grown in the field at Kimberly, Idaho and plants selected for large white great northern seed were harvested in bulk. A single-plant selection for large white great northern seed was made in F₅ in the field at Parma. The F₆ progeny-row was screened in the greenhouse for BCMV resistance and large white great northern seed, all plants harvested in bulk, followed by seed increase in the field at Kimberly. Six F₆ plants were screened each for the NY-15 and US-6 strains of BCMV, NL-3K strain of the BCMNV, and the races 38 and 53 of *U. appendiculatus* in the separate greenhouse nurseries.

Maturity. Sawtooth is a full-season cultivar, taking 91 to 103 days with mean of 96 days in southern Idaho in 2005 and 2006 compared with a range of 90 to 105 days and a mean of 97 days for UI 425. Sawtooth's maturity ranged from 92 to 102 days with a mean of 99 days compared with a range of 86 to 97 days and mean of 91 days for 'Orion' across five locations in the WRBT. In the CDBN, maturity of Sawtooth across 10 locations ranged from 84 to 116 days with a mean of 98 days compared with the respective values of 79 to 94 and 89 days for Matterhorn.

Seed Yield. Average yield of Sawtooth was 2219 lbs A⁻¹ compared with 2140 lbs A⁻¹ for UI 425 across 15 environments in southern Idaho in 2005 and 2006. In the WRBT, the average yield of Sawtooth was 2333 lbs A⁻¹ compared with 2086 lbs A⁻¹ for Orion. In the CDBN across six western states Sawtooth yielded 2995 lbs A⁻¹ compared with 3019 for Matterhorn in 2006. However, across all 10 locations in the U.S. and Canada the respective yields in the CDBN were 2239 lbs A⁻¹ and 2791 lbs A⁻¹.

Seed Weight. Mean weight of the 100 seeds of Sawtooth of 40 g was similar to that of UI 425 in the IDBT in Idaho in 2005 and 2006. In the WRBT in 2006, the respective values for Sawtooth and Orion were 45 g and 36 g. In the CDBN across 10 environments in 2006, 100 seeds of Sawtooth weighed 43 g compared with 34 g for Matterhorn.

Seed Status. Breeder and Foundation seed of Sawtooth will be maintained by the Idaho Foundation Seed Program under the direction of the Idaho Agricultural Experiment Station, University of Idaho, Moscow, ID 83844. However, a small quantity of seed of Sawtooth for research purposes is available from S. Singh for the first five years. Appropriate acknowledgement of its developers and the University of Idaho for the use of Sawtooth as germplasm would be highly appreciated. The PVP for Sawtooth is pending.

Acknowledgement. We thank David Webster and Carl Strausbaugh for the help with some disease evaluations; to Phillip Miklas, Howard Schwartz, Mark Brick, and Carlos Urrea for the WRBT data; and to all participants of the CDBN for data and collaboration. Financial support from the Idaho Bean Commission and the College of Agriculture and Life Sciences, University of Idaho are gratefully acknowledged.

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RELEASE OF PINTO ‘SHOSHONE’ DRY BEAN
Shree P. Singh, Henry Terán, Marie Dennis, Margarita Lema,
Richard Hayes and Craig Robinson

University of Idaho, Kimberly and Parma Research and Extension Centers, Idaho

Abstract. Shoshone is a high yielding, broadly adapted, and medium maturing cultivar that has a recessive resistance to BCMV (NY-15, US-6) and BCMNV (NL-3K). Shoshone is also resistant to the race 38 of *Uromyces appendiculatus*. However, Shoshone exhibits an intermediate reaction or small pustules when exposed to the rust pathogen race 53. Shoshone is moderately tolerant to Fusarium root rot, BCTV, heat and drought. Shoshone has an indeterminate semi-prostrate growth habit Type III with small to medium length vine.

Shoshone was tested in the Idaho Bean Adaptation Nursery (IBAN), Idaho Dry Bean Trial (IDBT), Western Regional Bean Trial (WRBT), and National Cooperative Dry Bean Nursery (CDBN) as UIP15-53G-4G-1, ABL 8, or 06I4 from 2003 to 2006.

Pedigree and Breeding History. Shoshone was selected from the multiple-parent population UIP15 = H9657-42-2/3/ ‘Poncho’/G 17341// ‘Kodiak’/BelDakMi-RMR-14 made in 1999-2000. Breeding line H9657-42-2 with a tall upright growth habit Type II and large pinto seed was developed at the USDA-ARS, Prosser, Washington (P. Miklas, unpublished). Pinto Poncho is a Rogers/Syngenta cultivar with an indeterminate growth habit Type III which is susceptible to BCMV (US-6 strain), BCMNV (NL-3K) and the race 53 (Middle American) of *U. appendiculatus*. G 17341 was selected at the Centro Internacional de Agricultura Tropical (CIAT), Palmira, Colombia from a population developed at Cornell University by R.E. Wilkinson (unpublished). G 17341 has an intermediate level of resistance to common bacterial blight [caused by *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye and *X. campestris* pv. *phaseoli* var. *fuscans*] and a growth habit Type III (Lema et al., 2007). The small semi-shiny pinto seed of G 17341 is variable for a slow darkening trait that allows for improved storage and reduced degradation of color. Stavely et al. (1998) at the USDA-ARS, Beltsville, Maryland, and North Dakota and Michigan Agricultural Experiment Stations cooperatively developed pinto germplasm line BelDakMiRMR-14. BelDakMiRMR-14 has growth habit Type III and pyramided resistance to all known strains of BCMV and BCMNV, and all races of *U. appendiculatus* in the United States. The Michigan Agricultural Experiment Station released pinto Kodiak (Kelly et al., 1999). Kodiak has an indeterminate upright or erect growth habit Type II with small to medium length vine in southern Idaho. Kodiak carries the *I* gene resistance to the US-6 and NY-15 strains of the BCMV. In addition, Kodiak exhibits local necrosis or pinpoint lesions when inoculated with the NL-3K strain of the BCMNV. Kodiak is resistant (no disease symptoms) to the race 38 (Andean) of *U. appendiculatus* however exhibits an intermediate reaction or small pustules when inoculated with the race 53 of the pathogen.

The multiple-parent F_1 was screened for the US-6 strain of BCMV in the greenhouse at Kimberly, Idaho. An early maturing BCMV resistant plant with light-colored pinto seed was harvested and the F_1 -derived F_2 ($F_{1:2}$) progeny-row was planted in the field at Kimberly following the gamete selection procedure (Asensio-S.-Manzanera, 2006). Selection was made for early maturing slow darkening light pinto seed color, and all selected plants were harvested in bulk. Six plants were screened each for BCMV (US-6), BCMNV (NL-3K), and the races 38 and

53 of *U. appendiculatus* in the greenhouse. An early maturing BCMV and rust resistant plant with light-colored slow darkening pinto seed was harvested. The F_{3:4} plant-to-progeny-row was grown in the field at Parma, Idaho where all early maturing plants with light pinto seed color were harvested in bulk followed by seed increase in the greenhouse and then in the field at Kimberly. Six plants were screened each for BCMV (NY-15, US-6), BCMNV (NL-3K), and the races 38 and 53 of *U. appendiculatus*.

Maturity. Shoshone is a medium maturing cultivar, taking 89 to 101 days with mean of 94 days compared with a range of 86 to 101 and mean of 95 days for Bill Z in the IDBT in southern Idaho in 2005 and 2006. Its maturity ranged from 86 to 95 days with a mean of 90 days compared with a range of 90 to 95 days and mean of 93 days for Bill Z across four locations in Colorado, Idaho, Nebraska, and Washington in the WRBT in 2006. In the CDBN, maturity of Shoshone across nine locations ranged from 82 to 99 days with a mean of 90 days compared with the respective values of 78 to 98 and 86 days for Othello.

Seed Yield. Average yield of Shoshone was 2132 lbs A⁻¹ compared with 2023 lbs A⁻¹ for Bill Z across 14 environments in the IDBT in southern Idaho in 2005 and 2006. In the WRBT across five environments, the average yield of Shoshone was 1852 lbs A⁻¹ compared with 1848 lbs A⁻¹ for Bill Z in 2006. In the CDBN across 10 locations in the U.S. and Canada in 2006, Shoshone yielded 2607 lbs A⁻¹ compared with 2439 lbs A⁻¹ of Othello.

Seed Weight. Mean weight of the 100 seeds of Shoshone was 36 g compared with 34 g for Bill Z in the IDBT in Idaho in 2005 and 2006. In the WRBT in 2006, the respective values for Shoshone and Bill Z were 36 g and 35 g. In the CDBN across 10 environments in 2006, 100 seeds of Shoshone weighed 36 g which was similar to that of Othello.

Seed Status. Breeder and Foundation seed of Shoshone will be maintained by the Idaho Foundation Seed Program under the direction of the Idaho Agricultural Experiment Station, University of Idaho, Moscow, ID 83844. However, a small quantity of seed of Shoshone for research purposes is available from S. Singh for the first five years. Appropriate acknowledgement of its developers and the University of Idaho for the use of Shoshone as germplasm would be highly appreciated. The PVP for Shoshone is pending.

Acknowledgement. We thank David Webster and Carl Strausbaugh for the help with some disease evaluations; to Phillip Miklas, Howard Schwartz, Mark Brick, and Carlos Urrea for evaluations in the WRBT; and to all participants of the CDBN for data and collaboration. Financial support from the Idaho Bean Commission and the College of Agriculture and Life Sciences, University of Idaho are gratefully acknowledged.

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RELEASE OF COMMON BEAN GERMPLASM LINE HR67

Soon J. Park*, Kangfu Yu, Shuyu Liu and Terry Rupert
Agriculture and Agri-Food Canada, Greenhouse and Processing Crops Research Centre, Harrow,
Ontario, Canada N0R 1G0, *Corresponding author, e-mail: parks@agr.gc.ca

Common bean (*Phaseolus vulgaris* L.) germplasm line HR67 has resistance to common bacterial blight (CBB) [*Xanthomonas campestris* pv. *phaseoli* (Smith) Dowson]. Developed at the Agriculture and Agri-Food Canada Greenhouse and Processing Crops Research Centre (AAFC GPCRC), Harrow, Ontario, Canada, HR67 was selected from cross W1675 between ‘Centralia’ and H1333-15158, made in 1988. Centralia, a mid season navy bean cultivar, was used for its good agronomic characteristics, including high yield, indeterminate growth habit, and resistance to bean common mosaic virus races 1 and 15 and to anthracnose races 17 and 23. H1333-15158 is an F₃ navy bean line with resistance to CBB selected from the cross HR13-621//OAC Rico/XAN159, made in 1986. CBB resistance is derived from germplasm line XAN 159.

The F₁ of cross W1675 were grown in the greenhouse in the fall of 1988. F₂ plants were advanced in the spring greenhouse at Harrow in 1989. Then, F₃ plant rows were grown in a CBB nursery in 1989 and a line selected and further advanced in the CBB nursery in 1990 and 1991. CBB resistance was confirmed in 1992 spring greenhouse test. The bulked line (W1675-56455) was selected for its medium maturity, upright plants with semi-determinate growth habit (II^a), high yield potential, and resistance to blight.

The line W1675-56455 was tested in a preliminary yield trial in blight nursery in 1992 and advanced performance trial in the blight nursery in 1993. The common bacterial blight nursery was established by growing breeding lines surrounded by CBB susceptible and resistant check lines and inoculated by high pressure sprayer with an inoculum prepared with a mixture of local isolates of *X. phaseoli* (Park and Dhanvantari, 1987).

It was tested as HR67-1675 for registration in the Ontario cooperative white bean cultivar registration trials in 1994 by the Ontario Pulse Committee. The trials were conducted at 8 sites in SW Ontario. HR67 was canned to evaluate processing quality at GPCRC quality lab, Harrow. The tests were taste panel evaluation for appearance, flavour and texture, and canned bean colour by Hunter Labscan colourimeter. Also it was tested for hydration coefficients and percent solid/drain weight, and texture of the canned beans measured by the Instron texture measurement system for firmness in newton (N mm⁻¹) and plateau force in N. (Voisey 1971). Tests for resistance to anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) and bean common mosaic virus (BCMV) were conducted under controlled conditions in the growth cabinet by artificial inoculation at GPCRC Screening for common bacterial blight [*Xanthomonas campestris* pv. *phaseoli* (Smith) Dowson] resistance was conducted by artificial inoculation in growth room (by multiple needle technique) and also field nurseries (high pressure sprayer inoculation).

HR67 has an average yield potential with medium maturity in southwestern Ontario. It yielded 2357 kg ha⁻¹ which was less than the average of four check cultivars, ‘Centralia’ ‘Dresden’, ‘Schooner’, and ‘Midland’ with an average yield of 2535 kg ha⁻¹ in 8 trials in 1994. It matured

about a day later (100 d) than the checks. HR67 has slightly larger seed mass (20.8 g 100 sds⁻¹) than the checks (18.7 g).

HR67 has acceptable cooking and canning quality (with organoleptic test score of 8.4) in comparison with six checks (8.4 with a range of 8.0-9.2), 'OAC Seaforth', 'Envoy', 'Mitchell', 'Dresden', 'Midland' and 'OAC Gryphon' and it has better canning quality than Envoy. Its hydration coefficient and washed/drain weight were 1.92 and 59.1 %, respectively, very similar to the check averages. HR67 has fairly firm texture, similar to the checks.

HR67 is resistant to bean common mosaic virus race 1 and 15, and to anthracnose races 17 (α) and 23 (δ), but susceptible to race 89 (α B). It is tolerant to white mould caused by *Sclerotinia sclerotiorum*, probably due to a tolerant parental line, OAC Rico. It is highly resistant to common bacterial blight similarly to CBB resistant germplasm line HR45 (Park and Dhanvantari, 1994). HR67 has been used as a parental source for CBB resistance and also for molecular studies. The major resistance QTL in HR67 is tightly linked to UBC 420 SCAR and it also carries SW13 marker for BCMV resistance 'I' gene. Other molecular markers tightly linked to the major CBB resistance QTL, PV-tttc001, STS333, STS183, may be used for transferring the CBB resistance gene effectively through molecular marker assisted selection technique. A BAC library of HR67 is also available.

HR67 has green hypocotyls and white flowers. Pods are light tan coloured with absence/slight ventral curvature and short straight beaks when ripe. Seeds are oval shaped with dull seed coat lustre with white hilum. It has semi-determinate growth habit (II^a) of upright plant type with short vine and is also suited for narrow bean production and direct combine harvest.

Stock seed will be maintained by the Agriculture and Agri-Food Canada Greenhouse & Processing Crops Research Centre, Harrow, Ontario N0R 1G0. Small quantities of seed for research purpose may be obtained upon request from the corresponding author.

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SUBJECT MATTER INDEX - Volume 50

Acutifolius sp.....	9, 11, 13
Angular Leaf Spot	91, 93, 95, 97, 99, 101, 103
Anthracnose	85, 87, 89, 99, 103
Breeding, Recurrent Selection	29, 105, 107, 155
Coccineus sp.....	7, 135
Common Bacterial Blight, Xanthomonas.....	105, 107, 109, 111, 113, 115, 117
Cooking, Canning, Processing Quality	31, 33, 35, 37, 39, 41
Drought, Water Stress	153, 157, 159, 161, 163, 171
Economics	199
Embryogenesis	15
Fertility, Fertilization, Green Manure, Growth Regulators, Rhizobium	43, 65, 141, 147, 165, 167, 179, 181, 183
Gene Flow	3
Genetics	25
Germplasm, Diversity, Core Collection	19, 21, 27
Interspecifics.....	1, 5, 7, 9
Irrigation	197
Markers & Marker-Assisted-Selection	17, 71, 73, 75, 77, 109, 111
Models	185
Nematodes.....	83
Nutrition, Phenolics, Phytates, Micronutrients.....	43, 45, 47, 49, 51, 53
Organic	145
Participatory, Farmer Adoption	187, 189, 201, 203
Phaseolin.....	69, 71
Phytophthora.....	143
Roots	149, 151
Roots Rots	139, 141, 145
Rust & Soybean Rust	103, 119, 121, 123, 125, 127
Salinity.....	11, 13
Seed Characteristics, Green Shell	55, 57, 59, 61
Seed Quality	113
Snap Beans	63, 65, 67, 79, 145, 175, 177
Temperature Stress	199
Varietal Testing & Releases.....	191, 193, 195, 205, 207, 209, 211, 213, 215, 217, 219, 221
Viruses	77, 79, 81
Weed Control.....	169, 173, 175
White Mold, Sclerotinia	129, 131, 133, 135, 137
Wild Species.....	1, 3, 23, 69

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George Abawi
Dept. of Plant Pathology
NYSAES Cornell University
630 W. North St.
Geneva, NY 14456
Phone 315-787-2374
Fax: 315-787-2389
E-mail: gsa1@cornell.edu

Jorge A. Acosta Gallegos
INIFAP, Campo Exp. Bajio
Km. 6.5 Carr. Celaya-San Miguel
Allende
CP 38010 Celaya, GTO MEXICO
Phone 461-661-5023 ext. 164
Fax: 461-661-5431
E-mail: jamk@prodigy.net.mx

M.W. Adams
5607 Colby Rd.
Crystal, MI 48818
Phone 989-235-6561
Fax: 989-235-6561

ADM –Edible Bean Specialties,
Inc.
PO Box 117
Huston, ID 83630

African Cntr. Crop Improvement
University of Kwazulu-Natal
P Bag X01, Scottsville 3209
Kwa Zulu Natal SOUTH AFRICA
Phone 27-33-2605274
Fax: 27-33-2605580

AG BIOTECH INC.
PO Box 1325
San Juan Bautista, CA 95045
Phone 831-635-0206
Fax: 831-635-0572
E-mail: info@agbiotech.net

AGRO–HARIBEC Inc.
919 Route 239
St. Aime, Quebec
J0G 1K0 CANADA
Phone 450-788-2196
Fax: 450-788-2709
E-mail: Pierre@haribec.com

Homero Aidar
EMBRPA Arroz e Feijao
C. Postal 179
75375-000 Santo Antonio deGoias, GO
BRAZIL

Cathie Aime
USDA, ARS, SBML
Rm 319, B011A
10300 Baltimore Ave.
Beltsville, MD 20705
Phone 301-504-5758
E-mail: Cathie@nt.ars-grin.gov

Richard F. Allison
Dept. of Plant Biology
Michigan State University
East Lansing, MI 48824-1312
Phone 517-432-1548
Fax: 517-353-1926
E-mail: allison@msu.edu

Antonio Alvarez Tortosa
Seminis Vegetable Seeds Iberica
PO Box 175
04700 El Ejido Almeria SPAIN
Phone 34950580012
Fax: 34950581162

Maurilio Alves Moreira
Instituto de Biotecnologia Aplicada a
Agropecuaria da UFV
Vicoso, M. G.
36571-000 BRAZIL
Phone 55-31-3855-2977
Fax: 55-31-3855-2864
E-mail: moreira@ufv.br

Ana Lilia Alzate Marin
Rue Guatambu 626
Bairro Jardim Recreio
CEP 14040-160 Ribeirao Preto
Sao Paulo BRAZIL
Phone 55-16630-8556
E-mail: anaalzate@rge.fmrp.usp.br

Manuel Amané
Coordinacao, Programa Feijao
I.N.I.A.
C.P. 3658 Mavalane,
Maputo MOZAMBIQUE

Warren Anderson
Campus Box 5, AgriBus & AgriSci
Middle Tennessee State University
Murfreesboro, TN 37132
Phone 615-898-2408
Fax: 615-898-5169
E-mail: wanderso@mtsu.edu

Steve Antonius
600 N. 15th St.
Rochelle, IL 61068
Phone 815-562-1345
Fax: 815-562-1319
E-mail:
Steve.antonius@delmonte.com

Carlos Manuel Araya F.
Univ. Nacional/Escuela de Ciencias
Agrarias, Lab. De Fitopatologia
Apartado 86-3000, Heredia
COSTA RICA
Phone 506-277-3301
Fax: 506-261-0035
E-mail: caraya@una.ac.cr

Pedro Antonio Arraes Pereira
5601 Sunnyside Ave.
Room 4-1193
Beltsville, MD 20705-5741
Phone 301-504-4556
Fax: 301-504-4528
E-mail: paa@ars.usda.gov

J. W. Aylesworth
Box 98
Woodslee, Ontario
N0R 1V0 CANADA
Phone 519-975-2557
Fax: 519-975-1045

Parthiba M. Balasubramanian
Agriculture & Agri- Food Canada
Morden Res. Stat.
Unit 100 – 101 Route 100
Morden, MB R6M 1Y5 CANADA
Phone 204-822-7229
Fax: 204-822-7207
E-mail: parthibab@agr.gc.ca

Pedro Alberto Balatti
Inst. de Fisiologia Vegetal
Universidad Nacional de La Plata
CC327 LaPlata CP 1900 ARGENTINA
Phone 54-221-4236618
Fax: 54-221-4233698
E-mail: pbalatti@ceres.agro.unlp.edu.ar

Jim Ballerstein
NYSAES Dept. of Hort. Sci.
PO Box 462
Hedrick Hall
Geneva, NY 14456-0462
Phone 315-787-2223
Fax: 315-787-2216
E:mail: jwb2@cornell.edu

Mark J. Bassett
2900 NW 32nd St.
Gainesville, FL 32605
Phone 352-374-8264
mark@righteousindignation.com

Messias Jose Bastos de Andrade
Departamento de Agricultura
Univ. Federal de Lavras
CP 37, CEP 37200-000
Lavras-MG BRAZIL
Phone 35-3829-1327
Fax: 35-3829-1301
E:mail: mandrade@ufla.br

Jean-Pierre Baudoin
Faculté Univ. des Sciences Agron.
Unité de Phytotechnie tropicale et d'Hort
Passage des Déportés
2 à B.5030 Gembloux BELGIUM
Phone 32081-622112
Fax: 32081-614544
E:mail: baudoin.jp@fsagx.ac.be

Jeannette Sofia Bayuelo-Jimenez
Inst. de Investigaciones Agro. Y For.
Univ. Michoacana de San Nicolas de
Hidalgo, Km. 9.5 Carretera Morelia-
Zinapécuaro, Tarimbaro, Michoacan CP
58880 MEXICO
Phone 54-443-2958323
Fax: 52-443-2958324
E:mail: Jsbayuelo2002@aol.com

Priscila Bazin
EMBRPA Arroz e Feijao
C. Postal 179
75375-000 Santo Antonio deGoias, GO
BRAZIL

Bean Coordinator
EARO Melkassa Research Center
P. O. Box 436
Nazreth, ETHIOPIA
Phone 251-2-112186
Fax: 251-2-113777

Bean Research Group
Awassa Research Center
P. O. Box 6
Awassa, ETHIOPIA

Bean Research Group
KARI-Katumani
Dryland Farming Res Sta
P. O. Box 340
Machakos, KENYA

Bean Research Group
KARI Reg. Research Centre
P. O. Box 27
Embu KENYA

Bean Research Group
KARI Reg. Research Centre
P. O. Box 169
Kakamega, KENYA

Bean Research Group
Min. of Agric. Research and Training Inst.
MARTI Uyole
P. O. Box 400
Mbeya TANZANIA

Bean Research Group, Director of Research
Alemaya Univ. of Agriculture
PO Box 138
Dire Dawa ETHIOPIA

James S. Beaver
Dept. of Agronomy & Soils
Univ. of Puerto Rico, Mayaguez
PO Box 9030
Mayaguez, PR 00681-9030
Phone 787-832-4040/2566
Fax: 787-265-0220
E:mail: j_beaver@hotmail.com

Steve Beebe
CIAT
7343 NW 79th Terrace
Medley, FL 33166-2211
Phone 650-833-6625
Fax: 650-833-6626
E:mail: s.beebe@cgiar.org

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Maurice R. Bennink
FSHN, MSU
106F Trout Food Sci. Bldg
East Lansing, MI 48824-1224
Phone 517-355-8474 ext 103
Fax: 571-353-8963
E:mail: mbennink@msu.edu

Rick Bernsten
411 E Agriculture Hall
MSU
East Lansing, MI 48824
Phone 517-355-3449
Fax: 517-432-1800
E:mail: bernsten@msu.edu

Matgorzata. Berova
Agricultural University
Menolelev St. 12
4000 Plovoliv BULGARIA
Phone 359326126
Fax: 35932832004

Kirstin Bett
Dept. of Plant Sciences
University of Saskatchewan
51 Campus Dr.
Saskatoon, SK S7N 5A8 CANADA
Phone 306-966-4947
Fax: 306-966-5015
E:mail: k.bett@usask.ca

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Matthew W Blair
CIAT-Intl. Center for Tropical Agric.
7343 NW 79th Terrace
Medley, FL 33166-2211
Phone 650-833-6625
Fax: 650-833-6626
E-mail: m.blair@cgiar.org

Fred A. Bliss
Seminis Vegetable Seeds
37437 Highway 16
Woodland, CA 95695
Phone 530-669-6154
Fax: 530-666-6791
E-mail: fred.bliss@seminis.com

Roberto Bollini
Istituto Biologia e Biotechnologie, CNR
Via Bassini 15
20133 Milano, ITALY
Phone 390223699435
Fax: 390223699411

Lech Boros
Dept. of Seed Science & Technology
Plant Breeding Institute
05-870 Blonie POLAND
E-mail: l.boros@ihar.edu.pl

Lluís Bosch
CEIB-ESAB
Urgell, 187
Barcelona 08036 SPAIN
Phone 34-934137553
Fax: 34-934137501
E-mail: lluis.bosch-roura@upc.es

Andrea Brady, ADM
861 Sandcherry St.
London, Ontario N6H 5V1 CANADA
Phone 519-657-9064
Fax: 519-657-3837
Email: Andrea_brady@admworld.com

Mark A. Brick
Dept. of Soil & Crop Sciences
Colorado State University
Fort Collins, CO 80524
Phone 970-491-6551
Fax: 970-491-0567
E-mail: mbrick@lamar.colostate.edu

Osmar Rodrigues Brito
Universidade Estadual de Londrina
Dept de Agronomia
Londrina, Parana 86051-970 BRAZIL
Phone 552143371-4555
Fax: 552143371-4697
E-mail: osmar@uel.br

Jan Bert Brouwer
PO Box 910
Horsham,
Victoria 3402 Australia
Phone 61353846293
E-mail: janbertb@wimmera.com.au

Steve Brown
Jack's Bean Company LLC
402 N. Interocean, Box 327
Holyoke, CO 80734
Phone 970-854-3702
Fax: 970-854-3707
E-mail: steve@jacksbean.com

Louis Butare
Chief, Programme Legumineuses
ISAR Rubona, B.P. 138
Butare RWANDA

Bruno Campion
C.R.A. Inst. Sperimentale Per L'Orticoltura
via Paullese 28, 26836 Montanaso
Lombardo Lodi ITALY
Phone 39-0371-68171
Fax: 39-0371-68172
E-mail: bruno.campion@virgilio.it

Francesc Casanas Artigas
Escola Superior Agricultura Barcelons
Campus de Baix Llobregat
Avinguda del Canal Olímpic s.n.
08860 Castelldefels SPAIN
Phone 34-935521215
Fax: 34-935521001
E-mail: francesc.Casanas@upc.edu

Oscar Checa
CIAT
C/O M. Blair
7343 NW 79th Terrace
Medley, FL 33166-2211
E-mail: ochk@latinmail.com

Chief., Programme Legumineuses
FOFIFA
B.P. 1444 Ambatobe
Antananarivo 101
MADAGASCAR

Chief
Programme Haricot
ISABU-Mosso, BP 795
Bujumbura, BURUNDI

Dr Rowland Chirwa
Coordinator, SABRN
Chitedze Res. Stat.
P. O. Box 158
Lilongwe, MALAWI
Phone 781-182-76722
Fax: 781-182-782835

CIAT PAN-AFRICA Coordinator
Kwanda Agric. Research. Inst.
P. O. Box 6247
Kampala, UGANDA
Phone 256-41-567670
Fax: 256-41-567635

CIAT Regional Bean Programme
PO Box 2704
Arusha TANZANIA

Karen Cichy
Michigan State University
Plant & Soil Science Bldg
E. Lansing, MI 48824
Phone 517-355-6884
Fax: 517-337-6782
E-mail: cichykar@msu.edu

Robert B. Colville
1127 Westview Drive
Rochelle, IL 61068-1205
Phone 815-562-2980
E:mail: rbcysc@rochelle.net

Robert L. Conner
Morden Research Station
Unit 100 - 101 Route 100
Morden, Manitoba R6M 1Y5 CANADA
Phone 204-822-7221
Fax: 204-822-7207

Coordinator
Agric. Techn. Dev. Transfer
PO Box 255
Butare RWANDA

Coordinator, Bean Programme
KARI Hort Research Station
P. O. Box 220
Thika, KENYA

Jeremy Davidson
32 Blind Creek Rd.
R.D. 3
Blenheim NEW ZEALAND
Phone 03-570-5382
E:mail: Davidson.j@xtra.co.nz

Carlos Alberto De Bastos Andrade
Universidade Estadual de Maringa
Bairro Jardim Universitario
Av. Colombo 5790
CEP 87020-900
Maringa, PR BRAZIL
Phone 55442614407
E:mail: cabandrade@uem.br

Itamar Pereira de Oliveira
Embrapa Arroz e Feijao
Caixa postal 179
75375-000 San Antônio de Goiás/GO
BRAZIL
Phone 55-62-833-2181
Fax: 55-62-833-2100
E:mail: itamar@cnpaf.embrapa.br

Trazilbo Jose De Paula, Jr.
EPAMIG
Vila Gianetti 47
Vicoso, MG 36570-000 BRAZIL
Phone 55-313891-2646
Fax: 55-313899-5224

Antonio M. De Ron
Dept. of Plant Genetic Resources
PO Box 28
36080 Pontevedra SPAIN
Phone 34-986-854800
Fax: 34-986-841362
E:mail: amderon@mbg.cesga.es

Leslie L. Dean
Idaho Seed Bean Co., Inc.
P. O. Box 1072
Twin Falls, ID 83303-1072
Phone 208-734-5221
Fax: 208-733-1984
E:mail: LLBDean@mindspring.com

Daniel G. Debouck
CIAT
7343 NW 79th Terrace
Medley, FL 33166-2211
Phone 415-833-6625
Fax: 415-833-8826
E:mail: d.debouck@cgiar.org

Maria Jose del Peloso
EMBRAPA Arroz E Feijao
C. P. 179 75 375-000 Santo Antonio De Goias
BRAZIL
Phone 55-62-3533-2158
Fax: 55-62-3533-2100
E:mail: mjpeloso@cnpaf.embrapa.br

Luis del Rio
Plant Pathology, NDSU
306 Walster Hall
Fargo, ND 58105
Phone 701-231-7073
Fax: 701-231-7851
E:mail: Luis.delRio-Mendoza@ndsu.nodak.edu

Alfonso Delgado-Salinas
Inst de Biologia, UNAM
Herbario Nac de Mexico, Apdo Pos 70-367
04510 Mexico, DF MEXICO
Phone 525-5424-3867
adelgado@servidor.unam.mx

Patricia DeMark
ADM-JRRRC
1001 N Bruch College Rd
Decatur, IL 62521
Phone 217-451-2060
Fax: 217-451-2457
E:mail: Patricia_DeMark@admworld.com

Brett Despain
ADM -Edible Bean Specialties, Inc.
PO Box 117
Huston, ID 83630
Phone 208-455-7728
Fax: 208-459-3370
E:mail: brett_despain@admworld.com

Lucy Diaz
CIAT
Intl. Center for Tropical Agric.
7343 NW 79Th Terrace
Medley, FL 33166-2211
E:mail: l.diaz@cgiar.org

Michael H. Dickson
77 White Springs Lane
Geneva, NY 14456
Phone 315-789-1996
E:mail: mandjdickson@aol.com

Dobroudja Agricultural Institute
Biblioteka
9520 GENERAL TOCHEVO
BULGARIA
Phone 359-58-879234
Fax: 359-5731-4448

Kirk Dolan
Food Science & Human Nutrition
Michigan State University
135 Trout Bldg.
East Lansing, MI 48824
Phone 517-355-8474 X119
Fax: 517-353-8963
E:mail: dolank@msu.edu

Robert Duncan
Plant Pathology
UC-Davis
One Shields Ave
Davis, CA 95616
Phone 530-752-8321
Fax: 530-752-5674
E:mail: rwduncan@ucdavis.edu

Shigehiko Ebe
Kitami Agri. Expt. Station
52 Yayoi
Kunneppu-cho, , Hokkaido 099-1406
JAPAN
Phone 81-157-47-2146
Fax: 81-157-47-2774
E:mail: ebesg@agri.pref.hokkaido.jp

J. Alberto Escalante Estrada
Especialidad de Botanica
IRENAT
Montecillo, Mex 56230 MEXICO
Phone 595-2-0247
Fax: 595-20247
E:mail: jasee@colpos.mx

Estacion Experimental Agropecuaria Salta
INTA
Maria Elisa Maggio
Casilla de Correos 228, Salta 4400
ARGENTINA
Phone 54-387 4902214
Fax: interno 212/E:mail:
memaggio@correo.inta.gov.ar

Kathryne Everts
27664 Nanticoke Rd.
Salisbury, MD 21801
Phone 410-742-1178
Fax: 410-742-1922
E:mail: keverts@umd.edu

Carlos Esteban Falconi
Mimbela n23-151 y La Gasca
Quito ECUADOR

Luis Claudio Faria
EMBRPA Arroz e Feijao
C. Postal 179
75375-000 Santo Antonio deGoias, GO
BRAZIL
E:mail: lcfaria@cnpaf.embrapa.br

Juan Jose Ferreira Fernandez
SERIDA Apdo 13
Villaviciosa, Asturias SPAIN
Phone 34-85890066
Fax: 34-85891854
E:mail: jjferreira@serida.org

Iraja Ferreira Antunes
EMBRAPA / CPACT
Caixa Postal 403
96001-970 Pelotas, RS, BRAZIL
Phone 53-275-8434
Fax: 53-275-8412
E:mail: iraja@cpact.embrapa.br

Deidre Fourie
ARC-Grain Crops Institute
Private Bag X1251
Potchefstrom 2520 REPUBLIC OF SOUTH
AFRICA
Phone 27-18-299-6312
Fax: 27-18-297-6572
E:mail: FourieD@arc.agric.za

Gary D. Franc
College of Agric.-Plant Sciences
1000 E. University Ave, Dept. 3354
University of Wyoming
Laramie, WY 82071
Phone 307-766-2397
Fax: 307-766-5549
E:mail: francg@uwyo.edu

George F. Freytag
3808 Crescent Drive
Fort Collins, CO 80526
Phone 970-223-2189

Incoronata Galasso
CNR Instituto di Biologia e Biotecnologia
Agraria,
Via Bassini15
20133 Milan ITALY
Phone 39-0223699435
Fax: 39-0223699411
E:mail: galasso@ibba.cnr.it

Nadejda Ganeva
IZK "Maritza" - Biblioteka
UL, "Brezovsko Shosse" 32, KL 3
PK 20, 4003 Plovdiv BULGARIA

Gautier Semences SAS
Rte d' Avignon
B. P. No. 1
13630 Eyragues FRANCE
Phone 04-90-240-270
Fax: 04-90-240-271

Robert J. Gehin
Harris Moran Seed Co.
1677 Muller
Sun Prairie, WI 53590
Phone 608-837-6574
Fax: 608-837-3758

Jean Robert Gelin
Plant Sci. Dept.
NDSU
360B Loftsgard Hall
Fargo, ND 58105-5051
Phone 701-231-8148
Fax: 701-231-8474
E:mail: Jean.gelin@ndsu.nodak.edu

Dimitar Genchev
Dobroudja Agricultural Institute
9520
General Tochevo BULGARIA
E:mail: genchev@dai-gt.dobrich.net

Paul Gepts
Dept. of Plant Sciences/MSI
Crop & Ecosystem Sciences
One Shields Avenue
University of California
Davis, CA 95616-8780
Phone 530-752-7743
Fax: 530-752-4361
E:mail: plgepts@ucdavis.edu

Robert L. Gilbertson
Dept. of Plant Pathology
University of California
1 Shields Ave
Davis, CA 95616
Phone 916-752-3163
Fax: 916-752-5674
E:mail: rlgilbertson@ucdavis.edu

Chris Gillard
Ridgetown College/Univ. of Guelph
120 Main St., E.
Ridgetown, ON N0P 2C0
CANADA
Phone 519-694-1632
Fax: 519-674-1600/ E:mail:
cgillard@ridgetownc.uoguelph.ca

Ramon Giraldez
Dept. de Biologia Funcional
Universidad de Oviedo
33006 Oviedo SPAIN
Phone 34-985103594
Fax: 34-985103534
E:mail: giraldez@uniovi.es

Graciela Godoy-Lutz
406 Plant Science
Department of Plant Pathology
University of Nebraska
Lincoln, NE 68583-0722
Phone 402-472-5759
Fax: 402-472-2853
E:mail: ggodoy@unlnotes.unl.edu

Everaldo Goncalves de Barros
BIOAGRO . UFV
36571-000
Vicosa M. G., BRAZIL
Phone 55-31-899-2151
Fax: 55-31-899-2864
E:mail: ebarros@ufv.br

Maria Celeste Goncalves Vidigal
Av. Colombo 5790-cep:87020-900
Univ. Estadual de Maringa
Maringa, Parana
87020-900 BRAZIL
Phone 442635036
Fax: 442615599
E:mail: mcgvidigal@uem.br

Kenneth F. Grafton
Department of Plant Sciences
North Dakota State University
Fargo, ND 58105
Phone 701-231-8145
Fax: 701-231-8520
E:mail: k.grafton@ndsu.nodak.edu

Phillip Griffiths
NYSAES
302 Hedrick Hall
Geneva, NY 14456
Phone 315-787-2222
Fax: 315-787-2216
E:mail: pdg8@cornell.edu

Robert Hall
Dept. of Environmental Biology
University of Guelph
Guelph, Ontario N1G 2W1 CANADA
Phone 519-824-4120
Fax: 519-837-0442
E:mail: rhall@uoguelph.ca

Donald Halseth
15D Plant Sci Bldg
Cornell University
Ithaca, NY 14853-0327
Phone 607-255-5460
Fax: 607-255-9998
E:mail: deh3@cornell.edu

An N. Hang
Washington State University
24106 N Bunn Rd
Prosser, WA 99350
Phone 509-786-9201
Fax: 509-786-9370
E:mail:

Richard M. Hannan
Plant Introduction, USDA
Rm. 59, Johnson Hall, WSU
Pullman, WA 99164
Phone 509-335-3683
Fax: 509-335-6654
E:mail: hannan@wsunix.wsu.edu

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Bob Henson
Carrington Research Extension Center
PO Box 219
Carrington, ND 58421
Phone 701-652-2951
Fax: 701-652-2055/ E:mail:
bhenson@ndsuxext.nodak.edu

Sanjuana Hernandez Delgado
Centro de Biotechnologia Genomica-IPN
Blvd. Del Maestro esq. Elias Pina s/n Col.
Narcisco Mendoza
88710 Reynosa
Tamaulipa MEXICO

David Holland
Washington State University
Dept. of Agric. & Resource
Economics
Box 646210
Pullman, WA 99164-6210

Gerrit Hoogenboom
Dept. of Biological & Agric.Eng.
University of Georgia
Griffin GA 30223
Phone 770-229-3438
Fax: 770-228-7218
E:mail: gerrit@uga.edu

George L. Hosfield
208 Artists Alley
Blowing Rock, NC 28605
Phone 828-295-6727
E:mail: georgelouis@earthlink.net

Khwaja Hossain
Division of Science & Math
Mayville State University
330 3rd Street, NE
Mayville, ND 58257
Phone 701-788-4728
E:mail: k_hossain@mayvillestate.edu

Francisco J. Ibarra-Perez
INIFAP-pfocomas Centrales
Av. Progreso No. 5 Col. Del Carmen, Del.
Coyoacan, D.F. 04110 MEXICO
Phone 52-55-5484-1967
E:mail: Ibarra.francisco@inifap.gob.mx

Inst. for Wheat & Sunflower
"Dobroudja" - Biblioteka
Near General Toshevo
BULGARIA

Vanjak-Kvaic Institute of Field &
Vegetable Crops
Maksima Gorkog 30
21000 Novi Sad SERBIA AND
MONTENEGRO
Phone 381-21-4898142
Fax: 381-21-624212
E:mail: tanja@ifvcns.ns.ac.yu

Instituto di Virologia Vegetale
Strada delle Cacce 73
10135 Torino ITALY
Phone 011-348-99-51

Carmen Jacinto-Hernandez
Apartado Postal 10
56230 Chapingo, Mex, MEXICO
Phone 595-4-2877
Fax: 595-4-6528

Antony Jarvie
PANNAR Research Services Pty
(LTD), Box 19
Greytown 3250 REPUBLIC OF
SOUTH AFRICA
Phone 033 4131131
Fax: 033 4171208
E:mail: antony.jarvie@pannar.co.za

Frans J. Jongeleen
SVS Holland BV
JPO Box 22
1600 AA Enkhuizen NETHERLANDS
Phone 0220-357000
Fax: 0220-357035

Yu Kangfu
Agriculture and Agri-Food Canada
Greenhouse and Processing Crops Research
Centre
Harrow, Ont. N0R 1G0 CANADA
Phone 519-738-2251 ext. 479
Fax: 519-738-2929
E:mail: yuk@agr.gc.ca

Lawrence Kaplan
26 Parker St
Newton Centre, MA 02459
Phone 617-527-3449
Fax:
E:mail: lawrence.kaplan@umb.edu

Ed Kee
16483 County Seat Hwy.
Georgetown, DE 19947
Phone 302-856-7303
Fax: 302-856-1845
E:mail: kee@udel.edu

John Keenan
5202 Fawn
Farmington, NM 87402
Phone 505-327-4219
Fax: 505-327-4219
E:mail: jgkeenan@sisna.com

James D. Kelly
Dept. of Crop & Soil Sciences
Michigan State University
East Lansing, MI 48824
Phone 517-355-0271 x1181
Fax: 517-353-3955
E:mail: kellyj@msu.edu

Paul M. Kimani
Dept of Crop Science-Kabete
Univ. of Nairobi
P. O. Box 30197
Nairobi, KENYA
E:mail: kimanipm@nbnet.co.ke

Joao Kluthcouski
EMBRPA Arroz e Feijao
C. Postal 179
75375-000 Santo Antonio deGoias, GO
BRAZIL

Ken Kmiecik
7202 Portage Rd.
DeForest, WI 53532
Phone 608-842-1411
Fax: 608-846-7892
E:mail: ken.kmiecik@seminis.com

Josué Kohashi Shibata
Centro de Botanica, Col. de Postgr.
Montecillo, Edo. de Mexico
C.P. 56230 MEXICO
Phone 1-595-9520200 ext 1318
Fax: 1-595-9520247
E:mail: jkohashi@colpos.mx

Chet Kurowski
Harris Moran Seed Company
9241 Mace Blvd
Davis, CA 95616
Phone 530-747-3216
Fax: 530-756-1016
E:mail: c.kurowski@harrismoran.com

Richard Larsen
USDA ARS
24106 N. Bunn Rd
Prosser, WA 99350
Phone 509-786-9259
Fax: 509-786-9277
E:mail: rlarsen@tricity.wsu.edu

Colin Leakey
The Close, 15 Cambridge Road
Girton
Cambridge CB3 0PN ENGLAND
Phone 1223-276866
Fax: 1223-565215
E:mail:

Margarita Lema-Marquez
Kimberly Research & Extension Center
University of Idaho
3793 North 3600 East
Kimberly, ID 83341-5076
Phone 208-423-6624
Fax: 208-423-6637
E:mail: mlema@kimberly.uidaho.edu

Librarian
CSIRO Livestock Industries
Queensland Biosci. Precinct
306 Carmody Rd.
St. Lucia QLD 4067 AUSTRALIA
Phone 6173214-2555
Fax: 6173214-2911
E:mail: qstcp@csiro.au

Library/Bibliotheque
Agriculture & Agri-Food Canada
Edifice Sir John Carling Building
Ottawa, ON K1A 0C5 CANADA

A.J. Liebenberg
ARC-Grain Crops Institute
Private Bag X1251
Potchefstroom 2520 REPUBLIC OF SOUTH
AFRICA
Phone 27-18-299-6326
Fax: 27-18-297-6572
E:mail: LiebenbergA@arc.agric.za

Merion M. Liebenberg
ARC-Grain Crops Institute
P. Bag X1251
Potchefstroom 2520 REPUBLIC OF
SOUTH AFRICA
Phone 27-18-299-6311
Fax: 27-18-297-6572
E:mail: LiebenbergM@arc.agric.za

Dale T. Lindgren
461 W University Dr.
University of Nebraska - West Central
Center
North Platte, NE 69101
Phone 308-696-6706
Fax: 308-696-6780
E:mail: dlindgren1@unl.edu

Lucia Lioi
Istituto Di Genetica Vegetale-CNR
Via Amendola 165/A
70126 Bari, ITALY
Phone 39080-5583400
Fax: 39080-5587566
E:mail: Lucia.lioi@igv.cnr.it

Richard Lowe
Pure Line Seeds, Inc.
P. O. Box 8866
Moscow, ID 83843
Phone 208-882-4422
Fax: 208-882-4326
E:mail: pure@moscow.com

Ted Lund
Brotherton Seed Company
Box 1136
Moses Lake, WA 98837
Phone 509-765-1816
Fax: 509-765-1817
E:mail: ted@brothertonseed.com

Devanand Luthria
Food Composition Lab
Bldg. 161, BARC (East)
Beltsville, MD 20705
Phone 301-504-7247
Fax: 301-504-8314
E:mail: luthriad@ba.ars.usda.gov

Jonathan Lynch
Dept. of Horticulture
Penn State University
221 Tyson Building
University Park, PA 16802-4201
Phone 814-863-2256
Fax: 814-863-6139
E:mail: Jpl4@psu.edu

Robert Mabagala
Tanzania Bean CRSP, Crop Science
Sokoine Univ. of Agr, P.O. Box 3005
Morogoro, TANZANIA
Phone 255-56-3661
Fax: 255-56-4645
E:mail: mabagal@suanet.ac.tz

F. Magayane
Sokine Univ. of Agriculture
Dept. of Crop Sci. & Prod.
Box 3005, Subpost
Morogoro TANZANIA

Steve Magnuson
1509 Stadium Ct
Lehigh Acres, FL 33971
Phone 239-693-5153
239-810-2944
E:mail: d.s.magnuson@att.net

Magrath Library
Serials Dept.
Univ. of Minnesota
1984 Buford Ave
St. Paul, MN 55108-1012

George Mahuku
CIAT
7343 NW 79th Terrace
Medley, FL 33166-2211
Phone 572-445-000
Fax: 572-455-0073
E:mail: g.mahuku@cgiar.org

Roxanne Mainz
Syngenta Seeds, Inc.
317-330th St.
Stanton, MN 55018-4308
Phone 507-663-7612
Fax: 507-645-7519/ E:mail:
roxanne.mainz@syngenta.com

Godwill Makunde
Crop Breeding Institute
PO Box CY 550
Harare ZIMBABWE
E:mail: mgodwill@hotmail.com

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Acquisition Division
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Charles Masangano
Bunda College of Agriculture
Box 219
Lilongwe
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Netzahualcoyotl Mayek Perez
Centro de Biotecnologia Genomica-IPN
Blvd. Del Maestro esq. Elias Pina s/n Col.
Narcisco Mendoza
88710 Reynosa
Tamaulipa MEXICO
Phone 899-9243627
Fax: 899-924-3627
E:mail: nmavekp@hotmail.com

M. Mbikayi Nkonko
Chief D' Antenne
PNL/INERA MULUNGU
BP 327
Changugu RWANDA

Phil McClean
Department of Plant Sciences
North Dakota State University
Fargo, ND 58105-5051
Phone 701-231-8443
Fax: 701-231-8474
E:mail: mcclean@beangenes.
cws.ndsu.nodak.edu

Debra McLaren
Agric & Agri-Food Canada
Brandon Research Center
PO Box 1000A, RR3
Brandon, MB R7A 5Y3 CANADA
Phone 204-578-3561
Fax: 204-728-3858
E:mail: dmclaren@agr.gc.ca

Rex Metzger
Kelley Bean Company
1520 Ave "B"
PO Box 83
Scottsbluff, NE 69361
Phone 308-635-2338
Fax: 308-635-2339
E:mail: rexmetzger@kelleybean.com

Thomas Michaels
Dept. of Horticultural Sci.
University of Minnesota
1970 Folwell Ave.
St. Paul, MN 55108
Phone 612-624-7711
Fax: 612-624-4941
E:mail: michaels@umn.edu

Charlotte M.S. Mienie
ARC-Grain Crops Institute
Private Bag X1251
Potchefstroom 2520 REPUBLIC OF SOUTH
AFRICA
Phone 27-18-299-6315
Fax: 27-18-297-6572
E:mail: MienieC@arc.agric.za

Edison Miglioranza
Universidad Estsadal de Londrina
Depto de Agronomia
Londrina Parana 86051-970 BRAZIL
Phone 43-371-4697
E:mail: emiglor@uel.br

Phil Miklas
USDA, ARS, IAREC, WSU
24106 No. Bunn Road
Prosser, WA 99350-9687
Phone 509-786-9258
Fax: 509-786-9277
E:mail: pmiklas@pars.ars.usda.gov

Carol A Miles
WSU Vancouver REU
1919 NE 78th St
Vancouver, WA 98665-9752
Phone 3605766030
Fax: 3605766032
E:mail: milesc@wsu.edu

El Sadig S. Mohamde
Coordinator, Grain Legumes
Shambat Res. Sta.
P. O. Box 30
Khartoum N.
SUDAN

Kennedy Muimui
Misamfu Regional Research Cntr.
PO Box 410055
Kasama
ZAMBIA

James R. Myers
Dept. of Horticulture, ALS 4017
Oregon State University
Corvallis, OR 97331
Phone 541-737-3083
Fax: 541-737-3479
E:mail: myersja@hort.oregonstate.edu

Rosa Navarrete-Maya
Sur 121 MZ 17 L 14
Col. Juventino Rosas
D. F. 087000 MEXICO
Phone 6505975

Susan Nchimbi-Msolla
Sokine Univ. of Agriculture
Dept. of Crop Sci. & Prod.
Box 3005, Subpost
Morogoro TANZANIA

Dawn S. Neuman
Dept. of Biol Sci, 4505 Maryland Pkwy.
University of Nevada-Las Vegas
Las Vegas, NV 89154-4004
Phone 702-895-3390
E:mail: neuman@ccmail.nevada.edu

James Nienhuis
Dept. of Hort, 1575 Linden Drive
University of Wisconsin
Madison, WI 53706
Phone 608-262-6975
Fax: 608-262-4743
E:mail: nienhuis@wisc.edu

F. Nuez
ETSIA-Dept. Biotechnologia
Camino de Vera 14
46022-Valencia, SPAIN
Phone 34-6-387-74-21
Fax: 34-6-387-74-29

George Nyombaire
Food Science
MSU
129 Food Science Trout Bldg
E. Lansing, MI 48824
Phone 517-355-1261
E:mail: nyombair@msu.edu

Barry Ogg
Dept. of Soil & Crop Sciences
Colorado State University
Fort Collins, CO 80523-1170
Phone 970-491-6354
Fax: 970-491-0564
E:mail: beans@lamar.colostate.edu

Arie Oppelaar
SVS Holland Research Station
Wageningen
Wageningse Afweg 31
6702 PD Wageningen
HOLLAND

Pedro F. Ortega M.
Martires de Cananea 475
Col. Ley 57
Hermosillo, Sonora, 83110
MEXICO
Phone 52-662-261-0074
Fax: 52-662-261-0073
E:mail: ortega.pedro@inifap.gob.mx

Eteban S. Osuna-Ceja
Km. 32.5 Carretera AGS-ZAC
C.P. 20660, A.P. 20
Pabellon de Arteaga, AGS
MEXICO
Phone 46595 80167
Fax: 014659580167
E:mail: esosuna@yahoo.com

J. Saul Padilla-Ramirez
Revolucion 309 Col. Popular
Pabellon de Arteaga CP 20660
Ags MEXICO
Phone 465-958-0167
Fax: 465-958-0186
E:mail: jsaulpr@yahoo.com

James Palmer
Michigan Crop Improvement Assoc.
PO Box 21008
Lansing, MI 48909
Phone 517-332-3546
Fax: 517-332-9301
E:mail: palmerj@michcrop.com

Gloria Palomares
Dept. de Biotech, Univ. Politecnica
Camino de Vera 14
46022 Valencia, SPAIN
Phone 96-387-74-21
Fax: 96-3877429
E:mail: gpaloma@btc.upv.es

Soon O. Park
Texas Agricultural Res. Center
Texas A&M University
2415 East Highway 83
Weslaco, TX 78596-8399
Phone 956-969-5610
Fax: 956-969-5620
E:mail: so-park@tamu.edu

Soon Jai Park
Agriculture & Agri-Food Canada
Greenhouse & Crops Res. Centre
2585 County Rd 20
Harrow, Ontario NOR 1GO CANADA
Phone 519-738-2251
Fax: 519-738-2929
E:mail: parks@agr.gc.ca

Parks Library
Acquisitions Department
Iowa State University
Ames, IA 50011-2140
Phone 515-294-2749
Fax: 515-294-7411

Marcial A Pastor-Corrales
USDA-ARS-Vegetable Lab
10300 Baltimore Ave.
Bldg. 010A Rm. 240 BARC-West
Beltsville, MD 20705
Phone 301-504-6600
Fax: 301-504-5555
E:mail: pastorm@ba.ars.usda.gov

Calvin H. Pearson
Fruita Research Center
1910 L Road
Fruita, CO 81521
Phone 970-858-3629
Fax: 970-858-0461
E:mail: calvin.pearson@colostate.edu

Eduardo Peralta Idrovo
Panamerican Sur km 14
Estacion Expt. Santa Catalina
INIAP
Quito ECUADOR
Phone 59322693360
Fax: 59322693360
E:mail: Legumini@pi.pro.ec

Florence Picard
Ets Vilmorin
49250 La Menitre FRANCE
Phone 02-61-79-41-79
Fax: 02-41-79-41-21
E:mail: florence.picard@vilmorin.com

Vicki J. Pierce
Del Monte Corporation
6580 Furlong Avenue
Gilroy, CA 95020
Phone 408-842-4180
Fax: 408-847-2768

Angela Rosa Piergiovanni
Istituto Di Genetica Vegetale- CNR
Via Amendola 165/A
70126 Bari ITALY
Email:
Angelarosa.piergiovanni@igv.cnr.it

K. N. Pillay
Pannar Research Services Pty LTD
P. O. Box 19
Greytown, 3250 SOUTH AFRICA
Phone 27-33-4139645
Fax: 27-33-4171208
E:mail: kiru.pillay@pannar.co.za

Plant Pathology Library
Univ. of Wisconsin, 584 Russell Lab
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Madison, WI 53706
Phone 608-262-8698
Fax: 608-263-2626
E:mail: sclloyd@library.wisc.edu

Pop Vriend Seeds B.V.
P. O. Box 5
1619 ZG Andijk NETHERLANDS
Phone 31-22859-1462
Fax: 31-22859-3354
E:mail: rcdkroon@popvriendseeds.nl

Tim Porch
USDA ARS SAA TARS
2200 P.A. Campos Ave., Suite 201
Mayaguez, PR 00680
Phone 787-831-3435
Fax: 787-831-3386
E:mail: maytp@ars-grin.gov

Richard Pratt
Horticulture & Crop Science
OSU/OARDC
1680 Madison Ave.
Wooster, OH 44691
Phone 330-263-3972
Fax: 330-263-3887
E:mail: Pratt.3@osu.edu

Emmanuel Prophete
Ministry of Agriculture
PO Box 2363
Port-au-Prince, HAITI
Phone 509-404-2193
Fax:
E:mail: eprophete@hotmail.com

Rosario Provvidenti
115 Ent-Plant Path Bldg.
NYS Agr. Exp. Station
Geneva, NY 14456
Phone 315-787-2316
Fax: 315-787-2389
E:mail: rp13@cornell.edu

Magno Antonio Patto Ramalho
Dept. de Biologia - UFLA
Cx. Pos. 3037
37200-000 Lavras, M.G BRAZIL
Phone 035-829-1352
Fax: 035-829-1341
E:mail: magnoapr@ufla.br

Robert E. Rand
1630 Linden Drive
University of Wisconsin
Madison, WI 53706
Phone 608-262-3269
Fax: 608-263-2626
E:mail: rer@plantpath.wisc.edu

Thomas Randgaard
Faribault Foods Inc.
128 NW 15th St.
Faribault, MN 55021
Phone 507-331-1400
Fax: 507-331-1457
E:mail:
trandgaard@faribaultfoods.com

Jack Rasmussen
Plant Pathology
N.D. State University
306 Walster Hall
Fargo, ND 58105
Phone 701-231-1027
Fax: 701-231-7851
E:mail: Jack.Rasmussen@ndsu.nodak.edu

John Rayapati
JRRRC
1001 Brush College Rd.
Decatur, IL 62521-1656
Phone 217-451-4225
Fax: 217-451-4230
E:mail: rayapati@admworld.com

Ron Riley
Basin Seed Co.
1922 S Middleton Rd
Nampa, ID 83686
Phone 208-461-4656
Fax: 208-461-4439
E:mail: ron.riley@basinseed.com

A Paula Rodino
Dept of Plant Breeding
Carballeira 8-Salcedo
36 Pontevedra SPAIN
Phone 34-986-854800
Fax: 34-986-841362
E:mail: aprodino@mbg.cesga.es

Maria Teresa Rodriguez Gonzalez
Especialidad de Botanica
IRENAT
Montecillo, Mex 56230 MEXICO

Gonzalo Rojas-Cifuentes
Plant Sci. Dept
NDSU
360 B Loftsgard Hall
Fargo, ND 58105-5051
Phone 701-231-8148
Fax: 701-231-8474
E:mail: Gonzalo.rojas@ndsu.nodak.edu

Belinda Roman
Depto. De Biologia
UPR-Cayey
205 Avenida Antonio R. Barcelo
Cayey, PR 00736
Phone 787-517-5579
Fax:
E:mail: romanbelinda@hotmail.com

Rigoberto Rosales Serna
Cerrado del Amanecer #200
Fraccionamiento Real del Country
CP 34140 Durango, Durango MEXICO
Phone
Fax:
E:mail: rigoberto_serna@yahoo.com

Juan Carlos Rosas
EAP/ZAMORANO
P. O. Box 93
Tegucigalpa, HONDURAS
Phone 504-776-6140 ext 2314
Fax: 504-776-6242
E:mail: jcrozas@zamorano.edu

Gerrit Ruiter
Holland-Select B.V.
PO Box 27
1619 ZG
Andijk HOLLAND
Phone 228-291578
Fax: 228-591755
E:mail: info@holland-select.nl

Carmen Asensio Sanchez-Manzanera
SIDTA-Junta de Castilla y Leon
Ctra de Burgos km 118, Apdo. 172
47080 Valladolid SPAIN
Phone 34-983-414461
Fax: 34-983-414780
E:mail: asesanmr@itacyl.es

Marta Santalla
Mision Biologica de Galicia
PO Box 28
36080 Pontevedra SPAIN
Phone 34 -986-854800
Fax: 34-986-841362
E:mail: msantalla@mbg.cesga.es

Aloisio Sartorato
EMBRPA Arroz e Feijao
C. Postal 179
75375-000 Santo Antonio deGoias, GO
BRAZIL
Phone 055-062-8332171
Fax: 055-062-8332100
E:mail: sartorat@cnpaf.embrapa.br

Hitoshi Sato
Tokachi Agri. Expt. Station
S9-2 Shinsei Memuro-Town
Hokkaido 082-0071 JAPAN
Phone 81-155-62-2431
Fax: 81-155-62-0680
E:mail: satohhs@agri.pref.hokkaido.jp

Jim Schild
University of Nebraska
Planhandle Res. & Ext. Center
4502 Ave. I
Scottsbluff, NE 69361-4907
Phone 308-632-1481
Fax: 308-632-1481
E:mail: Jschild1@unl.edu

Roger A. Schmitt
Del Monte Corp. Agr Res Ctr
205 No. Wiget Lane
Walnut Creek, CA 94598
Phone 925-944-7312
Fax: 925-942-0940
E:mail: roger.schmitt@delmonte.com

Eduardo C. Schroder
Dept. of Agronomy & Soils
University of Puerto Rico
Mayaguez, PR 00681-9030
Phone 787-832-4040 x3471
Fax: 787-265-0860
E:mail: e_schroder@hotmail.com

Howard F. Schwartz
Dept. of Bioagr. Sci. & Pest Mgmt.
C205 Plant Sciences
Colorado State University
Fort Collins, CO 80523-1177
Phone 970-491-6987
Fax: 970-491-3862/ E:mail:
howard.schwartz@colostate.edu

Seminis Vegetable Seeds
Ruelle Simon
80500 Guerbigny FRANCE
Phone 011-33-3-22-37-5000
Fax: 011-33-33-22-37-4810

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Ron Shellenberger
Pro Vita
PO Box 628
Kuna, ID 83634
Phone 208-463-7624
Fax: 208-442-6433
E:mail: Ron@Provita-Inc.com

Shree P. Singh
Kimberly Research and Extension
University of Idaho
3793 N. 3600 East
Kimberly, ID 83341
Phone 208-423-6609
Fax: 208-423-6559
E:mail: singh@kimberly.uidaho.edu

Rusty Smith
USDA-ARS-CG&PR
PO Box 345
Stoneville, MS 38776
Phone 662-686-5499
Fax: 662-686-5218
E:mail: rsmith@ars.usda.gov

Raymond C. Smith
Sunland Seed Pty Ltd.
P. O. Box 7,
Cooperook 2426 NSW AUSTRALIA
Phone 61-265-563234
Fax: 61-265563234

Tom Smith
Plant Agriculture Dept.
Crop Science Bldg.
University of Guelph
Guelph, ON, N1G 2W1 CANADA
Phone 519-824-4120 ext58339
Fax: 519-763-8933
E:mail: thsmith@uoguelph.ca

Societe Generale
3, rue Martin Luther King
Boite Postale 242
24010 Avignon Cedex 1 France
Phone 049-080-5700

Svetla Sofkove-Bobcheva
Maritza Vegetable Crop Res. Inst.
32 Brezovsko Shosse Strb,
4003 Plovdiv BULGARIA
Phone 35932 650180
Fax: 35932 650177
E-mail: Svetlas_76@yahoo.com

Jose Eustaquio Souza Carneiro
Universidade Federal de Vicosa
Dept. de Fitotecnia
Vicosa, MG 36571-000
BRAZIL
E-mail: jesc@ufv.br

Pedro Crescencio Souza Carneiro
Departamento de Biologia Geral
UFV
Vicosa, MG 36570-000
BRAZIL
Phone 55133-8993368
Fax: 55313-8992549
E-mail: carneiro@ufv.br

Art Spaans
Bakker Bros
Oostelijke Randweg 12
1723 LH
Noord-Scharwoude HOLLAND
Phone 208-459-4420
Fax: 208-459-4457
E-mail: K2seeds@speedyquick.net

Francesca Sparvoli
Istituto di Biologia e Biotechn. Agraria,
CNR
Via Bassini15
20133 Milan ITALY
Phone 39-0223699435
Fax: 39-0223699411
E-mail:

Eben Spencer
ADM Edible Bean Specialties, Inc
Box 208
Oslo, MN 56744
Phone 218-695-5566
Fax: 218-695-5566
E-mail: spencer@invisimax.com

Doug Sprehe
Hylands Seeds Research
41703 US Hwy 2 SW
East Grand Forks, MN 56721
Phone 218-773-8834
Fax: 218-773-9809
E-mail: dsprehe@hylandseeds.com

Mark Stack
Southwestern Colo. Res. Center
Box 233
Yellow Jacket, CO 81335
Phone 970-562-4255
Fax: 970-562-4254
E-mail: mark.stack@colostate.edu

J. Rennie Stavely
2206 Apple Tree Lane
Silver Spring, MD 20905-4415
Phone 301-384-6853
E-mail: JRNGStavely@comcast.net

James R. Steadman
Dept. of Plant Pathology
University of Nebraska
406 PSH
Lincoln, NE 68583-0722
Phone 402-472-3163
Fax: 402-472-2853
E-mail: jsteadman1@unl.edu

Kathy Stewart-Williams
University of Idaho
3806 N. 3600 E.
Kimberly, ID 83341
Phone 208-423-6655
Fax: 208-423-6656
E-mail: williams@kimberly.uidaho.edu

Peter Stoffella
2199 South Rock Road
University of Florida
Fort Pierce, FL 34945-3138
Phone 772-468-3922
Fax: 772-468-5668

Diana Lilova Svetleva
25 'Gustav Vajgand' Str.
Plovdiv 4000
BULGARIA
Phone 359-32-227829
Fax: 359-32-633157
E-mail: svetleva@yahoo.com

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E-mail: info@us.swets.com

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Alan Taylor
Horticultural Sciences
630 North St
Geneva, NY 14456-0462
Phone 315-787-2243
Fax: 315-787-2320
E-mail: agt1@cornell.edu

Steven R. Temple
Plant Science Department, UC Davis
Mail Stop One
One Shields Ave.
Davis, CA 95616
Phone 530-752-8216
Fax: 530-752-4361
E-mail: srtemple@ucdavis.edu

John Theuws
Kempen Laan 7
B-3600 Genk
BELGIUM
Phone 32-89-85-2931
E-mail: johntheuws@versateladsl.be

Alyson Thornton
Harris Moran
1677 Muller Rd.
Sun Prairie, WI 53590
Phone 608-837-6574
Fax: 608-837-3758
E-mail: a.thornton@harrismoran.com

Michael D. T. Thung
Embrapa Arroz e Feijao
CXP 179
75375-000 Santo Antonio De Goias/GO
BRAZIL
Phone 5562 35332183
Fax: 55625332100

Adriana Tofino
C/O CIAT (M. Blair)
7343 NW Terrace
Medley FL
Phone 33166
E-mail: apt611@hotmail.com

Joseph Michel Tohme
C I A T
7343 NW 79th Terrace
Medley, FL 33166-2211
Phone 415-833-6625
Fax: 415-833-8826
E:mail: j.tohme@cgiar.org

Heloisa Torres da Silva
Rod. Goiania
St. Antonio de Goias, Go
CP 179
74001-970 Goiania, GO BRAZIL
Phone 062-8332150
Fax: 062-8332100
E:mail: heloisa@cnpaf.embrapa.br

S. J. Tsao
Dept. of Horticulture
#1 Sec 4 Roosevelt Rd.
Taipei, R.O.C. 106 TAIWAN
Phone 886-2-23630231 ext. 2526
Fax: 886-2-23625542
E:mail: jocelyn@ntu.edu.tw

Ray Tucker
Bush Bros. & Co
1016 East Wesgarber Rd
Knoxville, TN 37909-2683
Phone 865-909-8647
Fax:
E:mail: rtucker@bushbros.com

Mark A. Uebersax
FSHN
204 G. Malcolm Trout Bldg.
Michigan State University
East Lansing, MI 48824-1224
Phone 517-355-8474
Fax: 517-353-8963
E:mail: uebersax@msu.edu

Michael Ugen
NARO
NAARI
P. O. Box 7084
Kampala UGANDA
Phone 256-41-567635

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Bioscience & Natural Res.
2101 VLSB #6500
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Lincoln, NE 68588-0410

Juan-Tay Urbina
Estacion Exp. Quilamapu
Casilla # 426
Chillan CHILE
Phone 56-42-209714
Fax: 56-42-209720
E:mail: Jtay@quilamapu.inia.cl

Carlos Urrea
Panhandle Res. & Extension Center
University of Nebraska
4502 Avenue I
Scottsbluff, NE
Phone 308-632-0556
Fax: 308-632-1365
E:mail: Currea2@unl.edu

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Current Serial Records, Room 002
10301 Baltimore Ave.
Beltsville, MD 20705
Fax: 301-504-5243
E:mail: wgelenter@nal.usda.gov

John Van Herk Jr.
Hyland Seeds
P.O. Box 250, 2 Hyland Dr.
Blenheim, Ontario
N0P 1A0 CANADA
Phone 519-676-8146
Fax: 519-676-5674
E:mail: JVanHerk@Thompsonslimited.com

Bert Vandenberg
Dept. of Plant Sciences
51 Campus Drive, Univ of
Saskatchewan
Saskatoon, SK S7N 5A8 CANADA
Phone 306-966-8786
Fax: 306-966-5015
E:mail: bert.vandenberg@usask.ca

Patricia Vargas
Campo Exp. Valle de México
INIFAP-SAGARPA
PO Box 10
Chapingo, 56230
MEXICO

Greg Varner
Saginaw Valley Research Farm
3066 S Thomas Road
Saginaw, MI 48609
Phone 989-781-0260
Fax: 989-781-0260
E:mail: varnerbean@hotmail.com

Carmen Asensio Vegas
Instituto Tecnológico Agrario
Ctra de Burgos km 118
Apdo. 172
47080 Valladolid SPAIN
Phone 34-983-414461
Fax: 34-983-414780
E:mail: asevegma@itacyl.es

Pedro Soares Vidigal Filho
Av. Colombo 5790-cep:87020-900
Universidade Estadual de Maringa
Maringa, PR 87020-900 BRAZIL
Phone 21442634407
E:mail: psvfilho@uem.br

Rogério Faria Vieira
Grain Legume Researcher
EPAMIG - Vila Gianetti 47
Vicosa, MG 36571-000 BRAZIL
Phone 55-31-3891-2646
Fax: 55-31-3894-5224
E:mail: rfvieira@epamig.br

Clibas Vieira
Dept. de Fitotecnia
Univ. Federal de Vicosa
Vicosa, MG 36571-000 BRAZIL
Phone 55-31-3899-1169
E:mail: cbvieira@ufv.br

Oswaldo Voysest Voysest
1225 Bushnell St
Beloit, WI 53511
Phone 608-313-8606
E:mail: ovoysestv@aol.com

Wageningen UR Bibliotheek
66775
Postbus 9100
6700 HA Wageningen
NETHERLANDS

Dan Wahlquist
Syngenta Seeds, Inc.
6338 HWY 20 - 26
Nampa, ID 83687
Phone 208-465-8510
Fax: 208-467-4559
E:mail: dan.wahlquist@syngenta.com

J. G. Waines
Botany and Plant Sciences
University of California
Riverside, CA 92521-0124
Phone 909-787-3706
Fax: 909-787-4437
E:mail: giles.waines@ucr.edu

Ralph Waniska
TAMUS, Soil & Crop Sciences
370 Olsen Blvd., 429 Heep Center
College Station, TX 77843-2474
Phone 979-845-2985
Fax: 979-845-0456
E:mail: waniska@tamu.edu

Ralph Waniska
Crop & Soil Sciences
TAMU
2474 Heep Center
College Station, TX 77843-2474
Phone 979-845-2985
Fax: 979-845-0456
E:mail: waniska@tamu.edu

David Webster
Seminis Vegetable Seeds
21120 Highway 30
Filer, ID 83328-5508
Phone 208-326-6136
Fax: 208-326-4326
E:mail: David.Webster@seminis.com

Dr. Molly Welsh
Curator, Phaseolus Collection
WRPIS
59 Johnson Hall
Pullman, WA 99164-6402
Phone 509-335-3692
Fax: 509-335-6654
E:mail: mmwelsh@wsu.edu

Jeffrey White
ALARC, USDA-ARS
21881 North Cardon Lane
Maricopa, AZ 85239
Phone 520-316-6368
Fax: 520-316-6330
E:mail: jwhite@uswcl.ars.ag.gov

Irvin E. Widders
Bean/Cowpea CRSP
Michigan State University
321 Agriculture Hall
E. Lansing, MI 48824
Phone 517-355-4693
Fax: 517-432-1073
E:mail: widders@msu.edu

Dale Williams
ND Foundation Seedstocks
Dept. of Plant Sciences, NDSU
166F Loftsgard
Fargo, ND 58105-5051
Phone 701-231-8140
Fax: 701-231-8474
E:mail: Dale.Williams@ndsu.nodak.edu

Jim Wyatt
University of Tennessee
605 Airways Blvd.
Jackson, TN 38301-3200
Phone 901-424-1643
Fax: 901-425-4760
E:mail: jwyatt6@utk.edu

Dongfang Yang
Dept. of Life Science
Hebei Normal Univ. of Sci. & Tech.
Qinhuangdao, Changli 066600 PR CHINA
Phone 86-335-203-9046
Fax: 86-335-202-4487
E:mail: Yang_dongfang@hotmail.com

Yellowstone Bean Company
329 S. River Rd.
Rt. 1 - Box 1198
East Bridger, MT 59014
Phone 406-662-3622
Fax: 406-662-3679
E:mail: ybco@brmt.net

Mildred Zapata
Dept. of Crop Protection
Univ. of Puerto Rico
PO Box 9030
Mayaguez, PR 00680
Phone 787-265-8484
Fax: 787-265-3857
E:mail: Plant_Zapata@hotmail.com