**ANNUAL REPORT OF THE** 

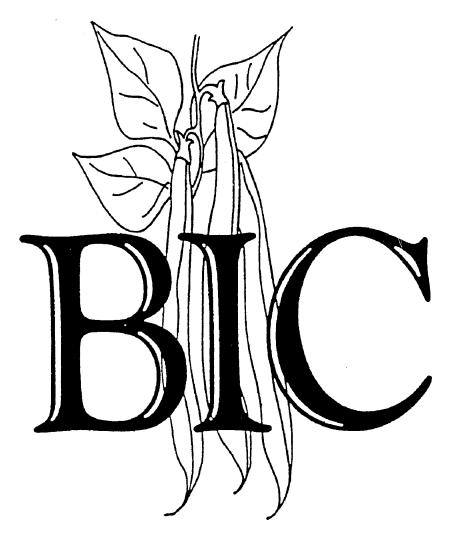
# BEAN IMPROVEMENT COOPERATIVE

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

> VOLUME 67 2024

ANNUAL REPORT OF THE

## BEAN IMPROVEMENT COOPERATIVE



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> VOLUME 67 2024



## THE LXVII

## Report of The

## **BEAN IMPROVEMENT COOPERATIVE**

No. 67

April 2024

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Kirstin Bett Karen Cichy Simon Chang Kelvin Kamfwa Phil Miklas Jim Myers Juan Osorno Peter Pauls Timothy Porch (President) Andrew Wiersma

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> http://www.bic.uprm.edu/ SITE REGULARLY UPDATED

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## **TABLE OF CONTENTS**

LXVII Annual Report of the Bean Improvement Cooperative	Page iv
BIC Coordinating Committee Membership - 1957 to 2024	
BIC Genetics Committee Minutes	
2023 BIC Award Recipients In Memory of Roger Kirkby	
	АЛТУ
<b>ORAL PRESENTATIONS (2023 BIC MEETING)</b>	
INTERSPECIFIC CROSSES IMPROVE DROUGHT TOLERANCE IN ANDEAN COMMON BEAN H. Buendia, C. Cadena, J. Aparicio, J. Cantor, S. Beebe, J. Wilker	1
A COLLABORATION TOWARDS THE COMPREHENSIVE IMPROVEMENT OF LIMA BEANS: ADDRESSING CONSUMER INFORMATION, PRE-BREEDING, AND GERMPLASM INFORMATION/UTILIZATION BOTTLENECKS Jaclyn A. Adaskaveg, R. Varma Penmetsa, Jenna Hershberger, Lyle Wallace, Sangita Subedi, Michelle Hee Robert Hanifin, Marilyn L. Warburton, Samuel Hokin, Andrew Farmer, Donna Winham, Philip Roberts, Emmalea Ernest, Sarah Dohle, Antonia Palkovic, Travis Parker, Paul Gepts, Christine Diepenbrock	
MARKER-ASSISTED SELECTION TO REDUCE POPULATION SIZES AND TIME NEEDED FOR BACKCROSSING TO PRODUCE NON-DARKENING CRANBERRY BEANS	5
GENOTYPE-SPECIFIC TRANSCRIPTIONAL DYNAMICS IN THE EARLY ROOT DEVELOPMENT OF COMMON BEAN V. Ortiz, K. Cichy, C.R. Buell, M. Haus	7
GENOME WIDE ASSOCIATION STUDIES (GWAS) FOR TRAITS RELATED TO UPRIGHT PLANT ARCHITECTURE IN DRY BEAN ( <i>PHASEOLUS VULGARIS</i> L.) Oscar Rodriguez, Jayanta Roy, Jose Figueroa, Juan M. Osorno, Philip E. McClean	9
<ul> <li>GENOME-WIDE ASSOCIATION STUDY AND IDENTIFICATION OF CAUSAL ALLELES AT</li> <li>UR-11 LOCUS CONTROLLING RUST RESISTANCE IN COMMON BEAN</li> <li>Mohammad Erfatpour, Kristin J. Simons, Jose Figueroa-Cerna, Rian Lee, Phillip E. McClean,</li> <li>Juan M. Osorno</li> <li>GENETIC ARCHITECTURE OF ANTHRACNOSE RESISTANCE IN THE YELLOW BEAN</li> <li>COLLECTION OF COMMON BEAN</li> </ul>	
Kuwabo Kuwabo, Swivia M. Hamabwe, Paul Kachapulula, Karen Cichy, Travis Parker, Chikoti Mukuma, Kelvin Kamfwa	15
IDENTIFYING RESISTANCE TO ANTHRACNOSE IN ANDEAN BUSH BEAN LINES WITH HIGH-VALUE MARKET GRAIN TYPE	15
V. Arredondo, E. Espitia, H.F. Buendia, E. Portilla, C. Cadena, G. Mosquera	
UNCOVERING NEW GENOMIC REGIONS ASSOCIATED WITH WHITE MOLD RESISTANCE IN DRY BEANS	17
Jose C. Figueroa-Cerna, Jayanta Roy, Kristin Simons, Phillip McClean, Phillip N. Miklas, Juan M. Osorno	17
INTEGRATION OF SENSING, CROP MODELING, AND GENOMICS IN A COMMON BEAN/TEPARY INTERSPECIFIC POPULATION TO IMPROVE PRODUCTIVITY AND QUALITY TRAITS IN US AND AFRICAN BREEDING CONTEXTS Jonathan M. Berlingeri1, Sassoum Lo, Troy Williams, Margaret Riggs, Edgar Cortes, Ameera Khan, Anton Palkovic, Travis Parker, Paul Gepts, Timothy Porch, Santos Barrera Lemus, Carlos Urrea, Karen Cichy, Phillip Miklas, Teshale Assefa, Clare Mukankusi, Brian Bailey, J. Mason Earles, Christine Diepenbrock	19 ia
QUANTITATIVE TRAIT LOCI ASSOCIATED WITH DROUGHT TOLERANCE IN AN ANDEAN MAPP POPULATION OF COMMON BEAN Swivia M. Hamabwe, Nicholas A. Otieno, Alvaro Soler-Garzón, Phillip N. Miklas, Travis Parker, David M Kramer, Abhijnan Chattopadhyay, Pride Cheelo, Kuwabo Kuwabo, Kelvin Kamfwa	21

DRY BEAN TOLERANCE TO SALINITY, WATERLOGGING, AND COMBINED CONDITIONS Audrey Rhodes, Thomas De Sutter, Juan M. Osorno, Barney Geddes	. 23
A GENOME-WIDE ASSOCIATION STUDY OF SNAP BEAN POD PRODUCTION UNDER IDEAL AND HEAT-STRESSED CONDITIONS Morgan Stone, Brian Ward, Shane Robinson, William C. Bridges, Sandra E. Branham	. 25
IMPROVING CONSUMER TRAITS IN DRY BEANS	. 27
UTILIZING BENCHTOP NEAR-INFRARED SPECTROSCOPY TO PREDICT LIMA BEAN ( <i>PHASEOLUS LUNATUS</i> L.) NUTRITIONAL COMPOSITION	. 29
APPROACHES TO DEVELOP ROOT-KNOT NEMATODE RESISTANT GREEN BABY LIMA VARIETIES Emmalea Ernest, Alyssa Koehler, Eboni Traverso	. 31
SCREENING DRY EDIBLE BEAN GERMPLASM FOR RESISTANCE TO SOYBEAN CYST NEMATODE Harkamal Kaur, Juan M. Osorno, Guiping Yan	. 33
PYRAMIDING OF GENES THAT CONFER MULTIPLE DISEASE RESISTANCE IN SNAP BEANS AND PERFORMANCE OF BREEDING LINES IN KENYA Edith Esther Arunga, Grace Watare, Serah Njau, Brian Wafula, Nancy Munubi	. 35
THE GENOME OF MESOAMERICAN COMMON BEAN OAC REX – EVIDENCE OF INTROGRESSION OF DISEASE RESISTANCE INTO <i>PHASEOLUS VULGARIS</i> FROM <i>PHASEOLUS ACUTIFOLIUS</i>	
GENES IN DUE TIME FOR COMING STRESSORS D.G. Debouck	. 39
USDA PHASEOLUS GERMPLASM COLLECTION, CURRENT AND FUTURE HAPPENINGS, COMMENT AND COLLABORATIONS WELCOME!	

## POSTER PRESENTATIONS (2023 BIC MEETING)

CANDIDATE GENES AND MARKERS FOR RESISTANCE IN THE BCMV/BCMNV HOST-PATHOGEN INTERACTION IN COMMON BEAN	3
POTENTIAL OF GENOMIC PREDICTION FOR WHITE MOLD IN DRY BEAN (PHASEOLUS VULGARIS L.)	5
GENE EXPRESSION ANALYSIS IN AMENDOIM CAVALO COMMON BEAN CULTIVAR CHALLENGED WITH COLLETOTRICHUM LINDEMUTHIANUM	7
RESISTANCE TO BACTERIAL BROWN SPOT IN ADZUKI BEAN	9
IDENTIFICATION OF EFFECTOR BINDING ELEMENTS LOCALIZED IN PROMOTER REGIONS OF GENES OF <i>PHASEOLUS VULGARIS</i> L TARGETS OF <i>XANTHOMONAS</i> SPECIES TRANSCRIPTION ACTIVATOR-LIKE EFFECTORS	1

STUDY OF IMPROVED BEAN VARIETIES, IN MONOCULTURE AND INTERCROPPED WITH MAIZE, AND OF THE RHIZOBIA-BEAN SYMBIOTIC SYSTEM UNDER DIFFERENT AGRONOMIC TREATMENTS	53
Antonio M. De Ron, A. Paula Rodiño, J. Leonardo Tejada-Hinojoza, Carmen Salinero	55
NITROGEN EFFECTS ON FIXATION IN COMMON BEAN	55
GENOME-ENABLED BREEDING ACROSS PHASEOLUS SPECIES	57
DRONE IMAGERY PHENOTYPING USING MACHINE LEARNING APPROACHES TO ESTIMATE HARVEST MATURITY IN DRY BEANS	59
MAPPING OF QUANTITATIVE TRAIT LOCI LINKED TO MORPHO-AGRONOMIC TRAITS IN RILS DERIVED FROM CDRK X YOLANO COMMON BEAN ( <i>PHASEOLUS VULGARIS</i> ) POPULATION	61
UNCOVERING INFLUENCES ON SEEDLING ESTABLISHMENT J.M. Sinnaeve, Viviana Ortiz, M.J. Haus	63
MARKET TRENDS OF NEW PULSE-BASED PRODUCTS LAUNCHED DURING 2012-2021 IN THE U.S DIVERSIFYING DEMANDS, CHALLENGES, AND OPPORTUNITIES Rie Sadohara, Karen Cichy, Mark A. Uebersax	
FARMER PARTICIPATORY VARIETY SELECTION AND SEED DISSEMINATION OF HIGH YIELDING FASTER COOKING AND IRON-RICH VARIETIES OF COMMON BEAN IN ZAMBIA Kelvin Kamfwa, Swivia Hamabwe, Kuwabo Kuwabo, Mwiinga Mulube, Jason Wiesinger, Raymond Glahn, Karen Cichy	

## **RESEARCH PAPERS FOR 2024**

PHENOTYPING A GENETIC DIVERSITY PANEL OF MESOAMERICAN COMMON BEAN FOR DROUGHT TOLERANCE	. 69
Elizeu David Santos, Lorenzo Francesco Poli Frederico, José Santos Neto, Daniel Soares Alves, Isabella Mendonça Arruda, Vania Moda-Cirino	
DROUGHT TOLERANCE OF NEW ANDEAN BEAN LINES IN KENYA Paul M. Kimani, Iddah Kabutbei, George Chemining'wa, Mary Mburu, John H. Nderitu	. 71
ANDEAN LINES SELECTED FOR HEAT TOLERANCE FROM BULK BREEDING PHASEOLUS IMPROVEMENT COOPERATIVE (PIC) POPULATIONS Tim Porch, Deidre Fourie, Phil Miklas	. 73
GLOBAL WARMING EFFECTS ON NATURAL POPULATIONS OF THE DURANGO RACE COMMON BEAN COMPLEX Rigoberto Rosales-Serna, César Manuel Reyes-Rodríguez, Norma Almaraz-Abarca, Donaji Sierra-Zurita, Jorge Luis Becerra-López, Saúl Santana-Espinoza, and Muhammad Ehsan	. 75
TEPARY BEAN ( <i>PHASEOLUS ACUTIFOLIUS</i> ) OUT-YIELDS COMMON BEAN ( <i>P. VULGARIS</i> ) IN SEMI-ARID ORGANIC CONDITIONS Travis Parker, Troy Williams, Mike Reeske, Sassoum Lo, Antonia Palkovic, Paul Gepts	. 77
FIELD EVALUATION OF THE SYMBIOTIC AND AGRONOMIC EFFECTIVENESS OF PRESELECTED LOCAL RHIZOBIUM ISOLATES ON THREE BEAN ( <i>PHASEOLUS VULGARIS</i> L.) VARIETIES Noupé Diakaria Coulibaly, Lassina Fondio, Christian Landry Ossey, André Gabazé Gadji, Aya Félicité N'Gaza, Mako François De Paul N'Gbesso, Louis Butare	. 79
ENHANCING WHITE MOLD RESISTANCE IN DRY BEAN THROUGH GENOMIC SELECTION Jose C. Figueroa-Cerna, Jayanta Roy, Kristin Simons, Phillip McClean, Phillip N. Miklas, Juan M. Osorno	. 81

3
5
7
9
91
3
95
97
9
)1
13
15
)7
19
1
3

INDIRECT SELECTION ON ROOT ARCHITECTURE IN THE MSU PINTO DRY BEAN BREEDING PROGRAM
RESPONSE OF THE TEPARY DIVERSITY PANEL TO COMBINED ASIAN BEAN FLOWER THRIP AND LEAFHOPPER PRESSURE
ASSESSMENT OF DAMAGE CAUSED BY PESTS TO THREE VARIETIES OF DRY BEAN IN THE CENTRE REGION OF COTE D'TVOIRE
COMPARATIVE STUDY OF THE EFFECTS OF GROWING GREEN BEANS ( <i>PHASEOLUS VULGARIS</i> L.) UNDER NETS AND WITHOUT NETS AND PHYTOSANITARY TREATMENTS ON DEVELOPMENT AND YIELD PARAMETERS
IDENTIFICATION OF GENOMIC REGIONS ASSOCIATED WITH GRAIN YIELD IN COMMON BEAN
Reberth Renato da Silva, Ednilson Barros Barroso, Maria Luíza Paiva de Oliveira, Jose C. Figueroa-Cerna, Juan Manuel Osorno, Elaine Aparecida de Souza
MEASURING CANOPY HEIGHT IN COMMON BEAN MICROPLOTS USING UAV DIGITAL PHENOTYPING METHODS
SYNERGISTIC APPROACH FOR DROUGHT/NON-DROUGHT CLASSIFICATION OF BEANS: HARNESSING THE POTENTIAL OF MULTISOURCE DATASETS USING MACHINE LEARNING
USING OCT TO MEASURE SEED COAT THICKNESS IN COMMON BEAN 129 Bella Amyotte, Kiana Karimi Shahmarvandi, Scott Noble, Kirstin E. Bett
LONGITUDINAL ASSESSMENT OF VARIABILITY COMPREHENSIVE ANALYSIS (YEAR 3 OF 3) 131 Scott Bales, Rie Sadohara, Karen Cichy
<ul> <li>WHITE BEANS ARE A PROMISING MARKET CLASS FOR DELIVERING MORE BIOAVAILABLE</li> <li>IRON TO CONSUMERS IN GHANA</li></ul>
ENHANCING THE IRON NUTRITION OF BEANS: UTILIZING THE SLOW AND NON-DARKENING TRAITS TO REDUCE PROANTHOCYANIDINS IN MULTIPLE MARKET CLASSES OF DRY BEANS 135 Raymond P. Glahn, Jason A Wiesinger, Karen A. Cichy, Kelvin Kamfwa, Juan M. Osorno, Phillip E. McClean, K. Peter Pauls
WATER UPTAKE PATTERNS AND HARD-TO-COOK PHENOMENA OF BEAN VARIETIES AND ADVANCED LINES BRED IN EASTERN AFRICA
P.M. Kimani, P. Komu, A. Warsame, J. Mudiope, P. Githuka
<ul> <li>P.M. Kimani, P. Komu, A. Warsame, J. Mudiope, P. Githuka</li> <li>IMBIBITION AND GERMINATION IN <i>P. COCCINEUS</i> L. SEEDS</li></ul>
IMBIBITION AND GERMINATION IN P. COCCINEUS L. SEEDS

NUTRITIONAL QUALITY OF BEANS FROM DIFFERENT COLLECTIONS IN DURANGO, MEXICO14 Ixchel Abby Ortiz-Sánchez, Sonia Valdez-Ortega, Rigoberto Rosales-Serna, Cynthia Adriana Nava-Berumen, Héctor Fabián Martínez-Pérez, Norma Almaraz-Abarca	15
STARCH GRANULES IN COTYLEDON OF DOMESTICATED AND WILD GERMINATING SEEDS OF <i>PHASEOLUS VULGARIS</i> L	17
NOTICE OF NAMING AND RELEASE OF PID 2, A PINTO CULTIVAR WITH HIGH YIELD AND ENHANCED GRAIN QUALITY	19
EVALUATION OF THE AGRONOMIC PERFORMANCE OF FOUR VARIETIES OF COMMON BEAN IN CENTRAL COTE D'IVOIRE	51
GRAIN YIELD AND ITS RELATIONSHIP WITH ITS COMPONENTS IN CLIMBING PHASEOLUS SPECIES	53
DYNAMICS OF POD PRODUCTION, YIELD AND BEAN COMPONENTS IN WARM CLIMATE	55
INCREASE IN BEAN PRODUCTIVITY BY ESTABLISHING DOUBLE CROPPING IN THE VALLE DELMEZQUITAL, HGO., MEXICO	57
CLOSE-SPACED ARRANGEMENT TO INCREASE SEED YIELD IN COMMON BEAN IMPROVED LINES	59
YIELD RESPONSE OF DRY BEANS TO ORGANIC AND INORGANIC FERTILIZERS AND BIOFERTILIZER	51
<ul> <li>COMPARATIVE STUDY OF THE NUTRITIONAL COMPOSITION OF <i>PHASEOLUS VULGARIS</i></li> <li>(COMMON BEAN), <i>VIGNA UNGUCULATA</i> (COWPEA) AND <i>VIGNA RADIATA</i> (MUNG BEAN)16</li> <li>Noupé Diakaria Coulibaly, Aya Félicité N'Gaza, Christian Landry Ossey, André Gabazé Gadji, Mako François De Paul N'Gbesso, Lassina Fondio and Louis Butare</li> </ul>	53

## 2024 RESEARCH TECHNIQUE

BREEDING COMMON BEANS FOR RESISTANCE TO BRUCHIDS	165
James S. Beaver, Timothy G. Porch, Juan Carlos Rosas, Kelvin Kamfwa, Juan M. Osorno, Maria Mazala	
SUBJECT MATTER INDEX	180
2024 MEMBERSHIP DIRECTORY	181
2023 FINANCIAL STATEMENT	200
Cover: Photos by Kelvin Kamfwa and Maria Mazala showing development of lines resistant to the common be	ean

weevil (*Acanthoscelides obtectus*)

#### THE 67th ANNUAL REPORT OF THE BEAN IMPROVEMENT COOPERATIVE

**The Bean Improvement Cooperative (BIC)** celebrated its 32<sup>nd</sup> Biennial Meeting from the 6-8 of November 2023 at Clemson University in Greenville, South Carolina. We thank the NAPIA and Dr. Dil Thavarajah, Tristan Lawrence and Summer Chandler for the excellent planning and execution of the Conference and for their warm reception. There was a large contingent of scientists and students at the meeting. To offset the costs of their travel and conference costs, 11 students were selected for travel award funding from Zambia (2), Canada (2), and the U.S. (7). Given the success of this meeting and the response of the participants, we plan to continue in the near term with concurrent BIC/NAPIA meetings, with the BIC organizing the next meeting in Lincoln, Nebraska in 2025, hosted by Dr. Carlos Urrea.

We thank the BIC sponsors for their generous support of the BIC 2023 meeting including Provita and Limagrain/Vilmorin providing Gold level sponsorship, Syngenta (Silver), Pop Vriend Seeds B.V. (Copper), Pure Line Seeds (Bronze), and Nebraska Dry Bean Commission (Friend). These conferences depend on these contributions and collaboration.

A significant number of our colleagues were recognized for their service to the BIC community through their exemplary research, with their biography information presented on the next pages. Special recognition through the Frazier - Zaumeyer Distinguished Lectureship was given to Dr. Karen Cichy, Research Geneticist, USDA-ARS, East Lansing, Michigan, and Raymond Glahn, Research Physiologist, USDA-ARS, Ithaca, New York. We thank them for their contributions and for the large advances in quality and nutrition that they have helped to facilitate. Meritorious service awards were presented to Daniel G. Debouck, CIAT, Cali, Colombia; and Antonio M. De Ron, Spanish National Research Council (CSIC), Pontevedra, Spain. The Distinguished Achievement Award was presented to Emmalea Ernest, University of Delaware, Newark, Delaware; Consuelo Estévez de Jensen, University of Puerto Rico, Juana Diaz, Puerto Rico; and Aldemaro Clara, CENTA, San Salvador, El Salvador. The Technical Merit Award was presented to Antonia Palkovic, University of California, Davis, California. We thank each of them for their contributions and to those colleagues who recognized them.

Given the importance of research methods on the BIC website and their importance for maintaining techniques from one generation to the next, the Coordinating Committee has decided to include a section in the BIC report dedicated to an updated or new research method. This year we have included the first such report "Breeding common beans for resistance to bruchids" by Beaver et al. We invite volunteers to submit ideas for a technique to include in the next BIC report. Please share information about the BIC with interested colleagues who might like to attend the 2025 meeting or join as members. Also, feel free to contact us with any new ideas, contributions, or updates for the BIC website or this Annual Report. The BIC continues to conduct business by email, postings on the webpage, and through the online publication of this Annual Report.

The BIC Coordinating Committee welcomes Dr. Andrew Wiersma as a new member and thanks Dr. Ken Kmiecik for his contributions and service on the Coordinating Committee. This volume is my last as President. I have enjoyed working with you during my term of service, and thank each of you for your contributions that have enriched our efforts to serve farmers, consumers, and science. I want to welcome Dr. Juan Osorno who will begin serving as BIC President in 2025!

-We wish you a fulfilling and successful year.

Warm regards,

#### **Tim Porch, BIC President**

#### BIC COMMITTEE MEMBERSHIP - 1957 to 2024

**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, Covne, Dean, Jorgensen, Polzak, Zaumeyer 1972 Burke, Covne, Dean, Jorgensen, Kiely, Polzak, Zaumeyer Ballantyne, Bravo, Burke, Coyne, Dickson, Emery, Evans, Kiely, Saettler, Zaumeyer 1974 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 Covne, Dickson, Mok, Saettler, Schoonhoven, Schwartz, Silbernagel, Steadman, Wallace 1988 Brick, Dickson, Emery, Magnuson, Roos, Schwartz, Singh, Steadman, Uebersax 1992 Dickson, Emery, Grafton, Magnuson, Schwartz, Singh, Stavely, Steadman, Uebersax 1994 Antonius, Dickson, Grafton, Magnuson, Park, Schwartz, Singh, Stavely, Uebersax 1996 Antonius, Grafton, Park, Schwartz, Singh, Stavely, Myers, Kotch, Miklas, Riley 1998 Antonius, Park, Schwartz(ex officio), Singh, Myers, Kotch, Miklas, Riley, Beaver, Vandenberg, Kelly 2000 Antonius, Beaver, Kelly, Kotch, Miklas, Myers, Park, Riley, Schwartz, Singh, Vandenberg 2001 Antonius, Beaver, Kelly, Kotch, Miklas, Myers, Park, Riley, de Ron, Schwartz, Vandenberg 2003 Beaver, Kelly, Kmiecik, Kurowski, Miklas, Myers, Park, Riley, de Ron, Schwartz, Vandenberg 2007 Beaver, Kelly, Kmiecik, Miklas, Myers, Park, Riley, de Ron, Schwartz, Shellenberger, Vandenberg 2008 Beaver, Kelly, Kmiecik, Miklas, Myers, Pauls, Riley, de Ron, Schwartz, Shellenberger, Vandenberg 2010 Beaver, Kelly, Kmiecik, Miklas, Myers, Pauls, Riley, de Ron, Schwartz, Shellenberger, Vandenberg 2011 Bett, Kelly, Kmiecik, Miklas, Myers, Osorno, Pastor-Corrales, Pauls, Riley, de Ron, Wahlquist 2015 Bett, Cichy, Kelly (ex officio), Kmiecik, Miklas, Myers, Osorno, Pauls, Souza, Trapp, Wahlquist 2020 Bett, Cichy, Kmiecik, Miklas (ex officio), Myers, Osorno, Pauls, Porch, Souza, Trapp, Wahlquist 2021 Bett, Cichy, Kamfwa, Kmiecik, Miklas, Myers, Osorno, Pauls, Porch, Souza, Wahlquist 2023 Bett, Chang, Cichy, Kamfwa, Kmiecik, Miklas, Myers, Osorno, Pauls, Porch 2024 Bett, Chang, Cichy, Kamfwa, Miklas, Myers, Osorno, Pauls, Porch, Wiersma

#### **Awards Committee:**

- 1971 Baggett, Briggs, Burke, Dean, Wallace
- 1973 Burke, Dean, Mauth, Zaumeyer
- 1975 Ballantyne, Frazier, Mauth
- 1977 Ballantyne, Curme, Frazier, Schuster
- 1979 Ballantyne, Schuster, Silbernagel, Temple
- 1981 Abawi, Bliss, Monis, Silbernagel
- 1983 Adams, Bliss, Burke, Dean, Morris
- 1985 Emery, Hagedorn, Sandsted, Schwartz
- 1987 Emery, Hagedorn, Sandsted
- 1989 Coyne, Silbernagel, Wallace

- 1995 Coyne, Dickson, Stavely
- 1997 Coyne, Schwartz, Stavely
- 2001 Hosfield, Magnuson, Schwartz
- 2004 Hosfield, Schwartz, Singh
- 2012 Noffsinger, Schwartz, Singh
- 2014 **Beaver**, Noffsinger, Urrea
- 2015 **Beaver**, Myers, Urrea
- 2023 Myers, Urrea

#### **Genetics Committee**

- 2004 Bassett (Chair), Beaver, Blair, Gepts, McClean, Miklas, Welsh (ex officio)
- 2005 Beaver (Acting Chair), Blair, Gepts, McClean, Miklas, Porch, Welsh (ex officio)
- 2007 Beaver, Blair, Gepts, McClean, Miklas, Porch (Chair), Welsh (ex officio)
- 2008 Bett, Blair, Gepts, McClean, Miklas, Porch (Chair), Urrea, Welsh (ex officio)
- 2014 **Bett** (Chair), Ferreira, Gepts, Goncalves-Vidigal, Kalavacharla, Kelly, Kisha (ex officio), McClean, Osorno, Porch, Urrea
- 2018 Bett, Ferreira, Gepts, Goncalves-Vidigal, Kalavacharla, Kelly (Chair), Kisha (ex officio), McClean, Osorno, Porch, Urrea
- 2020 Bett, Ferreira, Gepts, Goncalves-Vidigal, Kalavacharla, Kelly, McClean, Miklas (Chair), Osorno, Porch, Urrea
- 2021 Bett, Ferreira, Gepts, Goncalves-Vidigal, Kalavacharla, Kelly, McClean, **Miklas** (Chair), Osorno, Parker, Porch, Urrea
- 2022 Brown, Dohle, Ferreira, Gepts, Gomez, Goncalves-Vidigal, Kelly, McClean, **Miklas** (Chair), Osorno, Parker, Porch, Urrea
- 2023 Brown, Dohle, Ferreira, Gepts, Gomez, Goncalves-Vidigal, Kelly, McClean, Miklas, Osorno, **Parker** (Chair), Porch, Urrea

## **BIC GENETICS COMMITTEE MEETING MINUTES**

Location: The Westin Poinsett Greenville Hotel, Greenville, SC

Date: Wednesday November 8, 2023, 12:30 to 1:15 PM EST

**Committee Members:** Brown, Dohle, Ferreira, Gepts, Goncalves-Vidigal, McClean, Miklas (Chair), Osorno, Parker, Porch, Urrea

Members Present: Dohle, Goncalves-Vidigal, Miklas (Chair), Osorno, Parker, Porch, Urrea

#### A. Old Business:

1. The Genetics Committee 2022 meeting minutes were approved by email and published in the 2023 BIC v66.

#### **B.** New Business:

- 1. Review gene description changes to BIC Gene List—noted in text below with deletions (strikethrough) and additions (in gray) for *fin*, *ppd*, *stringless*, *Bct*, etc. (Gepts/Parker, Miklas/Soler-Garzón).
  - a. *Fin Finitus* (Latin): indeterminate vs. *fin* determinate plant growth (Lamprecht 1935b; Rudorf 1958); long vs. short internode; later vs. earlier flowering. *Fin* is 1 eM from Z (Bassett 1997e) and located on Pv01 (Koinange et al. 1996; Freyre et al. 1998). Corresponds to *PvTFL1y* (Phvul001G189200, Kwak et al 2008, 2012; Repinski et al. 2012).
  - b. *ppd* (*neu*) *photoperiod-insensitive* gene found in 'Redkloud' with a syndrome of effects (Wallace et al. 1993); an allele-specific associated primer is now available for *ppd* (Gu et al. 1995); probably the same locus as *Neu*<sup>+</sup> for short day vs. *neu* for day *neutral* flowering response to length of day of Rudorf (1958). The red/far-red photoreceptor gene PHYTOCHROME A3 (PHYA3, Phvul.001G221100) was identified as the *ppd* gene on Pv01 (Kamfwa et al., 2015; Weller et al., 2019).
  - c. *St stringless* pod; *st* gives a complete string (Prakken 1934). Believed to result from gene duplication of *PvINDEHISCENT* (*PvIND*, Phvul.002G271000) and retrotransposon insertion between the tandem repeats, leading to *PvIND* overexpression in stringless types (Parker et al. 2022). Has modifiers.
  - d. ..., *V* is located on Pv06 (McClean et al. 2002). *V* encodes flavonoid 3'5' hydroxylase (F3'5'H, Phvul.006G018800), a P450 enzyme required for the expression of dihydromyricetin-derived flavonoids in the flavonoid pathway (García-Fernández et al., 2021; McClean et al., 2022).
  - e. *Bct* (*Ctv-1*) a gene conditioning resistance to *beet curly top virus* discovered by Schultz and Dean (1947). The *Ctv-1* symbol was proposed by Provvidenti (1987) and updated to *Bct* by Larsen and Miklas (2004). *Bct* is located near 2,943,470 to 3,001,466 bp (G19833 v2.1) on Pv07 (Soler-Garzón et al. 2023), and linked markers are listed in the Beyond SCARs table (http://www.bic.uprm.edu/?page\_id=91).

**Decision**: The gene description amendments were accepted. Juan Osorno motioned, Tim Porch seconded, and all were in favor. Travis Parker and Phil Miklas will update the text and include information about the modifiers, reference genome for gene models, chromosomes, as needed.

2.  $bc-u^d$  and  $bc-u^r$  (formerly bc-4) are alleles (manuscript sent) noted in text below with deletions (strikethrough) and additions (in yellow).

 $bc-u^d$  ..... Originally named bc-u by Drijfhout (1978b) but renamed by Soler-Garzón et al. (2021b) to reflect Durango race origin and absence from host groups (HG) 2, 4, 5, 7 and presence in HG-10. Gene model Phvul.005G125100, a Vps4 AAAC ATPase ESCRT protein, was identified (Soler-Garzón et al. 2024) as the candidate gene for  $bc-u^d$  bc-4, and a marker for the putative causal mutation is listed in the Beyond SCARs table (http://www.bic.uprm.edu/?page\_id=91).

 $bc-u^r - bc-4$  (previously bc-4) (Soler-Garzón et al. 2021b) when combined with bc-2 provides resistance to all BCMV (except PG-5) but not BCMNV (Soler-Garzón et al. 2024) pathogroups. Gene model Phvul.005G125100, a Vps4 AAAC ATPase ESCRT protein, was identified as the candidate gene for bc-4  $bc-u^r$  (the 'r' superscript acknowledges discovery in Robust navy bean) and a SNP marker for the putative causal mutation is listed in the Beyond the SCARS Table (http://www.bic.uprm.edu/?page\_id=91).

**Decision**: The gene description and updates for the bc-u locus in the BIC Genes List were approved. Travis Parker motioned, Tim Porch seconded, and all were in favor.

- 3. **Membership** updates
  - a. Phil Miklas will be rotating off as BIC Genetics Committee Chair. We thank him for his service!
  - b. Travis Parker was nominated as the new Chair by Phil Miklas, Carlos Urrea seconded and all were in favor.
- C: **Other**: How to handle genes that do not fit qualitative conventions (T. Parker will look into this and report back in 2024)
  - a. Seed dormancy pectin acetylesterase-8-2 (*Phvul.003G277500*) underlies seed coat impermeability, and 5-bp insertion reduces function, increasing water uptake (Soltani et al. 2021).
  - b. Pod indehiscence *PvPDH1 (Phvul.003G252100)* and other QTL (Parker et al. 2020)

**Discussion**: Perhaps such genes could be included and maintained on the gene list in a separate section for now.

c. For such genes would classical descriptors or ortholog names be better?

**Discussion**: Most were in favor of using the ortholog name versus classical descriptors since it aids in comparisons across species. However, gene orthologs for traits with established gene symbols (such as those in the Gene List) should reference the classical descriptor in any publications. Can continue this discussion in the next meeting.

## THE BEAN IMPROVEMENT COOPERATIVE

Proudly Presents the

## **Frazier - Zaumeyer Distinguished Lectureship**

То

## Karen Cichy

Research Geneticist USDA-ARS East Lansing, Michigan

&

## **Raymond Glahn**

Research Physiologist USDA-ARS Ithaca, New York

The **Frazier - Zaumeyer Distinguished Lectureship** was established in 2001 to recognize and honor a distinguished colleague who will present the keynote opening address at the biennial BIC meeting. The individual selected will have made outstanding and pioneering contributions to science that led to the advance of bean research. The Lecture will focus on current topics relevant to the BIC membership. The Lectureship is distinct from the other BIC career Awards such as the Distinguished Achievement and Meritorious Service Awards. Holders of these awards are not excluded from being awarded the Frazier-Zaumeyer Distinguished Lectureship. The name for the Lectureship honors the original BIC founder members, the late William A. 'Tex' Frazier, distinguished bean breeder and the late William 'Bill' Zaumeyer an equally distinguished bean pathologist. Dr. Tex Frazier working at Oregon State University is recognized for his pioneering work in developing the famous Bush Blue Lake snap bean and related germplasm. Dr. Bill Zaumeyer, USDA-ARS is recognized for his outstanding efforts in bean pathology.

#### KAREN CICHY

Dr. Karen Cichy is a USDA-ARS research geneticist in East Lansing, Michigan, working on pulse crop quality and nutrition at Michigan State U. Dr. Cichy received a B.S. degree in Horticulture with a minor in International Agriculture from the Pennsylvania State U. in 1998. She received both M.S. and Ph.D. degrees in Plant Breeding and Genetics from Michigan State U. in 2002, under the direction of Dr. George Hosfield, working on mineral content in dry beans, and in 2006 studying bean root traits in low phosphorus soils with Drs. Sieg Snapp and James Kelly as co-advisors. She was awarded a Fulbright Scholarship to conduct part of her doctoral studies at CIAT, Colombia under the direction of Dr. Matt Blair. Following graduation, she received a two-year USDA postdoctoral position to study low phytic acid mutants in barley with Dr. Victor Raboy at the USDA lab in Aberdeen, Idaho. She returned to MSU in 2009 to assume her current position as research geneticist in the USDA-ARS Sugarbeet and Bean Research Unit.

Dr. Cichy has established a highly respected research program focusing on genetic enhancement of pulse crop nutritional and processing qualities that is sought by other researchers including: graduate students and postdocs (14), visiting scientists (16), industry partners, international partners, and grantors (~\$3M just in the last five years). Her research on characterization and improvement of nutritional quality, consumer acceptance, and utilization of dry beans as a food source is expansive. She has contributed significantly to the genetic characterization and understanding of bean seed nutritional value, seed mineral bioavailability, digestibility, cooking time, flavor, seed coat traits, and canning quality, among other traits. Her characterization of cooking time has revealed faster cooking beans which increases nutritive value and reduces fuel costs. Much of her work involves multi-location trials to determine genotype, environment, and genotype x environment effects on target traits. She has been active in characterizing qualities of bean flours, bean puree, pastas, and other food products. Dr. Cichy has an active dry bean breeding program that is in the process of releasing new fast cooking 'Manteca' and 'Mayocoba' yellow beans that have enhanced iron content and iron bioavailability. Her genetic research also contributes to the characterization and understanding of agronomic traits including biological nitrogen fixation, low soil fertility tolerance, root rot resistance, anthracnose resistance, seed yield, and many others.

Dr. Cichy's collaborative spirit and scientific prowess has resulted in the publication of six book chapters and 87 peer reviewed journal articles. Since 2017 Karen has participated as PI, Co-PI, or collaborator on 20 grants and has received over 20 invitations as an invited speaker. Her vision and expertise are highly sought after at scientific meetings and conferences hosted by the processing industry. She has been active in the W-4150 Multistate Research Project, Bean Improvement Cooperative Coordinating Committee, Phaseolus Crop Germplasm Committee, and Michigan Dry Bean Commission, as well as serves on steering, oversight, and working groups for various entities, grant review panels, and is an Associate Editor for the Legume Science journal. Karen also works closely with the international community; especially in the East African nations of Tanzania, Zambia, and Uganda, where much of her time and resources are devoted to training young scientists on the agronomic and genetic techniques needed for improving pulse crop quality and nutrition. The Bean Improvement Cooperative recognized her contributions with the Distinguished Achievement award in 2015. The dry bean research community and industry partners have benefitted immensely from Dr. Cichy's collaboration, council, and scientific contributions in the critically important field of nutrition and processing quality, which she has helped to promote and expand in the state of Michigan and beyond.

#### **RAYMOND GLAHN**

Dr. Raymond Glahn is a Physiologist with USDA-ARS in Ithaca, New York, working on nutrition physiology including mineral biofortification and bioavailability. Dr. Glahn received his B.S. in 1983, M.S. in 1986, and Ph.D. in 1989 from the Pennsylvania State U. Kidney physiology and health was Dr. Glahn's area of expertise during his formative graduate and post-graduate studies. His dissertation was on 'Causes and treatment of urolithiasis in single comb white leghorns' under Dr. Wideman, Jr. Between B.S. and M.S. degrees, he spent a year at Bethesda Naval Hospital, in Bethesda, Maryland, as a Research Associate in the Department of Critical Care Medicine. After graduation, he was a Research Associate in the Department of Poultry Science at the U. of Arkansas in Fayetteville from 1989 to 1990. He spent two years from 1990 to 1992 as a Research Fellow in the Nephrology Research Unit, Department of Physiology at the Mayo Clinic and Foundation, in Rochester, Minnesota. In 1992 Dr. Glahn began with USDA-ARS Plant, Soil, and Nutrition Research Unit, Robert Holley Center for Agriculture and Health, in Ithaca, New York.

Over the course of his career, Dr. Glahn has established a renowned research program on micronutrient human nutrition. Today, he's one of the world's authorities on iron nutrition and bioavailability in a wide range of foods that include fish, infant formula, maize, raisins, rice, soybean, spinach, wheat, dry bean, and pulses. His most recent efforts have focused on studies involving mineral biofortification and bioavailability in lentils and dry beans, including black, carioca, pinto, slow-darkening pintos, red, white, and yellow beans. His research examines and characterizes which factors influence iron bioavailability of grain legumes, including storage, processing, cooking time, mineral accumulation, seed structure, and many others. Dr. Glahn's research discovered that phenolic compounds in the seed coats of black beans reduce available iron while beans with white seed coats exhibit more available iron than colored beans. Thanks to his research, and in collaboration with other bean scientists, we know today that certain market classes of beans such as yellow, white, and slow-darkening pintos offer the highest levels of iron bioavailability. Dr. Glahn was an important contributor to the HarvestPlus Global Research Initiative to improve and promote biofortified beans in Rwanda and other East African Countries. His research eventually led to a better understanding of the independence between iron biofortification and iron bioavailability, demonstrating that higher seed iron content doesn't necessarily translate into higher iron absorption/utilization in human nutrition, and how bioavailability can be addressed as a potential breeding target. These findings and others are relevant to human nutrition and health. Identifying molecular targets breeders can use to enhance the iron bioavailability of staple food crops like dry beans can have a profound impact on the health and well-being of subsistence farmers in Africa, Latin America, and elsewhere.

Dr. Glahn is a highly effective and productive scientist with 175 peer-reviewed publications, attesting to his collaborative spirit. Since 1998, Dr. Glahn has served as an affiliate Professor in the Division of Nutritional Sciences and in the Department of Food Science, at Cornell U., where he taught a Current Readings in Iron Bioavailability course, and contributes to undergraduate and graduate research, and as a guest lecturer. He was recognized by his peers with Excellence in Research Awards from Poultry Science Association in 1987 and 1988, and from American Physiology Society in 1992. Dr. Glahn received the Early Career Scientist of the Year award from USDA-ARS, North Atlantic Area, in 1999. He is currently the Research Leader for the Plant, Soil and Nutrition Research Unit in Ithaca, New York. Dr. Glahn is an active and valued participant in the dry bean and pulse research communities through his many collaborations and contributions to nutrition physiology and trace mineral absorption and bioavailability.

## THE BEAN IMPROVEMENT COOPERATIVE

Proudly Presents the

Meritorious Service Award

to

## **Daniel G. Debouck**

CIAT Cali, Colombia

## Antonio M. De Ron

Spanish National Research Council (CSIC) Pontevedra, Spain

## **Distinguished** Achievement Award

to

## **Emmalea Ernest**

University of Delaware Newark, Delaware

## Consuelo Estévez de Jensen

University of Puerto Rico, Mayaguez Juana Diaz, Puerto Rico

## **Aldemaro Clara**

CENTA San Salvador, El Salvador

## **Technical Merit Award**

to

Antonia Palkovic University of California, Davis

Davis, California

in recognition of outstanding accomplishments relating to bean (Phaseolus) improvement

#### **DANIEL G. DEBOUCK**

Dr. Daniel Debouck is a CIAT emeritus scientist globally recognized for his efforts in germplasm conservation of Neotropical crop species, but more importantly, for his efforts made in the collection and study of Phaseolus germplasm across almost his entire career. Born in Belgium, Dr. Debouck obtained his Ing. (Ingénieur Agronome, 1976), M.S. (Tropical Plant Sciences, 1979), and Ph.D. (Plant Physiology with a minor in Ethnobotany and Plant Ecology, 1983) degrees from Gembloux Agro-Bio Tech (Université de Liège) in Belgium.

In June 1977, Dr. Debouck arrived at CIAT in Cali, Colombia, as a FAO Associate Expert in the Genetic Resources Unit, with main responsibilities in plant exploration and germplasm management. He conducted multiple germplasm collections mostly in Mexico, while training germplasm curators of national programs. In 1979, he went back to Belgium to finish his Ph.D. After graduation in 1985, Dr. Debouck returned to CIAT as a Post-Doctoral Fellow, where he carried out multiple germplasm collections in ~10 countries in Latin America and the United States. He was later promoted to Senior Research Fellow and chaired important global committees/groups devoted to the conservation of genetic resources (FAO, CGIAR, UNCED, UNEP, among others). By 1992, he was promoted to Senior Scientist, with notable scientific accomplishments across South and Central America. In 1996, he was given the great responsibility of managing the Genetic Resources Unit at CIAT, a germplasm bank with more than 67k accessions of beans, cassava, and tropical forages and a staff of more than 70 people. This is one of the most important and best-managed germplasm banks worldwide, and its impact across the globe and breeding programs has been demonstrated. Dr. Debouck's passion for germplasm, his outstanding management skills, and his attention to detail are reflected in the quality of the gene bank materials and their documentation in an online database. He also served as thesis advisor to numerous students from Latin America. He successfully managed this unit until his retirement in 2019. However, retirement is probably not the right term to use, given that Dr. Debouck continues to be actively engaged in research, germplasm exploration, teaching, advocating, and writing, in addition to some advising roles across several countries. In recent years, Dr. Debouck has been central in upgrading the CIAT germplasm bank to international standards, resulting in the building of Future Seeds (the new CIAT gene bank), which opened in March 2022.

For those who have not had the pleasure to work with Dr. Debouck, here are some accomplishments that illustrate the profound impact of his career: 41 plant explorations in 14 Latin American countries from 1977-2019; collection of ~3,300 samples new to world gene banks; author or co-author of 1 monograph (published in 2002 after 14 years of work, it is the most upto-date taxonomic catalog of *Phaseolus* species), 25 book chapters, 115 research papers, 12 conference proceedings, and 37 international reports; author of the largest database on Phaseolus species in the public domain of the world; 26 awards across the globe; significant field work in Ecuador and NW Peru that led to the identification of an ancestral branch of wild P. vulgaris, eventually acknowledged as a sister species of common bean (P. debouckii A. Delgado); described for the first time nine new species of bean in Mexico, five in Costa Rica, and one in Guatemala; contributions to document the presence of seven races in common bean; documented the fifth domesticated species in the genus Phaseolus based on field work in Guatemala; contributed to establish a double domestication in Lima bean because of field work in Central America, Ecuador and Peru; instrumental in the revision and rebuttal of a patent on a yellow bean; and many more...The Bean Improvement Cooperative acknowledges the accomplishments and his many contributions to the bean community, and his world-wide impact on germplasm conservation.

#### ANTONIO M. DE RON

Antonio De Ron was born in Lugo, Spain in 1952 and is an *Ad Honorem* Professor of the Spanish National Research Council (CSIC) at the Misión Biológica de Galicia (MBG) in Pontevedra, Spain. He completed his undergraduate and PhD degrees at the University of Santiago de Compostela (Spain) in 1974 and 1987.

Dr. De Ron began working as an INIA Postgraduate Researcher at the Forestry Center in Pontevedra and teacher in a Secondary School in Pontevedra. He started his career in the MBG as a Tenured Scientist in 1988, and was was promoted to Scientific Researcher in 2004, and Research Professor in 2008. He founded the Genetics and Breeding of Legumes research group in 1988 and later the Biology of Agrosystems group in MBG. In parallel, he was a lecturer at the U. of Santiago de Compostela (1990-2004), at the National Open U. (1991-2014) and professor of the Master's Degree in Genomics and Genetics at the U. of Santiago de Compostela-U. of Vigo.

He served as President of the Spanish Association for Legumes (2006-2012), was Coordinator of the CSIC in Galicia, Spain (2019-2021), and was President of the Science Society of Galicia. Internationally, he was active in Europe and America. In Europe, Dr. De Ron coordinated the PHASELIEU project (1998-2002), is a member of the European Association for Research in Plant Breeding (EUCARPIA) as Leader of the Protein Crops Working Group, and organized the Protein Crops Symposium in 1998 and 2015. Currently, he is an active member of the Grain Legumes Working Group of the European Cooperative Programme for Plant Genetic Resources involving scientists from European countries in an evaluation initiative for legumes, including common bean. In America, Dr. De Ron belongs to the Bean Improvement Cooperative and served on the Coordinating Committee (2001-2015). He cooperated for years with Argentina in bean germplasm and breeding and in the research of the bean-rhizobia symbiosis. He has played an important role in collecting and conserving the MBG's legume germplasm collection.

Dr. De Ron focused his scientific career on plant genetic resources, particularly in legume crops such as common bean, peas, and cowpeas. In these species, he selected cultivars to be transferred to the agri-food and feed sector. The main goal of this research on the interactions of bean-soil symbiotic microbiota is to produce new biofertilizers to improve N fixation in bean production, reduce the emission of greenhouse gases, prevent crop diseases and promote plant growth. He made international and national expeditions to collect legume varieties and thanks to him there is at the MBG an important collection of legumes, especially beans, in Spain. Dr. De Ron was one of the authors who identified novel genetic variation in bean from the Iberian Peninsula (Spain and Portugal), characterized by morphological traits, phaseolin protein, and allozymes. Obvious signs of introgression between the two gene pools of bean (Mesoamerican and Andean) were observed mainly among white-seeded genotypes. The intermediate forms adapted to the Iberian Peninsula could have emerged from initial recombination between the gene pools, and the Iberian Peninsula could be a secondary center of genetic diversity.

He is a co-author of more than 200 papers in both international and national scientific journals and is an editor of some national and international journals such as Frontiers in Plant Science. In 2015, he edited the book Grain Legumes, with 78 co-authors. Dr. De Ron has supervised many MS students and 16 PhD students, some of whom have gone on to successful careers. Finally, an important aspect of Dr. De Ron is his dedicated effort to disseminate scientific and technical knowledge to society, as an example, he has organized "Science Week" in Pontevedra since 2010.

#### **EMMALEA GARVER ERNEST**

Dr. Emmalea Ernest grew up in Lancaster County, Pennsylvania with an interest in all kinds of plants from a young age. Her first job at the age of 12 was at a native plants nursery as well as volunteering at the Landis Valley Museum Heirloom Seed Project. Before college, she worked at a number of regional nurseries as well as the Longwood Gardens. She received her B.S. degree from The Pennsylvania State University in 2001 in Horticulture. She then attended Michigan State University where she received an M.S. degree in 2004 in Plant Breeding and Genetics for efforts to improve Ecuadorian bush beans for anthracnose resistance using a farmer participatory approach. She received her Ph.D. in Plant Science from the University of Delaware in 2020 for studies on the "Physiological effects of high temperature and the genetic architecture of heat stress response in lima bean."

She has been in the Cooperative Extension Vegetable and Fruit Program since 2004, first as an Extension Associate, then as an Associate Scientist and Scientist. In July 2023, she was appointed as an assistant professor and Extension Vegetable Specialist. The Bean Improvement Cooperative was responsible for her seeking out her current position in Delaware. While a graduate student attending MSU, she heard Ed Kee speak at the Sacramento BIC meeting, and the rest is history.

Although she is a newly minted assistant professor, Dr. Ernest has served the bean community in many capacities since obtaining her M.S. at MSU. She likely knows more about lima beans than anyone else in the bean community. She works on many different vegetable and fruit crops in the DelMaVa region, but her lima bean research has been the most extensive and impactful. She supports a substantial processed lima bean production acreage in the region. After a lapse of 15 years, she reinitiated one of the few lima bean breeding programs in the U.S. in 2004. This program focuses on genetic improvement of both baby and Fordhook types with an emphasis on heat stress tolerance and various biotic stresses including downy mildew, nematodes, and white mold. Her lines are being trialed in California, the Midwest, Canada and along the eastern seaboard. She has promising baby lima lines to be released in the next two years.

Applied and basic lima bean research has been strengthened by the presence of Dr. Ernest's research program. She has been part of the effort to bring lima bean into the genomics era with the publication of a genome sequence of a baby lima type in 2021. She has several papers on downy mildew in lima beans and its control through genetic and cultural means. Dr. Emmalea Ernest has made many significant service and research contributions to the world common bean community, and the community is distinguished by awarding her the BIC Meritorious Service Award.

#### **CONSUELO ESTEVEZ DE JENSEN**

Dr. Consuelo Estevez de Jensen is a Professor and Plant Pathologist at the University of Puerto Rico, Mayaguez and is stationed at the Fortuna Station in Juana Diaz, PR. She was born in Quito, Ecuador and completed her undergraduate degree in Agronomy at Universidad Central del Ecuador in Quito. She is currently one of the few active common bean pathologists worldwide and has contributed extensively to the field.

Dr. Estevez was one of the first Bean/Cowpea Collaborative Research Support Program (CRSP) trainees, completing her M.S. degree at the University of Minnesota in St. Paul, MN with Dr. Peter Graham and subsequently returning to Ecuador to serve as the host country principal investigator from Ecuador on a CRSP project titled "Improving the symbiotic nitrogen fixation of cultivars of Phaseolus vulgaris under low-resource conditions." She served on the Technical Committee of the Bean/Cowpea CRSP and was nominated for the Outstanding Latin American Scientist award in 1993. From 1992 to 1995 she served as the Department Head of the Crop Protection Department in the National Agricultural Research Institute (INIAP) at the Santa Catalina Experimental Station. Dr. Estevez completed her Ph.D. and a Postdoc at the University of Minnesota. Her dissertation research showed that the combination of Rhizobium and Bacillus improves root rot control, nodulation, and nitrogen fixation in common bean.

In 2003, Dr. Estevez joined the Agricultural Research Station at the University of Puerto Rico where she has served as a leader in the study of biological nitrogen fixation (BNF) in common bean. She conducted workshops in several countries including Angola, Dominican Republic, Ecuador, Haiti, Honduras, Puerto Rico, and Mozambique. Her training and institutional capacity development efforts in Haiti resulted in the production of peat-based inoculants for over 20,000 subsistence farmers. Dr. Estevez has published on BNF methodology and the response of common bean to Rhizobium inoculation and continues to generate information needed for the release of improved germplasm and cultivars.

Her contributions to the common bean community include collaborations on diseases such as the root rot disease complex, common bacterial blight, angular leaf spot, and powdery mildew. She has served as a Co-PI in Bean/Cowpea CRSP and Legume Innovation Lab Projects in Central America and Sub-Saharan Africa. Her seminal work on root rots in common bean and her continued collaboration with researchers in this area have led to significant advances in our understanding of common bean resistance and the genetic structure of pathogens such as *Fusarium* spp. and *Macrophomina phaseolina*. In recognition of these achievements, she was awarded a Certificate of Appreciation in 2015 at the Common Bean Disease workshop in South Africa. Her close collaboration with breeders in the bean community and participation in the W-4150 has strengthened and accelerated the breeding efforts in the U.S. and in the Caribbean and Central American region.

Dr. Estevez established the first Plant Diagnostic Clinic (PRPDC) at the UPR Juana Diaz Experiment Station, a monumental task. The laboratory is fully accredited in the Plant Diagnostics Network and serves numerous crops produced in Puerto Rico, including common bean, and the winter nursery industry. She coordinates closely with the USDA-APHIS in Puerto Rico and the Virgin Islands to provide education on plant diseases in the region and was awarded for these contributions. In addition to common beans, Dr. Estevez conducts research and supervises the thesis research of students dealing with citrus greening and soybean diseases.

#### ALDEMARO CLARA

Mr. Aldemaro Clara has been the Salvadorian dry bean leader at the National Center for Agricultural and Forestry Technology (CENTA) since 2006. Aldemaro earned his B.Sc. in Agronomy starting at the Universidad Autonoma Antonio Narro (UAAAN) de Saltillo, Coahuila, Mexico, and finalizing his degree at the Universidad Tecnica Latinoamericana de El Salvador in 1996.

From 1996 to 2006, he participated in government and private company projects, providing technical assistance to subsistence farmers in the cultivation of corn, beans, sorghum, and vegetables, living in rural communities, and facilitating the management of crops. He also collaborated as a technician in projects with the FAO and USDA on soil conservation and organic management of basic grain crops and silvopastoral systems.

Aldemaro is a member of the Central American Bean Network (representing El Salvador). He collaborates closely with Zamorano University in conducting trials and nurseries with the goal of common bean improvement. He is also a member of the Latin American network of legumes, LatinRed, based in Bolivia. Aldemaro has effectively coordinated with international collaborators from CIAT Colombia, Zamorano University in Honduras, the University of Puerto Rico, and Michigan State University, where common bean improvement projects have been coordinated with funds such as CIAT Agro Health, Zamorano-UPR Climate Change, and Tortillas on the Roaster-CIAT.

Aldemaro presents his research regularly on genetic improvement in beans at the Programa Cooperativo Centroamericano para el Mejoramiento de Cultivos y Animales (PCCMCA) since 2008. His research includes selection of breeding lines beans with high iron and zinc content, evaluation of common bean germplasm for high yield with tolerance and resistance to diseases, and improvement of beans for drought in the dry corridor of El Salvador.

Aldemaro has a dynamic dry bean breeding program. He has developed new bean varieties formally released by CENTA. Aldemaro directly participated in the improvement of the small red varieties CENTA Ferromás (high in iron and zinc and tolerant to BGYMV), and CENTA Chaparrastique (heat tolerant and BGYMV resistant). Most recently, he released CENTA EAC "Enrique Alvares Córdova" resistant to angular leaf spot, web blight, and BGYMV, and tolerant to high temperatures and low fertility. Other releases are CENTA Drought (tolerant to drought and resistant to BGYMV), CENTA Costeño 2 (resistant to BGYMV), and the black seeded CENTA Tacuba (resistant to BGYMV and tolerant to high temperatures). With the release of these bean varieties, farmers have been assisted in improving their sustainable practices by reducing chemical applications to control pests and diseases, and increasing yields.

#### **ANTONIA PALKOVIC**

Antonia Palkovic completed her BS in Political Science at SUNY Purchase College in 2003 and MSc in International Agricultural Development at UC Davis in 2012, with subsequent work as a Junior Specialist in the rangeland watershed and agroecology labs at UC Davis. Antonia has worked with the Dry Bean Breeding Program at UC Davis for more than 10 years, as an Assistant Project Scientist and Associate Project Scientist. Since March 2016, she has also worked part-time on the Student Collaborative Plant Breeding Education (SCOPE) project, which was recently renewed.

Antonia's accomplishments have been both broad and deep with regards to their nature and impact. Antonia has been a co-author on several publications since her appointment in the dry bean program >10 years ago, which is indicative of her expertise in dry bean breeding and her consistent and longstanding contributions to dry bean research at UC Davis. Antonia is also skilled in data analysis and the writing-up of research results in a way that is clear and appropriately targeted for the respective audience. She is also of course consistently maintaining and advancing materials in all stages of the breeding process (from new crosses to advanced breeding lines with use of relevant checks).

She has also taken on a substantial role within a USDA NIFA Specialty Crop Research Initiative project in lima bean that Dr. Paul Gepts is leading, and has been making fantastic use of that opportunity to expand upon our pre-breeding/genetics work in collaboration with other lima bean researchers in the U.S. Antonia is an expert in the agronomy and breeding program field operations of multiple dry bean species, including limas, garbanzos, and common bean. She regularly makes well-attuned and appropriately nuanced recommendations with regards to management based on factors such as weather, goals of a given experiment, and availability of equipment or funding.

Antonia is highly professional and collegial when discussing with growers, field station managers, greenhouse managers, project team members, and students, among others. Antonia's power of observation is strong, and she has independently identified interesting germplasm and interesting phenomena therein. For example, a lima population with potentially helpful end-of-season characteristics that she has developed; a nested design that would complement an existing population; and relationships between pod size and seed size. She and team have also developed interesting lima bean cultivars through SCOPE that are being explored further.

To summarize, Antonia has exhibited tremendous breadth and depth of knowledge and experience with regards to the production and (relatedly) biology of grain legumes. She is generous in sharing her insights and does so in timely fashion even when balancing multiple tasks, often in multiple locations. She is an excellent mentor of students who interface with the breeding program. She has also conducted significant service—e.g., as a long-time member of the field facilities committee.

## **IN MEMORY ROGER KIRKBY**

Roger Kirkby was Director for CIAT Africa and an outstanding scientist specializing in agronomy. Early in his career he served in a project in Ecuador in Latin America, but the major part of his career was spent on the African continent.

In partnership with national research programs, sub-regional organizations, and funders, he greatly contributed to the establishment and growth of the Pan-African Bean Research Alliance (PABRA). His leadership of PABRA in its early years laid a foundation of partnership based on integrity and trustworthiness that permitted PABRA to grow into a participatory network involving some twenty-eight countries.

His work and dedication have left an indelible mark on bean research and development. His contributions to the bean research have been of great importance for advancing sustainable agriculture, food and nutrition security for millions of small holders in Africa. Based at one time in Ethiopia, he finished his career in Uganda where he retired and spent his last years.

In addition to his professional accomplishments, Roger will be remembered as a kind person, passionate about his work, and committed to the welfare of the scientific community. His ability to inspire others, his mentoring of young scientists, and his generosity in sharing knowledge were admirable qualities that will always be remembered. His contribution to science and his passion for bean research will continue to be a source of inspiration for future generations.

## INTERSPECIFIC CROSSES IMPROVE DROUGHT TOLERANCE IN ANDEAN COMMON BEAN

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**INTRODUCTION:** Common bean (*Phaseolus vulgaris*, L.) is the most important protein crop grown worldwide for human consumption, and approximately 60 % of world bean production is threatened by terminal and intermittent drought stress (Beebe et al, 2013). Drought conditions are projected to worsen in the coming years and are likely to have a disproportionately large detrimental effect on the world's poorest farmers. Common bean is sensitive to climatic and environmental fluctuations, whereas related species *P. montanus* and *P. acutifolius* evolved in harsh environments and exhibit drought tolerance capacity (Cruz et al, 2023). The CIAT bean program employs different breeding strategies to improve drought tolerance: intra- and inter-gene pool crosses among *P. vulgaris*, and interspecific crosses with species in the secondary and tertiary gene pools.

**MATERIALS AND METHODS:** For the present analysis, a total of 791 advanced lines were evaluated under irrigated and drought conditions in 2022 at CIAT Palmira (Colombia). The trial included 260 lines of the Andean elite nursery (VEF), 308 inter- or intra-gene pool lines (IGP), 223 interspecific lines (INT), and 4 Andean drought check lines. Grain yield and pod harvest index (PHI) were assessed.

**RESULTS AND DISCUSSION:** Grain yield in irrigated and drought environments was positively correlated and ranged between  $R^2=0.09$  for the IGP materials and  $R^2=0.39$  for the INT materials. Similarly, PHI in irrigated and drought conditions was positively correlated and ranged between  $R^2=0.64$  for the VEF and  $R^2=0.79$  for the INT. These results indicate that selection for performance traits under drought conditions can also result in good performance in favorable environments. The higher  $R^2$  values obtained for the INT materials indicate their superior performance under drought stress. Indeed, grain yield in 13 INT lines exceeded the average yield of the controls by 20 % under drought, and 33.6 % of lines performed better than the check genotype CAL 96. We surmise that drought conditions resulting in more stable yield performance than in the VEF or IGP materials across favorable and harsh environments. Incorporating interspecific germplasm into advanced breeding lines will improve drought tolerance in common bean and contribute to food and nutritional security in the face of climate change.

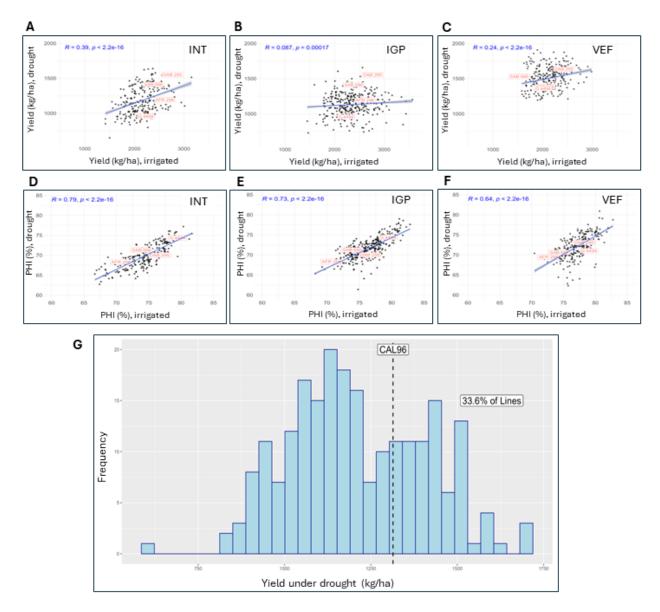


Figure 1. Yield (kg/ha) under irrigated versus drought conditions for three breeding strategies, A. interspecific (INT), B. inter- and intra- genepool (IGP), C. Andean elite nursery (VEF). Pod harvest index (PHI) under irrigated versus drought conditions for three breeding strategies, D. interspecific (INT), E. inter- and intra- genepool (IGP), F. Andean elite nursery (VEF). AFR298, SAB686, DAB295 and G4494 are drought check genotypes. G. Histogram of yield (kg/ha) under drought conditions for the inter- and intra- specific genepool genotypes. CAL96 is a check commercial cultivar.

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## A COLLABORATION TOWARDS THE COMPREHENSIVE IMPROVEMENT OF LIMA BEANS: ADDRESSING CONSUMER INFORMATION, PRE-BREEDING, AND GERMPLASM INFORMATION/UTILIZATION BOTTLENECKS

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Lima bean (*Phaseolus lunatus* L.), also known as butter bean, is one of five domesticated species of *Phaseolus* beans (Parker and Gepts 2021). Limas were independently domesticated in the Andes (flat, large-seeded types) and Mesoamerica (round, small-seeded or flat, medium-seeded types) (Gutiérrez Salgado et al. 1995) and are generally adapted to warmer climates, from dry to wet. Limas are part of the quaternary gene pool for common bean (*P. vulgaris*), with no reports of successful crosses between the two species (Porch et al. 2013). However, the two species are highly syntenic, with only a few major structural rearrangements in sequenced genomes (Garcia et al. 2021, Wisser et al. 2021). Limas are cultivated both as bush and vine types in multiple regions of the U.S. For example, California growers produce large- and baby-seeded dry limas, whereas mid-Atlantic growers produce succulent limas (primarily for frozen or canned consumption, with smaller-scale fresh-market production; USDA-NASS 2019). In a project funded by the USDA NIFA Specialty Crop Research Initiative, we are addressing three major bottlenecks (as depicted and described below) that currently constrain lima production/consumption and breeding.

**Objective 1: Consumer Information Bottleneck:** Investigate consumer views of lima varieties as dry beans or processed/fresh-market vegetables and promote awareness of their nutritional and culinary qualities.

Surveys are being prepared to assess perceptions of limas by growers, processors, culinary/ food service professionals, and



consumers, among other agricultural and food systems practitioners. Collaborations are also in the planning stage for sensory evaluation and/or consumer research of varieties and advanced-generation lines from participating breeding programs (for both dry and succulent limas) and of diverse accessions from the USDA National Plant Germplasm System (NPGS) collection. The project team is conducting education/outreach in partnership with growers, extension specialists, and culinary and communications experts. The breeding programs will be incorporating feedback from surveys alongside sensory evaluation and/or consumer research as they select parents and

crosses for use in their programs.

**Objective 2: Pre-Breeding Bottleneck:** *Develop early-generation, adapted breeding pools through germplasm conversion.* Approximately 90% of the lines within the USDA NPGS and other germplasm collections (e.g., Monteros-Rojas et al. 2013) are thought to be photoperiod-sensitive—i.e., will not flower under long days (standard growing conditions for limas in the continental U.S.). This biological constraint hinders the use of diverse lines in crosses and evaluation of those lines for potentially useful traits observable during or after flowering. Unadapted and adapted lines are being crossed to convert germplasm from photoperiod-sensitive to day-neutral. Progeny from these crosses will be selected using genetic markers developed in this project, building upon previous work in common bean (Parker & Gepts 2021) and lima bean (Lozano-Arce et al. 2023), for key adaptation/domestication traits including photoperiod insensitivity; bush, determinate growth habit; and white or green seed color (for dry and succulent limas, respectively).

**Objective 3: Germplasm Information/Utilization Bottleneck:** *Genomic, genotypic, and phenotypic characterization of the lima germplasm.* Genotyping and comprehensive field and laboratory-based phenotyping of the USDA NPGS lima collection are being conducted for agronomic (including key adaptation), grain nutritional quality (e.g., protein, fiber, starch, fat) and anti-nutritional (cyanogenic glucoside) traits, as well as nematode resistance. The photoperiod-sensitive nature of much of the collection has required additional steps: namely, multiple rounds of low-yielding seed increases in the greenhouse and testing of a potential winter nursery location in southern California (short-day conditions). Whole-genome sequencing of a small number of key lines is being conducted. The information will be integrated into the USDA-ARS Germplasm Resources Information Network (GRIN) and the Legume Information System (LIS).

<u>Within and across these objectives:</u> Activities taking place in this project that could feed into synergistic efforts as a broader bean community include nutritional quality phenotyping, genomic and phenomic selection, testing of genetic markers and/or sensing-based proxies for key adaptation genes/traits, establishment of a trait ontology, speed breeding, evaluation of progeny from unadapted-by-adapted crosses, and development of product profiles targeting producer and consumer acceptability of lima varieties.

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## MARKER-ASSISTED SELECTION TO REDUCE POPULATION SIZES AND TIME NEEDED FOR BACKCROSSING TO PRODUCE NON-DARKENING CRANBERRY BEANS

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#### **INTRODUCTION**

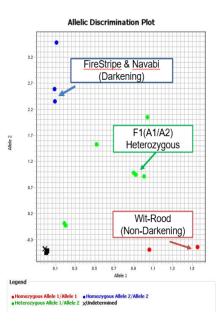
Post-harvest seed coat darkening is a detrimental trait that affects the appearance and cooking time of several market classes of beans, including cranberry beans. The dark background colour that develops in the bean during storage occurs in beans with a dominant allele for the Joker (J) gene (Basset 1996) that results in the accumulation proanthocyanidins (PA) in the seed coat (Beninger et al. 2005). Erfatpour and Pauls (2020) showed that the recessive allele in Witrood, a non-darkening genotype, was associated with a single nucleotide deletion in the Phvul010G130600 gene, which encodes a R2R3-MYB transcription factor that controls the expression of genes that encode enzymes in the phenylpropanoid pathway and developed a dominant marker for the dominant *J* allele. The current study describes the use of a biallelic KASP marker and background markers to select for heterozygous individuals with elevated levels of the recurrent parent genotype in a backcross crossing scheme to introgress the non-darkening trait into elite cranberry genotypes.

#### **MATERIALS AND METHODS**

F<sub>1</sub> progeny was obtained from crosses between high yielding, large seeded and disease resistant darkening cranberry beans lines (OAC Firestripe, and OAC Navabi) and the non-darkening cranberry bean (Witrood boontje). A Kompetitive Allele Specific PCR (KASP) co-dominant marker was developed from the SNP marker (G/-) developed by Erfatpour and Pauls (2020) for the darkening allele of Phvul.010G130600. Allele-specific forward KASP primers [A1 (with the fluorophore VIC to report the non-darkening allele), A2 (with FAM to report the darkening allele.)] and a common reverse primer were used. End-point fluorescent reading of the PCR products was performed with an Applied Biosystems QuantStudio instrument equipped with SNP allele calling software.

#### **RESULTS AND DISCUSSION**

A test of the KASP marker with parental DNA and DNA isolated from an  $F_1$  showed that the assay could discriminate between alleles and identify heterozygous individuals (Figure 1).



**Figure 1.** Allelic discrimination plot for KASP marker

The KASP assay of DNA isolated from BC1 individuals identified heterozygous individuals for the J gene (Figure 2).

The next step in the marker assisted selection is to conduct a background marker evaluation of the heterozygous (Jj) individuals by SNP profiling. Those with the largest percentage of recurrent parent alleles lines (from OAC Firestripe, and OAC Navabi) will be used for further rounds of backcrossing (Figure 3).

## CONCLUSIONS

With molecular markers for the nondarkening recessive allele of the J gene and SNP markers for the background alleles of

the recurrent parent(s) the non-darkening trait can be efficiently moved into elite cranberry germplasm.

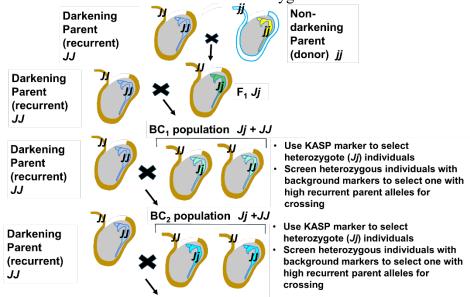
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Allelic Discrimination Plot FireStripe & Navabi 4.2 (Darkening) 3.7 3.2 2.7 BC1(A1/A2) Allele 2 Heterozygous 2.2 1.7 1.2 Wit-Rood 0.7 (Non-Darkening) 0.2 X Allele 1

**Figure 2.** KASP marker identification of BC1 individuals heterozygous for with J.



**Figure 3.** Genotypes of seedcoat and embryo tissues in a marker, assisted backcrossing scheme.

Changes in polyphenols of the seed coat during the after-darkening process in pinto bean (*Phaseolus vulgaris* L.). J Agric Food Chem 53:7777–7782

Erfatpour, M. and Pauls, K. 2020. A R2R3-MYB gene-based marker for the non-darkening seed coat trait in pinto and cranberry beans (*Phaseolus vulgaris* L.) derived from 'Wit-rood boontje'. Theoretical and Applied Genetics, 133(6), pp.1977-1994.

## GENOTYPE-SPECIFIC TRANSCRIPTIONAL DYNAMICS IN THE EARLY ROOT DEVELOPMENT OF COMMON BEAN

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## INTRODUCTION

The root system of common beans (*Phaseolus vulgaris*) is essential for plant growth and environmental interactions, especially during early development and seedling establishment where it must overcome various biotic and abiotic stressors. Root development involves complex cellular processes within specific developmental zones such as the meristematic, elongation, and differentiation zones. The apical unbranched zone, extending from the root tip to the emergence of lateral roots, encompasses these critical root zones and the length of the apical unbranched zone ( $L_{AUZ}$ ) acts as an indicator of growth rate (Pellerin and Tabourel 1995). Middle American genotypes, such as Stampede, tend to exhibit root traits conducive to drought and pathogen tolerance, including a larger root biomass, shorter  $L_{AUZ}$ , and increased adventitious roots when compared with Andean genotypes like Red Hawk (Strock et al. 2019; Haus et al. 2020). However, the underlying molecular mechanisms governing these distinctions across genotypes and developmental stages remain understudied. This study focuses on the genotype-specific transcriptional dynamics in the early development of the root apical unbranched zone in two common bean genotypes, Red Hawk and Stampede (dark red kidney and pinto, respectively).

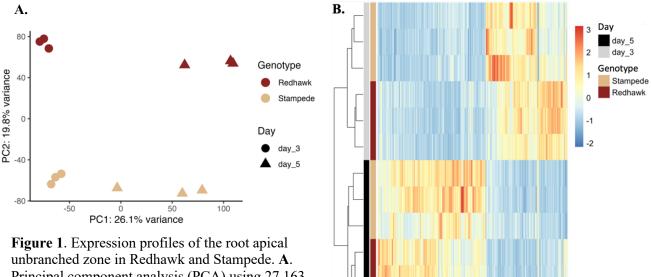
## **MATERIALS AND METHODS**

Redhawk and Stampede seeds were surface sterilized and grown in vermiculite in a Bioscience growth chamber under a 14-h light cycle at 25/20°C day/night. Plants were grown for 3 or 5 days in 48-cell pots and 1-gallon pots, respectively. Root sampling was performed each day between 1 and 3 pm. The apical unbranched zone was dissected, frozen in liquid nitrogen and ground for 35 seconds in a bead tissuelyser. Three replicates per timepoint and genotype were used, and four root systems were combined for each experimental replicate. Total RNA was extracted from the root tissues using a hot borate extraction protocol and RNA libraries were prepared using Lexogen's QuantSeq 3' mRNA-Seq Library Prep Kit FWD. For data analysis, we conducted gene expression quantification using the RNASeqV2 pipeline (https://github.com/pardojer23/RNAseqV2). Subsequently, differentially expressed genes across genotypes were identified utilizing the DESeq2 R package (Love, Huber, and Anders 2014). Functional enrichment analysis was conducted using Over Representation Analysis (ORA).

## **RESULTS AND DISCUSSION**

A total of 27,163 transcripts were used for gene expression analysis. Differentially expressed genes distinctly grouped by both developmental stage and genotype, with developmental stage accounting for most of the variance (Fig. 1). Comparative genotype analysis indicated a preservation of biological functions during development and simultaneously identified unique functional priorities by genotype. At day 3 post-germination, Red Hawk prioritized cell wall modification genes linked to reactive oxygen species (ROS), suggestive of an early developmental focus on cell elongation mechanisms. Expansins, which are proteins that facilitate cell wall

extension, and peroxidases, which play a role in cell wall loosening, were significantly enriched. Stampede prioritized biotic and abiotic stress responses at day 3, with an upregulation of genes in the disease-resistance protein (TIR-NBS-LRR class) and pathogenesis-related protein (PR10) families, including PvPR10-9 which has showed high expression in roots and association to abiotic stress and phytohormones responses in common bean (Feki et al. 2024). By day 5, the shift in Red Hawk towards cell-wall biosynthesis and signal transduction genes, including those for small GTPases involved in root tip growth, contrasted with Stampede's upregulation of disease resistance and regulatory genes.



unbranched zone in Redhawk and Stampede. **A**. Principal component analysis (PCA) using 27,163 transcripts. **B**. Heatmap representing 1,246 differentially expressed genes. The colored bar indicates normalized gene counts (Z-score values).

# CONCLUSIONS

This study underscores the dynamic nature of root development in common bean, revealing distinct transcriptional priorities at different developmental stages across genotypes. It also provides insights for crop improvement strategies within the bean community, particularly for breeding programs that consider root traits to enhance stress tolerance.

Day

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# GENOME WIDE ASSOCIATION STUDIES (GWAS) FOR TRAITS RELATED TO UPRIGHT PLANT ARCHITECTURE IN DRY BEAN (*PHASEOLUS VULGARIS* L.)

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**INTRODUCTION:** Growth habit is one of the most important domestication traits in dry bean (Phaseolus vulgaris L.). In the U.S., Type II, indeterminate upright varieties have helped farmers to switch to direct harvest during the last ~20 years. Previous work suggests that stem diameter and plant height are several of the most important traits to obtain upright plant architecture in some dry bean market classes. However, besides its phenotypic importance, knowing growth habit's genetic background is highly relevant to detect possible regions or genes related to the trait of interest. So, selection of upright plants is not only based on phenotypic traits, which could lead to errors, but is also based on genomic tools. Using genotypes with different growth habits and performing genome-wide association studies (GWAS), Moghaddam et al. (2016) found a strong signal on chromosome Pv01 for growth habit. The same peak was found using only Type I genotypes. However, when only indeterminate Type II and Type III genotypes were used, the most relevant peak was found on chromosome Pv07. This suggested different regions in the genome controlled determinate and indeterminate growth habits. Despite recent research, there is still a gap in understanding the influence on selection of upright genotypes in commercial breeding programs. Thus, understanding the genetic architecture of these traits would facilitate better upright plant selection based on phenotypic characteristics. Thus, this study aimed to find genetic regions related to plant height and stem diameter using a GWAS approach.

**MATERIALS AND METHODS:** A total of 262 black, navy, great northern, red and pink, pinto, and slow-darkening (SD) pinto beans breeding lines and cultivars were tested in replicated advanced yield trials (AYT) at different locations of North Dakota, during 2020, 2021 and 2022. Plant height (cm) and stem diameter (mm) measured right above the soil surface at maturity, were measured. DNA was isolated from all breeding lines and cultivars using the Mag-Bind® Plant DNA Plus kit. GBS libraries were developed using the protocol described by Schröder et al., (2016), and submitted for sequencing to HudsonAlpha institute for Biotechnology, Hunstville Alabama, USA. The reference genome UI 111 version 1.0 was used for this study. After filtering for SNP quality, 218 genotypes were used in the analysis. To perform the association mapping for both traits, the Genome-wide Efficient Mixed Model Analysis (GEMMA), and a compressed mixed linear model within the GAPIT R package were used. The lower mean square deviation (MSD) and q-q plots were considered to select the best models for further analysis. Evaluation of genes in the genomic regions  $\pm$  50 Kb from the significant SNP peaks were identified using the genome annotation of the V1.1 of the *P. vulgaris* UI 111 reference genome. Genes within those regions were investigated through literature searches.

**RESULTS AND DISCUSSION:** Based on the mean squared deviation (MSD) values and the qq plots, the best models were GAPIT MLM for plant height and GEMMA MLM for stem diameter. For plant height, significant peaks were found on chromosomes Pv03 and Pv07 (Figure 1).

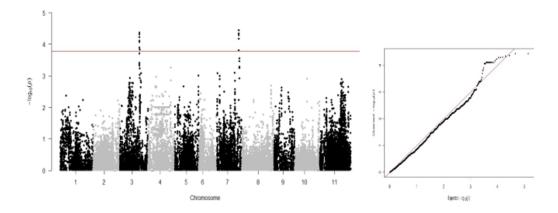


Figure 1. Manhattan plot and q-q plot for plant height using MLM in GAPIT

For plant height, the peak with the most significant signal was related with the SNP S07\_40'778.504 found on chromosome Pv07, between 40.7 and 40.8 Mb, and with a p-value of 3.60E-05. In this region, five SNPs surpassed the 9.29E-05 threshold. One of the most interesting gene models found in this region was PvUI111.07G214700.1, which is a growth-regulating factor. The second most important peak was found for the SNP S03\_36745938, with a p-value of 4.29E-05 located on chromosome Pv03, between 36.7 and 36.9 Mb. Some of the most important gene models located in this region were PvUI111.03G154900.1, related to a serine/threonine protein kinase, and PvUI111.03G157000.1 and PvUI111.03G157100.1, both associated with an F-box associated domain (FBA-3).

For stem diameter, significant regions were found on chromosomes Pv11 and Pv07 respectively. On chromosome Pv11, the SNP with the lowest P value (1.61 E-05) was S11\_8564171. Located around this position, gene models related to cyclin-dependent kinase regulatory subunit (CKS1) and apoptosis inhibitor//Ring//U-Box domain-containing protein were found. On chromosome Pv07, the region between 34.5 and 34.6 Mb showed genes models with functions related to gibberellin receptor GID1, WD repeat containing protein and leucine rich repeat proteins. Also, on Pv07, a single SNP with a p-value of 6.49E-05 was found in a region close to 31.4 Mb. In this region, some gene models encoded proteins related to cell division, ubiquitin, and serine/threonine protein kinase, respectively. In addition, the region ~40.7 Mb was also found to be significant. This region is highly significant for both stem diameter and plant height. Thus, this region becomes an interesting point to continue with further studies for plant architecture, especially for indeterminate growth in dry bean. This study was supported by the USDA-ARS Pulse Crop Health Initiative and Northarvest Bean Growers Association.

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#### GENOME-WIDE ASSOCIATION STUDY AND IDENTIFICATION OF CAUSAL ALLELES AT *Ur-11* LOCUS CONTROLLING RUST RESISTANCE IN COMMON BEAN

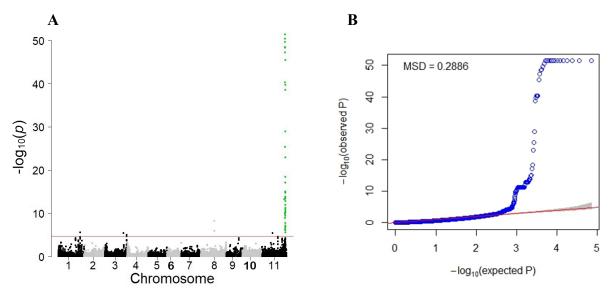
# Mohammad Erfatpour<sup>1</sup>, Kristin J. Simons<sup>2</sup>, Jose Figueroa-Cerna<sup>1</sup>, Rian Lee<sup>1</sup>, Phillip E. McClean<sup>1</sup>, Juan M. Osorno<sup>1</sup>

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INTRODUCTION: The Middle American common bean (Phaseolus vulgaris L.) rust resistance gene Ur-11 confers resistance to all known races of the pathogen Uromyces appendiculatus (Pers.: Pers.) Unger, except one (race 22-52, formerly race 108) (Pastor-Corrales et al. 2007). Therefore, deploying the Ur-11 gene in combination with other rust-resistance genes has been proposed as the most cost-effective strategy for controlling the highly variable rust pathogen in common bean. The Guatemalan black beans PI 181996 and PI 190078 are known as the original sources of the Ur-11 gene in the Middle American gene pool (Pastor-Corrales et al., 2007) and PI 181996 resistance was predominantly introgressed into common bean germplasm (Pastor-Corrales et al., 2003). The Ur-11 locus was mapped on chromosome Pv11 linked to Ur-3 (Miklas et al. 2006). Fine mapping localized Ur-3 at ~47 Mb on Pv11 of P. vulgaris G19833 reference genome (Hurtado-Gonzales et al. 2017). A genome-wide association study (GWAS) identified a genomic region associated with Ur-11 from 50.5 to 52.2 Mb on Pv11 of the G19833 reference genome v2.1 (Monclova-Santana 2019) which corresponds to a genomic region from 54 to 56 Mb on Pv11 of the *P. vulgaris* UI111 reference genome assembly v1.1. Even though great progress has been made in understanding the host:pathogen interactions between common bean and U. appendiculatus, the causal alleles of most rust resistance loci, including Ur-11, remain unknown. This study aimed to i) validate genomic regions associated with the Ur-11 locus conferring resistance to U. appendiculatus in Middle American beans, ii) determine a variant or set of variants in candidate genes that might identify it as the Ur-11 gene, and iii) develop a gene-based marker that can be utilized in marker-assisted selection in early stages of a breeding program for rust resistance.

**MATERIALS AND METHODS**: A panel of 352 Middle American type breeding lines from the North Dakota State University dry bean breeding program and 10 cultivars and germplasm lines of different market classes known to possess the *Ur-11* gene derived from PI181996 were evaluated for reaction to race 31-22 (previously known as race 67) in the greenhouse, as described by Acevedo et al. (2013). Rust severity from 14-day-old single plants was evaluated using a 1 to 9 scale (Van Schoonhoven and Pastor-Corrales 1987). A set of approximately 71k imputed SNPs from genotype-by-sequencing reads of 362 middle American-type bean genotypes was used for association mapping. The SNPs were filtered for a minor allele frequency of 0.05 for GWAS analysis. Manhattan and quantile-quantile plots were created using the R package qqman (Turner 2018). The Phytozome database was used to identify candidate genes (https://phytozome-next.jgi.doe.gov). Candidate genes were determined when the significant SNP was in the gene or located within a 100 Kb region from the gene. Multiple DNA sequence alignments were performed with Integrative Genomics Viewer.

**RESULTS AND DISCUSSION:** GWAS uncovered a significant locus on Pv11 tagged by 18 markers in the 55.16-56.33 Mb region of the P. vulgaris UI111 reference genome assembly v1.1, thereby confirming previous findings (Figure 1). Within the genomic interval, S11 55,167,642 and S11 55,458,849 showed the strongest association with the trait explaining 29% of the phenotypic variation. Candidate gene searches around the major locus revealed the presence of several leucine-rich repeats containing protein and receptor protein kinase coding genes. Multiple DNA sequence alignments of the candidate genes in 11 rust-resistant genotypes with 30 susceptible genotypes detected a missense mutation [c.1,328A>G] in the candidate gene PvUII11.11G202400 that results in the substitution of cysteine for tyrosine at position 443 of the protein coded by the allele in resistant genotypes. A PCR allele competitive extension (PACE) marker was developed and tested across a panel of ~650 bean genotypes composed of cultivars and breeding lines and ~300 Middle American Diversity Panel. No recombination event was observed for the marker among the test individuals; indicating that the polymorphism on which it is based is very close to or in the gene responsible for the resistance to rust pathogen race 31-22 in common bean. This marker can facilitate the selection of Ur-11 bean genotypes at the early stages of plant development.



**Figure 1.** (A) Manhattan plot highlighting SNPs associated with the *Ur-11* locus in the 55.16-56.33 Mb region of Pv11 of the *P. vulgaris* UI111 reference genome assembly v1.1, with (B) Q-Q plot showing substantial deviation from the diagonal for highly trait-associated SNPs. *P. vulgaris* chromosomes (1-11) are represented on the x-axis, and a -log 10 (p) values are shown on the y-axis. The red line indicates the threshold at a significance value of -log10(p)=4.7.

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# GENETIC ARCHITECTURE OF ANTHRACNOSE RESISTANCE IN THE YELLOW BEAN COLLECTION OF COMMON BEAN

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#### INTRODUCTION

Anthracnose caused by *Colletotrichum lindemuthianum* is a major disease of common bean (*Phaseolus vulgaris*) worldwide. Yellow beans are a major market class of common bean especially in eastern and southern Africa. The objectives of this study were i) evaluate the yellow bean collection for resistance to eight races of *C. lindemuthianum*, and ii) conduct genome-wide association analysis to identify genomic regions and candidate genes associated with resistance to eight races of *C. lindemuthianum*.

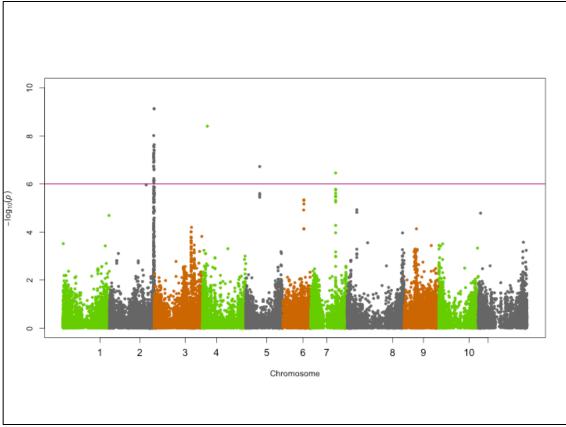
#### MATERIALS AND METHODS

In this study, the Yellow Bean Collection (YBC) consisting of 255 diverse genotypes with variable yellow seed colors, was utilized. The cultivars G2333 and Kabulangeti served as resistant and susceptible checks, respectively. Eight races (5, 19, 39, 51, 81, 183, 1050 and 1105) of *C. lindemuthianum*, characterized from isolates in Zambia, representing a broad virulence spectrum were used. Inoculation followed a standardized protocol, utilizing Styrofoam trays with a randomized design and three replications. Anthracnose severity was scored based on a CIAT scale. Statistical analysis of disease severity scores was performed using a mixed model approach in SAS 9.3. The YBC was genotyped with 72,866 SNPs using Genotyping by Sequencing. Population structure correction used Principal Component Analysis, and kinship was addressed with the Identical by Descent method. Association analysis for anthracnose resistance involved a Mixed Linear Model, with significant SNPs determined by Bonferroni-corrected p-values. Candidate genes within 400 kb of significant SNPs were identified from Phaseolus vulgaris v2.1 in Phytozome based on their functional roles in disease resistance.

#### **RESULTS AND DISCUSSION**

Identification of resistance sources within the yellow bean class is crucial for genetic enhancement considering the challenges in maintaining yellow color and seed size in progeny. The study confirmed substantial resistance in the YBC, with genotype YBC278 exhibiting exceptional resistance to all eight races, akin to the highly resistant check G2333. YBC278, presents a promising source for developing anthracnose-resistant yellow bean varieties. Additionally, YBC130 and YBC267 showcased notable resistance and could serve as alternative resistance sources. Genome-wide association analysis revealed major-effect loci on chromosomes Pv01, Pv02, Pv04, Pv05 and Pv07 as responsible for resistance in the YBC to the eight races. Notably, Pv01 harbored a major-effect QTL which overlapped with the Andean locus *Co-1* and conferred resistance to races 81, 1050 and 1105. Significant SNPs for resistance to race 39 were identified on Pv02. The genomic region on Pv04, which overlaps with known major-effect loci *Co-3*, *Co*-

*15*, *Co-16*, *Co-y* and *Co-z*, provided resistance to races 5, 19, 51 and 183. Additionally, novel major QTL on Pv05 and Pv07 were identified as additional genomic regions for resistance to race 39, complementing previously known resistance regions on Pv02 and Pv04. Plant resistance genes (R genes) with NB-ARC and LRR domains, which occurred in clusters, were identified as positional candidate genes for genomic regions on Pv02 and Pv04. These findings provide valuable insights for breeding programs targeting durable anthracnose resistance in yellow beans, with implications for broader disease resistance in common bean varieties.



**Figure 1**. Manhattan plot showing SNPs on chromosome Pv02, Pv04, Pv05 and Pv07 significantly associated with resistance to Andean anthracnose race 39 of the YBC genotypes evaluated in the greenhouse at University of Zambia, Lusaka, Zambia. The red solid horizontal line is the Bonferroni adjusted P-value (1.0E-06).

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# IDENTIFYING RESISTANCE TO ANTHRACNOSE IN ANDEAN BUSH BEAN LINES WITH HIGH-VALUE MARKET GRAIN TYPE

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## **INTRODUCTION**

Anthracnose, caused by the fungal pathogen *Colletotrichum lindemuthianum*, is a significant threat to common beans (*Phaseolus vulgaris*) worldwide, leading to substantial yield losses and threatening food security. Identifying resistance in Andean genotypes with market preferred commercial grain types has been a challenge. This study aimed to identify resistant genotypes in a panel of red and red mottled Andean bean lines developed by CIAT's Breeding Program, using greenhouse screening with individual anthracnose races. The results obtained showed that a reduced group of lines hold promising levels of resistance to multiple races and represent a valuable resource for regional breeders to improve the resistance level of the next generation of commercial varieties.

#### MATERIALS AND METHODS

Two hundred and twenty-nine advanced lines, including red and red mottled Andean bean genotypes, were infected under greenhouse conditions at CIAT-Palmira at the V2 stage using anthracnose strains race 7 (Cl2) and race 1097 (Cl114). La victorie was used as susceptible check, and severity was rated using the CIAT scale 1-9. A second trial was performed using a smaller group of 33 genotypes with resistant (score 1-3) and intermediate (score 4 -5) responses to Races 7 and 1097. This set was screened with race 393 (Cl48) and 19 (Cl326). Parental lines DAB583 and DAB65 were included, along with reference grain type genotypes CAL 143 and CAL 96.

#### **RESULTS AND DISCUSSION**

Results obtained from the first trial showed that Race 7 was the most aggressive, inducing susceptibility in 76% of the genotypes, with only 18.4% of the genotypes classified as resistant. On the other hand, 95% of the genotypes were resistant to Race 1097, demonstrating that the resistance gene(s) present in this nursery is more effective in controlling disease to Race 1097 than Race 7 (Figure 1). In the second trial, most of the tested lines were resistant to Race 19 and Race 393, although 9 lines were susceptible to Race 393 and only 2 were susceptible to Race 19, suggesting differences in the genetic base of the resistance to these two races.

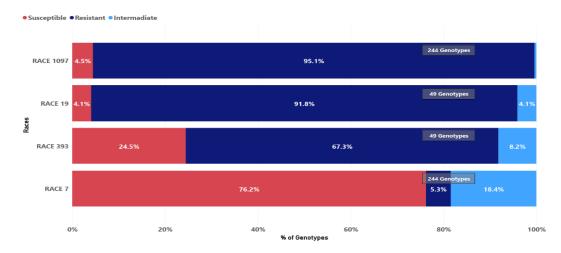


Figure 1. Number of genotypes resistant to different Anthracnose races.

The next step was to identify lines that could resist the infection of all 4 races. This comparison showed that 5 lines were resistant to all tested races with an average score <3.0 and all of them had a solid red grain color (Table 1). Surprisingly, although Race 7 seemed like the most aggressive, the Race7-resistance from two lines was broken by Race 393. Only 2 red-mottled lines were found to be highly resistant to 3 out of 4 races.

Genotype	Code	Race 1097	Race 7	Race393	Race 19	Pedigree-Description	Seed Color	Secondary color	Seed weight(100)
22ACC02517.000	ARD00070CIC	1.1	2.9	1.2	1.7	DAB600 x (BNA26 x BNA9)	6		49.4
22ACC02601.000	ARD00055CIC	1.4	1.9	1.5	1.3	BNA22 x BNA9	6		42.9
22ACC02843.000	AFR298	1.2	2.8	1.6	1.5	G6592 x A487	6		44.3
22ACC03251.000	ARD00033CIC	1.5	2.9	2.3	1.2	DAB65 x NAR10	6		47.7
22ACC03333.000	ARD00064CIC	1.1	2.9	1.3	2.0	DAA209 x (BNA22 x BNA9)	6		45.4
22ACC02199.000	ARD00062CIC	1.6	2.4	7.5	1.5	DAA209 x (NUA410 x DAB65)	6		51.1
22ACC02407.000	ARM00103CIC	1.5	2.8	3.4	1.6	DAB577 x (BNA22 x BNA4)	6	2	43.8
22ACC03243.000	ARM00104CIC	1.4	2.7	6.5	2.0	DAB65 x (NUA410 x DAB577)	6	2	39.7
22ACC03261.000	ARD00002CIC	1.3	2.6	4.2	1.3	DAB65 x G51144	6		39.4
CAL143		1.6	1.9	8.2	2.5	Commercial variety	6	2	35.0
DAB65		1.6	2.6	7.9	1.4	Parental Line	6		43.0
CAL96		1.3	8.9	1.2	1.6	Commercial variety	6	2	50.7
DAB583		1.5	3.9	1.8	1.4	Parental Line	5	7	42.4
La Victorie	N/A	8.8	8.7	8.7	7.9	Susceptible Check	N/A	N/A	N/A

 Table 1. Description of the most resistant Andean Bush bean lines.

# CONCLUSIONS

This study highlights the identification of promising anthracnose-resistant genotypes in the CIAT-Palmira Andean bush bean panel. Some of the lines will be evaluated in African countries to validate their resistance using their local anthracnose inoculum. These findings underscore the relevance of prioritizing Anthracnose screening that target lines with attractive commercial grain types.

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# UNCOVERING NEW GENOMIC REGIONS ASSOCIATED WITH WHITE MOLD RESISTANCE IN DRY BEANS

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# **INTRODUCTION**

Dry bean (*Phaseolus vulgaris* L.) is one of the major plant-based protein sources for human consumption. The US ranks third in production globally, with North Dakota as the top producer (~40%), mainly of pinto beans. Conversely, white mold (WM), caused by *Sclerotinia sclerotiorum* Lib de Bary, can significantly reduce seed yield of pinto beans, especially in humid temperate climates. Managing WM can incur high costs; thus, genetic resistance in new cultivars is crucial. To address this, a WM Multi-parent Advanced Generation Inter-cross (WM-MAGIC) population was developed at North Dakota State University (Escobar et al., 2022).

# MATERIALS AND METHODS

A population of 1040 lines from the WM-MAGIC population (Escobar et al., 2022) was used for this study. Phenotypic data was obtained under greenhouse conditions utilizing the seedling straw protocol by Arkwazee and Myers (2017). Genotypic data was obtained via genotype-bysequencing (GBS). Raw reads were processed to remove low-quality reads. High-quality reads were aligned to the common bean reference genome UI111 v1.1 ended with approximately 202,000 SNPs. The final HapMap was generated including SNPs with a minor allele frequency (MAF) > 0.01 and heterozygosity < 0.15. For GWAS, white mold ratings on a scale of 1-9 were transformed into two polynomial and one binomial phenotype distributions. Polynomial scale with scores of 1-5 and a scale 0-2 (resistant = 0, tolerant = 1, and susceptible = 2), were utilized. Additionally, a binomial distribution was employed to distinguish between resistant and susceptible genotypes. The genome-wide association study (GWAS) was performed with the genome-wide efficient mixed model association (GEMMA) software. Population relatedness and population structure (2 principal components) were included into the mixed linear model. The estimation of the cutoff threshold for detecting significant SNPs (*P-value*  $\leq 0.05$ ) was calculated by dividing the *P-value* with the effective number of independent tests (M<sub>eff</sub>), estimated with the equation proposed by Li and Ji (2005). Candidate genes within a genomic range of  $\pm 50$  Kb from the significant peak SNPs were examined utilizing the genome annotation of the UI111 v1.1 assembly.

# **RESULTS AND DISCUSSION**

After filtering based on genotype quality, heterozygosity, and MAF of 0.01, 908 genotypes with 55,578 SNPs were chosen for GWAS analysis. A total of 33 significant SNPs [-log (*P*-value)  $\geq$  4.42] were associated to WM resistance in dry beans across 12 genomic intervals (Table 1), where 7 of these genomic regions were found to colocalize with previous studies. These results were obtained using the UI111 v1.1 reference genome, marking a notable change from Escobar et al. (2022) findings. The genomic interval Pv05: 10.91 – 11.01 Mb explained the highest phenotypic variation (5.3%) with the peak SNP S05\_10957909. This region was previously reported containing the WM 5.4 QTL. The second most important region on Pv09: 4.34 – 4.44 Mb

explained 3.1%; however, no candidate genes were identified. The genomic region (Pv08:59.93-60.34 Mb) found at the end of chromosome Pv08 contained the highest number of gene models (52). The peak SNP in this region was found in the first exon of the gene model PvUI111.08G271200. In addition, three other candidate genes (PvUI111.08G271700, PvUI111.08G272000, and PvUI111.08G2741000) were found in this region. Three potential candidate genes PvUI111.01G048000, PvUI111.01G048500, and PvUI111.01G048600 were found in the genomic interval on Pv01: 5.20 - 5.30 Mb. No candidate genes were discovered within  $\pm 50$  Kb of the genomic intervals at Pv09: 37.34 - 37.44 Mb and Pv04: 33.59 - 33.69 Mb. Most of the candidate genes are related with kinesin, leucine rich repeat, and pentatricopeptide repeat (PPR) family proteins. Future work is needed to validate the five new genomic regions associated with WM in this research and their impact on resistance or tolerance to this disease.

		Interval		Cumulative			
Phenotypic distribution	Chr	Genomic interval or position	SNP	Base	-LOG <sub>10</sub> (P)	Variation (%)	variation (%)
	2	33.57	S02_33567785	A/G	4.70	2.5	
Quantitative	8	<b>59.93 - 60.34</b> *	S08_59930511	A/G	6.07	1.9	27.3
	11	17.84	S11_17837517	A/T	4.53	1.4	
Polynomial	5	$26.78^{*}$	S05_26778795	T/C	5.15	2.7	11.6
(1 - 5 scale)	8	59.93	S08_59930511	A/G	5.05	1.4	11.6
Polynomial (0 -2 scale)	9	4.39	S09_4395023	T/G	4.46	3.1	3.1
	1	5.25	S01_5249090	G/A	6.13	0.1	
	2	10.02	S02_10021126	G/A	4.64	0.8	
Din amial	4	33.64	S04_33644358	C/G	5.95	1.6	20.0
Binomial	5	$10.96^{*}$	S05_10957909	A/C	7.83	5.3	38.8
	9	32.79	S09_32791124	G/A	5.17	7 0.8	
* ~	11	23.59	S11_23586979	A/T	4.89	1.0	

**Table 1**. Significant (< 0.05) peak single-nucleotide polymorphisms (SNPs) associated with white mold resistance using multiple phenotypic distributions.

Note: \*Genomic intervals flanked by 4 or more SNPs; Chr =Chromosome; Genomic intervals highlighted in bold are new regions not previously reported.

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# INTEGRATION OF SENSING, CROP MODELING, AND GENOMICS IN A COMMON BEAN/TEPARY INTERSPECIFIC POPULATION TO IMPROVE PRODUCTIVITY AND QUALITY TRAITS IN US AND AFRICAN BREEDING CONTEXTS

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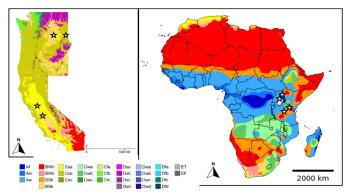
# **INTRODUCTION**

Common bean (*Phaseolus vulgaris* L.) is one of the most consumed grain legumes and an important source of dietary protein globally, but it is susceptible to drought and high-temperature stresses. Tepary bean (*P. acutifolius*), which is in the tertiary gene pool for common bean, is more adapted to hot and dry environments. Therefore, recent efforts to develop bean varieties tolerant to a combination of high temperatures and drought have focused on interspecific hybridization with closely related species that possess these and other beneficial traits (Porch et al., 2013; Cruz et al., 2023). In this large consortium project entitled "GxExM Innovation through Intelligence for Climate Adaptation" or GEMINI, we are developing a novel artificial intelligence-enabled sensing and 3-D biophysical modeling framework, integrated with genomics to dissect photothermal response and improve climate resilience of both productivity and quality in grain legumes.

#### **MATERIALS AND METHODS**

In this study, an interspecific population (composed of 314 lines) derived from crosses between common bean and tepary bean (Barrera et al., 2022) was evaluated for two summer field seasons (2022 and 2023) in each of two California locations (Parlier as a high-temperature stress environment in the reproductive stage and Davis as a lower-temperature environment). Additionally, in an effort to evaluate this population across an extensive environmental gradient (with contrasting daylengths, temperatures, and evaporative demands), these and other common bean, tepary, and interspecific lines are being grown in diverse environments in Uganda (Kawanda and Serere) and Tanzania (Moshi and Babati), and in well-watered vs. terminal drought conditions in Washington state (Prosser and Othello) (Figure 1).

Several agronomic traits, including flowering time, yield, and hundred-seed weight, have been assayed through traditional (groundtruth) assessments and from aerial- and rover-based sensing platforms and have been further evaluated *in silico* using a 3D biophysical crop model. Additionally, nutritional composition and cooking time have been assayed using near-infrared spectroscopy and an automated cooking time apparatus, respectively.



**Figure 1** The Koppen-Geiger climate classifications for each of the trial sites in their regional or continental contexts.

#### **Planned Analyses:**

Genotype-by-environment (GxE) analysis is being conducted to dissect the effects of G, E, and their interaction on priority traits (as scored from sensor data streams and via groundtruth methods). Best Linear Unbiased Predictors (BLUPs) and heritabilities will be calculated for each location for all agronomic, sensing-derived, physiological, and nutritional traits. Genome-wide association studies will be performed to identify genomic variants associated with priority traits. Further, through interdisciplinary collaborative partnerships, we are iteratively determining and testing the most impactful opportunities for, and most feasible implementations and outcomes of, pairwise and three-way integration of sensing, crop modeling, and genomics into the breeding of grain legume crops in U.S. and African contexts. Our initial results from these analyses across four Californian environments showed substantial variation in productivity across genotypes and locations and (in two environments tested thus far) moderate to high repeatabilities for groundtruth agronomic and sensing-derived traits.

#### **Outcomes:**

The main goal of this international effort from a team of diverse backgrounds is to hasten breeding progress for climate resilience of common bean productivity and quality profiles in the US and target geographies in Africa to help enhance food and nutritional security through this important protein staple.

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### QUANTITATIVE TRAIT LOCI ASSOCIATED WITH DROUGHT TOLERANCE IN AN ANDEAN MAPPING POPULATION OF COMMON BEAN

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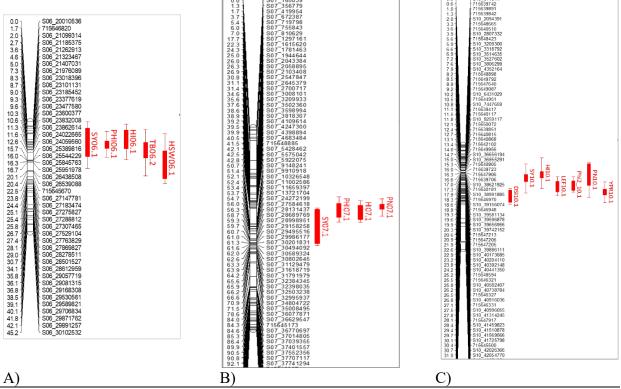
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**INTRODUCTION**: Drought is a major common bean (*Phaseolus vulgaris* L.) production constraint worldwide. There is a need to develop drought tolerant common been varieties to improve yield. The objective of this study was to identify the Quantitative Trait Loci (QTL) for drought tolerance in an Andean population of Recombinant Inbred Lines (RILs).

**MATERIALS AND METHODS**: A total of 155 F<sub>5:7</sub> RILs derived from a cross between Kijivu (drought tolerant) and Bukoba (drought susceptible) were evaluated for drought tolerance in field and pot experiments under drought stress (DS) and non-stress (NS) conditions. Four field experiments were conducted at three locations in Zambia in 2020 and 2021. All field trials were conducted under irrigation. The morphological, agronomic and physiological traits focused on included seed yield (SY), total plant biomass (TB), pods per plant (PN), hundred seed weight (HSW), harvest index (HI), and pod harvest index (PHI). Derived variables included drought susceptibility index (DSI) and yield percentage reduction (YPR). The pot experiment was done to collect data on photosynthetic traits including quantum yield of photosystem II (phi2) and linear electron flow (LEF) which were measured using the multispeQ device. The 155 RILs were genotyped with 11,292 single nucleotide polymorphism markers, and composite interval mapping was conducted to identify QTL for drought tolerance.

**RESULTS AND DISCUSSION**: There were no significant SY differences between Kijivu and Bukoba under NS. Under DS, the drought tolerant parent Kijivu had a significantly higher seed yield than Bukoba across all four trials confirming that Kijivu is more tolerant to drought than Bukoba, which is consistent with previous studies that identified Kijivu as drought tolerant. Significant differences were observed among RILs for all measured traits except under NS across all four field trials suggesting significant genetic variation between RILs in response to drought stress. Transgressive segregation was observed for the RILs for all traits.

A total of 60 QTL were identified for morphological, agronomic, and physiological traits under DS and NS conditions on all chromosomes except Pv11. The results showed a larger number of QTL identified in the DS than in NS conditions. There were 29 QTLs for the DS while 18 were identified in the NS trials and 13 were not specific to water treatment. The QTL coefficient of determination (R<sup>2</sup>) varied from 3.1 to 42.7% indicating that the identified QTL were comprised of both major and minor QTL. Drought tolerant parent Kijivu contributed positive alleles for most of the identified seed yield QTL under drought stress. However, Bukoba, the drought susceptible parent, did contribute positive alleles at a few QTLs, which could explain the transgressive segregation observed in the population for all traits measured. QTL 'hotspots' for drought tolerance were identified on chromosomes Pv06, Pv07, and Pv10 (Figure 1) where extensive co-localizations of SY with other agronomic and morpho-physiological traits under DS were observed. Additionally, these three QTL hotspots overlap with previously identified QTL for drought in both Andean and Middle American populations. This overlap demonstrates the stability of these QTL across environments, genetic backgrounds, and gene pools. The extensive co-localization on the QTL hotspots could be due to pleiotropic effect of the underlying gene/s for multiple agronomic and physiological traits involved in drought tolerance. Linkages among genes conditioning the different traits may also contribute to the three identified QTL hotspots. The three identified QTL hotspots together with the identified novel QTLs could be validated in different environments and genetic backgrounds to provide further insights into the magnitude of their effects and stability. If their effects and stability hold up in the validation studies, they could be targeted for marker-assisted selection for drought tolerance in common bean.



**Figure 1**. Linkage maps (in cM) showing drought tolerance QTL 'hotspots' of co-localized QTL for seed yield and other traits. A) Chromosome 6, B) chromosome 7, and C) chromosome 10

**CONCLUSIONS**: Three major QTL hotspots for drought tolerance were identified on chromosomes Pv06, Pv07, and Pv10.

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# DRY BEAN TOLERANCE TO SALINITY, WATERLOGGING, AND COMBINED CONDITIONS

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**INTRODUCTION:** No matter the growing region, salinity and waterlogging are threats to crop yield and global food security. Dry bean (*Phaseolus vulgaris* L.) is highly sensitive to both waterlogging and salinity, often having reductions in seed yield up to 100% under heavy stress conditions. The focused improvement of waterlogging/salinity tolerance in dry beans will allow producers to select dry bean market classes and cultivars tolerant to a given soil condition, as well as assist breeding programs in the breeding of cultivars. This project aims to improve cultivar selection of dry beans for waterlogged and saline soil conditions. Commercial pinto bean cultivars commonly grown in North Dakota were evaluated under greenhouse conditions.

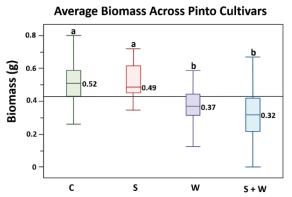
**MATERIALS AND METHODS:** A set of 20 pinto bean cultivars were evaluated in the greenhouse for tolerance to salinity, waterlogging, and the combined conditions. The experiment used a randomized complete block design with split plots and three replications for each treatment. Sodium sulfate and magnesium sulfate were mixed into the soil substrate prior to planting to replicate common North Dakota salts (Franzen, 2007). The target electrical conductivity (EC) for this experiment was EC1:1 2 dS/cm, as this was approximately the EC previously established as the 50% relative survivability (C50) for dry beans (Brogan et. al., 2011). Once seedlings reached the VC (unifoliate leaves visible) stage, containers were bottom capped and waterlogged for 10 consecutive days. After 10 days, chlorophyll content (SPAD), hypocotyl length (cm), dry upper biomass (g), and EC1:1 (dS/m) of the soil was collected. Data analysis was conducted with JMP Pro (version 17) to calculate an analysis of variance (ANOVA) through fit of least squares.

**RESULTS AND DISCUSSION:** There was no significant difference for the cultivar by treatment (CxT) interaction for the chosen metrics; however, there was a strong and significant distinction among the cultivars and the impact of the treatments individually (Table 1).

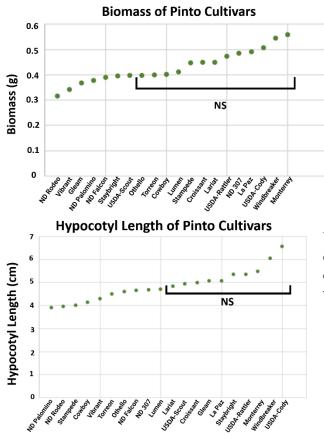
**Table 1.** Analysis of variance. Significance: '\*\*\*' = 0.001 '\*\*' = 0.01 '\*' = 0.05 'NS' = not significant

Effects	Biomass (g)	Chlorophyll (SPAD)	Hypocotyl length (cm)
Cultivar (C)	2.2e-07 ***	5.32e-07 ***	8.4e-07 ***
Treatment (T)	<2.2e-16 ***	<2.2e-16 ***	0.017 *
$\mathbf{C} \times \mathbf{T}$	0.06 NS	0.026 *	0.38 NS

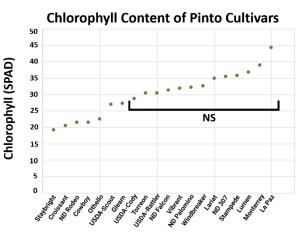
The presence of salinity did not significantly reduce the selected metrics individually or in combined conditions with waterlogging. Upper biomass was significantly reduced with the presence of waterlogged conditions; however, upper biomass was not significantly affected by salinity alone in this experiment (Figure 1).



**Figure 1.** Average upper biomass across pinto bean cultivars (means denoted by a different letter indicate significant differences between treatments) under different treatments: 'C' = control, 'S' = salts added, 'W' = waterlogged, 'S+W' = salts added and waterlogged.



Future experiments should include evaluating the given pinto bean cultivars to increased levels of salinity >2dS/m EC1:1 and ground truth the results in a field environment with natural salinity. Yield and seed metrics, such as seed size, should also be included in future trials as salinity has been shown to have a greater negative impact on these characteristics in field environments (Brogan et. al., 2011). Even though cultivars within the brackets were not significantly different from one another for the respective metric, the cultivars within the brackets appeared to perform statistically better for the respective metric within the given treatments, (Figure 2). This could suggest the possibility of cultivar specific differences to various stresses, such as salinity and waterlogging. These cultivars include: La Paz, Lariat, Monterrey, USDA Cody,



USDA Rattler, and Windbreaker, and will be evaluated in future field environments to confirm their increased tolerances to salinity and waterlogging.

**Figure 2.** Cultivar performances were assessed individually for each of the three metrics (from left to right: upper biomass, chlorophyll content, and hypocotyl length).

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# A GENOME-WIDE ASSOCIATION STUDY OF SNAP BEAN POD PRODUCTION UNDER IDEAL AND HEAT-STRESSED CONDITIONS

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### **INTRODUCTION**

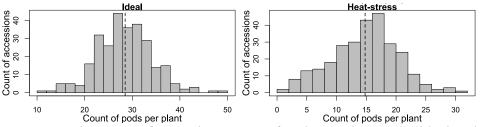
Snap bean pod production is hindered under heat stress during the flowering period when air temperatures exceed 30°C day/20°C night (Rainey and Griffiths, 2005, Vargas et al., 2021). Symptoms of heat stress include pollen sterility, flower abscission, malformation of pods, and embryo abortion or seed desiccation. Most common bean heat tolerance studies evaluated dry bean germplasm and the few reported for snap bean have been limited in terms of numbers of accessions and markers. Here we evaluated 266 accessions of the Snap bean Association Panel (SnAP) and 57 commercial cultivars for flowering and pod production traits in two years of field trials under ideal and heat-stressed conditions. Genotyping-by-sequencing (GBS) of the panel resulted in 28,978 SNPs, which were used for a genome-wide association study (GWAS) of days to flower, number of pods per plant, and the weight of pods per plant.

#### **MATERIALS AND METHODS**

The Snap Bean Association Panel (SnAP) consists of a total of 378 accessions; comprising of 150 accessions from Common Bean Coordinated Agriculture Project (Bean CAP) Snap Bean Diversity Panel (SBDP) and an additional 228 historical cultivars that were released/expired from Plant Variety Protection (PVP) (USDA-AMS, Myers and Celebioglu 2023). Field evaluations of the SnAP were limited to 266 determinate bush types, including green beans, yellow wax beans, purple-podded beans, and Romano beans. Evaluation of the SnAP was completed using a randomized complete block design (RCBD) with three replications and two planting dates, repeated over two consecutive years (2021-2022). The ideal planting dates (IPD) were in mid-April and the heat planting dates (HPD) were mid-May. Pods were systematically harvested from five representative plants within the central area of each plot. The total pod count was divided by the number of plants sampled, denoted as 'ppp' (pods per plant). Variants were called from genotyping-by-sequencing (GBS) data and used for GWAS of ppp under heat and ideal conditions. GWAS models FarmCPU (Liu et al., 2016) and BLINK (Huang et al., 2019) were run using GAPIT (Wang and Zhang, 2021).

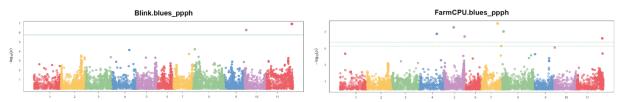
# **RESULTS AND DISCUSSION**

Heat stress was achieved in the field trials with the average ppp for ideal planting dates (IPD) at 27.6 pods, while the heat-induced planting dates (HPD) averaged 14.2 pods, representing a 48.75% decrease (Figure 1). A total of 28,978 SNPs were used for GWAS of ppp. FarmCPU had a total of six SNPs for ppp in the HPD, two of which were on chromosome Pv05. BLINK found two associations with ppp in the heat on chromosome Pv10 and Pv11 (Figure 2). There was only a single SNP identified by both FarmCPU and Blink which was associated with ppp in the HPD on chromosome Pv11 at position 51,156,816 bp. Vargas et al. (2021) found QTLs for the number of pods per plant in a dry bean population at Pv01, Pv04, and Pv08 under heat-stressed conditions.



**Figure 1**. Histogram of accession means of pods per plant under ideal and heat-stressed planting dates across years. The dotted line indicates the snap bean panel mean (across accessions).

Our results also identified significant SNPs on chromosomes Pv04 and Pv08 (S04\_34273966, S08\_4198189) in ppp under HPD. Oladzad et al. (2019) used a Bean Abiotic Stress Evaluation (BASE) panel with Middle American, Andean, and tepary bean genotypes, which found SNPs at Pv03, Pv08, and Pv11 for yield under heat stress (Oladzad et al. 2019). Similarly, our GWAS of ~300 lines from both Middle American and Andean origins found pod production SNPs at Pv08 and Pv11 under heat.



**Figure 2**. Manhattan plots for pods per plant in heat (ppph) using the BLINK and FarmCPU models.

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#### **IMPROVING CONSUMER TRAITS IN DRY BEANS**

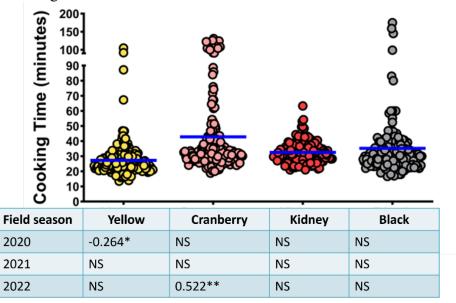
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**INTRODUCTION**: Consumer traits in dry beans include seed specific traits that improve ease of handling, processing, appeal, and nutritional value of products for end-users. Incorporating consumer traits into dry bean breeding programs is worthwhile as a means to maintain and/or increase demand for dry beans and food products developed from dry beans. Within the U.S. there is unrealized potential for dry bean consumption. While the U.S. Dietary Guidelines for Americans recommends 270 g cooked (1.5 cups) of pulses per week, consumers are eating less than half that (~140 g cooked) (Mitchell et al., 2021). The major barriers that consumers report inhibit them from eating more beans and other pulses are 1) lack of familiarity with eating and cooking pulses, 2) long cooking times, 3) preference for other foods, 4) unpleasant taste, and 5) that pulses cause digestive problems (Philips et al., 2015; Perera et al., 2020). Knowledge of these barriers can help identity traits to focus on to improve consumer demand for dry beans. The five major consumer traits evaluated by the USDA-ARS Food Legume Quality Genetics Lab are dry seed characteristics, canning quality, cooking time, functionality as a flour, and nutritional quality.

**RESULTS AND DISCUSSION:** Dry seed characteristics, including shape, size, color, fit within market class, resilience to seed coat damage during harvest and handling are valued by farmers, processers, and consumers. Nutritional quality characteristics, although often invisible for the consumer, are important to consider throughout breeding and processing since nutritional characteristics are impacted by genotype, environment, and processing method. Ideally, nutritional value should be optimized in products that reach consumers. Canning quality, cooking time, and use as flour, all share the underlying goal of improving the convenience of dry beans for consumers. Canning quality has been the most important end-use trait in U.S. bean breeding efforts since the 1970's. Canning quality evaluations have been carried out though pilot scale evaluations (Wang et al., 2022). These methods are effective but require specialized equipment which limit greater use among breeders. Black beans are the current market class with canning quality needs as there is genetic variability for color retention in canned black beans that has been challenging for the canning industry to address. Opportunities exist for phenotypic and genotypic prediction for canning quality, especially for color retention in black beans (Mendoza et al., 2014). Cooking time has had less focus in U.S. breeding programs. Technically, it easy to phenotype for cooking time with a Mattson pin drop cooker and the trait has high heritability (Cichy et al., 2019). In some cases, cooking time and seed yield are correlated (Figure 1). Cooking time is also related to canning quality, in that beans with shorter cooking times also need less retort processing time (Bassett et al., 2020). Nutritional benefits and tradeoffs have been identified in beans with shorter cooking times. Benefits include greater nutrient retention during the cooking process and tradeoffs include less insoluble dietary fiber in beans with shorter cooking times (Bassett et al., 2021). A new consumer trait for bean breeding is use as a flour. Key characteristics are milling quality, particle size, functionality, off-flavors, and color. Future directions in the area are to determine best screening characteristics for flour quality, develop high throughput methods to detect offflavors, lectin reduction, lab scale vs. commercial scale milling quality.

**CONCLUSIONS:** Canning quality, cooking time, and use as a flour are three consumer traits that can help improve consumer acceptability of dry beans by improving convenience related attributes. Canning quality has a long history of use in breeding, it currently involves very specialized phenotyping and there is a need to implement prediction tools. There is no strong relationship with seed yield, at least in black beans. Cooking time is not a major trait in the U.S., while it is easy to phenotype for cooking time. In some cases, there may be a negative seed yield relationship. Nutritional benefits and tradeoffs are associated with reduced cooking times. Use as flour is an up-and-coming consumer trait for dry beans. Future needs for this trait are to develop effective screening methods that are relatable to commercial needs.



**Figure 1.** Agronomic tradeoffs: Cooking times of breeding lines and correlation with seed yield. Dot plot depicting the cooking times of preliminary and advanced breeding lines from trials located at the Montcalm Research Farm and Saginaw Valley Research Extension Center in Michigan for field seasons 2020-2022. Each dot represents the mean of two field replicates. Pearson correlations with seed yield at \* is p-value 0.012 and \*\*0.005.

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# UTILIZING BENCHTOP NEAR-INFRARED SPECTROSCOPY TO PREDICT LIMA BEAN (*PHASEOLUS LUNATUS* L.) NUTRITIONAL COMPOSITION

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#### **INTRODUCTION**

Lima bean (*Phaseolus lunatus* L.) is an underutilized crop in the U.S. and worldwide with potential for diversifying food systems, especially under warming temperatures. In the U.S., California is the primary producer of dry limas, whereas the mid-Atlantic region is the primary producer of succulent limas sold as processed (frozen/canned) products (USDA-NASS 2019). Limas can be classified into two main market classes based on size (baby- and large-seeded) that typically correspond to their domestication locations (Mesoamerican and Andean, respectively) (Gutiérrez Salgado, Gepts and Debouck 1995). Near-infrared spectroscopy (NIRS) is a high-throughput, low-cost method that allows for the assessment of multiple macronutrient traits simultaneously when paired with appropriately calibrated prediction models. Here, we assessed the seed macronutrient composition of cultivars and advanced breeding lines varying in seed size. These efforts are part of a USDA-NIFA Specialty Crop Research Initiative-funded lima bean project to address bottlenecks in consumer information, pre-breeding, and germplasm information/utilization.

#### **MATERIALS AND METHODS**

We sampled 48 genotypes (70 samples total) composed of California-relevant cultivars, as well as advanced breeding lines from the UC Davis and University of Delaware breeding programs. Selected genotypes were grown across multiple years (2019-2023) and locations in California (Davis and Tulelake). Limas were categorized into baby-seeded (38 genotypes, n=43) or large-seeded (10 genotypes, n=27) classes, and hundred-seed weight was measured to assess the distribution within size classes. Seed samples were ground to a powder and scanned with the NIRS instrument (FOSS DS2500; Eden Prairie, MN) using the vegetal protein meals (VPM) calibration available from the instrument manufacturer to predict the percentages of protein, crude fiber, starch, fat, moisture, and ash in seeds. Variation in these traits within and between size classes was examined via summary statistics, correlation analysis, and principal component analysis.

#### **RESULTS AND DISCUSSION**

First, we examined the performance of the VPM calibration for predicting macronutrient composition in lima bean seeds. VPM calibration was developed using samples from multiple food legume species, including beans of unknown (to the user) type(s) as well as pea, soybean, and canola proteins/meals. We analyzed the following calibration statistics determined by: global H, a measure of the distance of a sample to the average of all samples in the calibration, and neighborhood H, a measure of the distance of a sample to the closest sample in the calibration. We found that the VPM calibration had low global and neighborhood H values for moisture, fat,

protein, crude fiber, and ash (total mineral content) for the lima bean samples assayed herein. However, the calibration had high global and neighborhood H values for starch (with notably less wet-chemistry data in the calibration for starch than for the other traits assayed), suggesting that the incorporation of additional lima bean samples in the calibration may improve model performance for starch in particular. Going forward, we will be further validating the predicted measurements from the calibration with wet-chemistry reference analyses (under way) and improving the calibration by incorporating these reference values into the calibration. We will test the performance of adapted pre-existing vs. *de novo* custom calibrations for limas, developed using partial least squares regression or artificial neural networks. We will also develop a model specifically for dietary fiber, rather than crude fiber.

We found the trait values predicted by the pre-existing calibration to be within the reported macronutrient values for limas (Adebo 2023). On average, the large-seeded limas surveyed had a higher protein percentage and lower starch and fat percentages compared to small-seeded limas. However, due to limitations in the availability of samples from large-seeded lines, this trend will need to be confirmed with a greater number of lines. We also found natural variation across genotypes for these traits; for example, a wide range of fiber percentages was observed in both size classes (from 5-13%). We found macronutrients also varied within a given genotype depending on the growing environment (e.g., location or year at a given location). Fiber and fat percentages were the main factors driving these differences, indicating these traits may be more strongly influenced by the growing environment than others.

#### CONCLUSIONS

Overall, our results indicate there are differences in macronutrients across market classes and variation in each class that can be utilized to breed for increased nutritional quality. The grain macronutrient data being generated herein will aid in selection of lines that will undergo further analysis in sensory trials, in addition to informing future crosses for improvement of seed macronutrient profiles alongside agronomic traits. Our results in diverse lima accessions will be utilized to conduct genetic mapping and genomic (and phenomic; based on NIR spectra) prediction for grain macronutrient traits with subsequent development of markers for the identified candidate genes. The main and interaction effects of genotype and environment on these traits will also be analyzed.

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# APPROACHES TO DEVELOP ROOT-KNOT NEMATODE RESISTANT GREEN BABY LIMA VARIETIES

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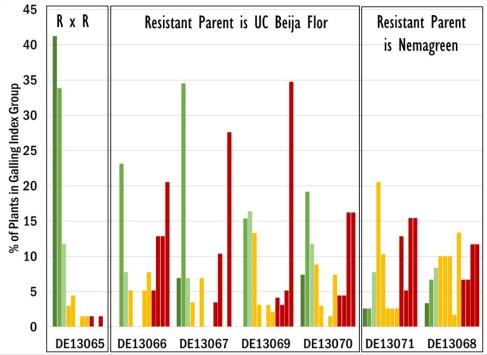
**INTRODUCTION**: Green-seeded baby lima bean (*Phaseolus lunatus*) is the predominant type grown for freezing and canning in the Mid-Atlantic region of the United States. Southern root-knot nematode (RKN), *Meloidogyne incognita*, is a growing threat to lima bean production in the sandy soils of the Delmarva Peninsula. Chemical and cultural controls for RKN are limited and field surveys have identified local populations as high as 10,000 RKN/500 cc soil, nearly 60 times the high damage threshold. None of the green baby lima varieties currently used for production in the Mid-Atlantic region are resistant to RKN. A program to breed new green baby lima varieties with RKN resistance was initiated at University of Delaware in 2013 to provide growers with an effective management strategy for this pest.

#### **MATERIALS AND METHODS**

Screening Strategies: Both field and greenhouse screening techniques have been used to select from breeding populations and to screen inbred lines for resistance. Field screens are conducted in an infested field that has been used for RKN management and screening trials for >10 years. Even though the field has a history of RKN, plots are inoculated at planting. Chopped RKN infested tomato roots obtained from greenhouse grown 'Marglobe' tomato plants are applied directly over the seeds before the furrow is closed. Evaluation of RKN galling and reproduction requires a destructive harvest, so breeding material is evaluated at maturity when dry seeds can be collected, yield trials at the succulent stage harvest and germplasm screens as early as 69 days after planting. The most severe galling symptoms are not evident until later in the season. Greenhouse screening is conducted in 164 ml "Cone-tainer" cells. Plants are grown in a media consisting of 50% autoclaved sand and 50% peat/perlite potting media (volume basis). Seedlings with fully expanded primary leaves are inoculated with 500 freshly hatched J2 stage RKN using the methods described by Atamian et al. (2012). Breeding material that is screened in the greenhouse is evaluated for RKN galling and reproduction about 90 days after planting once mature, dry seed is harvested from the Cone-tainer grown plants. For screens of inbred lines where seed collection is not necessary, plants can be evaluated as early as 60 days after planting. In both greenhouse and field screens, soil/media is rinsed from freshly dug roots and the galling severity is rated using the 0-10 Root Galling Index described by Bridge & Page (1980). RKN reproduction is evaluated using egg extraction and enumeration or by staining egg masses with 0.01% erioglaucine for 15 minutes (Atamian et al., 2012).

**Breeding Strategies**: In breeding for RKN resistance, advancing populations by single seed descent to the  $F_3$  or  $F_4$  generation before resistance screening has been the most successful approach for recovering nematode resistant lines that also have desirable quality and agronomic performance. Multiple genes are involved in resistance with galling response and resistance to RKN reproduction under separate genetic control (Roberts et al., 2008).  $F_3$  to  $F_5$  generation lines are screened for galling severity and  $F_6$  and later generation lines are screened for galling and nematode reproduction. Resistant lines from the breeding program have been yield trialed in

uninfected fields yearly since 2018 and inoculated yield trials have been conducted yearly since 2021. Two resistant cultivars, 'UC Beija Flor' and 'Nemagreen', were used to develop the greenseeded RKN resistant varieties for production in Delaware. The different distribution pattern of galling ratings among F<sub>3</sub> populations developed from these two parents and the presence of susceptible plants in the resistant x resistant cross suggests that Nemagreen and UC Beija Flor carry different galling resistance genes (Figure 1).



**Figure 1.** Distribution of  $F_3$  Galling Index Ratings for seven populations. Green bars are 0-2 ratings considered resistant. Yellow bars are 3-6 ratings considered susceptible. Red bars are 7-10 ratings considered very susceptible.

**Screening Diverse Germplasm to Identify New Sources of Resistance:** To identify new sources of resistance for the breeding program, a panel of 255 diverse lima bean genotypes was greenhouse screened in 2021-2022. The most resistant lines and susceptible checks (60 total genotypes) were field and greenhouse screened for galling and reproduction in 2022 to confirm their RKN response. Four small-seeded genotypes had very low galling and reproduction in all screens: PI 347779, PI 347784, UC Beija Flor and Cariblanco N. Three large -seeded genotypes had very low galling and reproduction in all screens: PI 256848 and two heirloom pole lima beans collected from Delaware growers.

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# SCREENING DRY EDIBLE BEAN GERMPLASM FOR RESISTANCE TO SOYBEAN CYST NEMATODE

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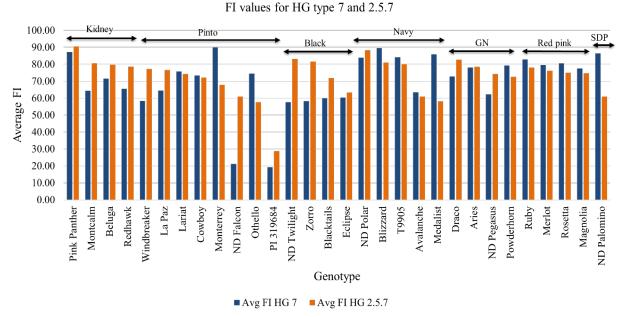
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**INTRODUCTION**: Dry bean (*Phaseolus vulgaris* L.) is one of the most important grain legume crops in the world. North Dakota is the top producer of dry beans in the USA, accounting for >40% of the total production. Soybean cyst nematode (*Heterodera glycines* Ichinohe; SCN) was first detected in North Dakota in 2003 in Richland County. Various populations of SCN, known as HG types, have been characterized in different counties of North Dakota, with HG type 0 being the most prevalent, followed by HG types 7 and 2.5.7 (Chowdhury et al., 2021). Screening studies with HG type 0 have shown that SCN can infect and reproduce on dry beans, leading to significant seed yield losses (Poromarto and Nelson, 2009; Poromarto et al., 2010), and a few resistant and tolerant genotypes were identified. However, there is a need to evaluate dry bean germplasm for multiple SCN HG types characterized in the region. Therefore, the objective of this study was to evaluate the response of dry bean genotypes to two additional SCN HG types (HG type 7 and 2.5.7) in a greenhouse screening experiment.

**MATERIALS AND METHODS**: To evaluate the reaction to soybean cyst nematode (SCN) infection, 30 commonly grown North Dakota dry bean cultivars from seven market classes were chosen. One dry bean PI line, PI 319684, and the susceptible Barnes soybean standard were also included. The SCN types 7 and 2.5.7 were collected from North Dakota soybean fields and maintained in the greenhouse using soybean Barnes. Dry bean seeds were surface sterilized with 1.0% NaOCl, washed with water, and germinated on filter paper afterward. The planting and inoculation of dry bean seedlings was done according to the procedure described by Acharya et al. (2022). The experiment was repeated once for both HG types. Plants were grown under controlled greenhouse conditions and were harvested to extract SCN females using standard sieving protocol (Krusberg et al., 1994). Females were counted, and those numbers were used to calculate Female Index (FI) values for each genotype. FI values were later used to classify the dry bean genotypes based on their resistance levels according to the soybean resistance rating scale (Schmitt and Shannon, 1992).

**RESULTS AND DISCUSSION**: SCN reproduced all the dry bean genotypes in both trials for HG type 7 and 2.5.7. and most dry bean genotypes were classified as susceptible (FI>60%) or moderately susceptible (FI=30-60%). Kidney bean cultivar Pink Panther showed the highest susceptibility to SCN HG type 2.5.7 with an FI of 90%, and pinto bean cultivar Monterrey had the highest susceptibility to HG type 7 with an FI of 90%. Only the pinto germplasm accession, PI 319684, was classified as moderately resistant (FI=10-30%) to both SCN HG types 7 and 2.5.7, with an average FI of 19% and 28%, respectively. The pinto bean cultivar ND Falcon, previously found to be resistant to HG type 0 (Osorno et al., 2020), was classified as moderately resistant to HG type 7 in this study with an average FI of 22%. Poromarto and Nelson (2009) suggested that the Mesoamerican gene pool (black, pinto, and navy beans) had higher levels of SCN resistance compared to the Andean gene pool (kidney and snap beans). While black and pinto bean cultivars

were observed to be resistant to moderately susceptible for HG type 0 in their experiments, they were found to be susceptible or moderately susceptible when tested against SCN HG types 7 and 2.5.7 in this study. The average FI for black beans was 16%, while pinto beans had an average FI of 39% for SCN HG type 0 in their study. When averaged over two trials in this study, black beans showed FI values of 59% and 75% for SCN HG types 7 and 2.5.7. Meanwhile, pinto beans had an average FI of 59% and 64% for HG type 7 and 2.5.7. Since most Andean and Mesoamerican genotypes showed a susceptible reaction in this study, this could suggest that HG type 7 and 2.5.7 might be more aggressive than HG type 0. Field trials are needed to further confirm the resistance and susceptibility of these genotypes under natural disease pressure conditions. Nonetheless, this study can potentially be helpful in breeding programs aiming to develop SCN-resistant dry bean cultivars. For example, PI 319684 has previously been observed as resistant to HG type 0 (Jain et al., 2019), making it a promising candidate for developing cultivars with resistance to multiple HG types. Breeding efforts with PI 319648 have already been initiated at NDSU. It also provides valuable information for growers to help them make informed decisions about cultivar selection and ultimately manage SCN in fields.



**Figure 1**. Average Female Index (FI) of all the dry bean genotypes in two trials of SCN HG types 7 and 2.5.7.

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# PYRAMIDING OF GENES THAT CONFER MULTIPLE DISEASE RESISTANCE IN SNAP BEANS AND PERFORMANCE OF BREEDING LINES IN KENYA

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# **INTRODUCTION**

The production of snap beans (*Phaseolus vulgaris* L.) in Kenya is mostly carried out by smallscale farmers and the produce is mainly for the export market, thus enhancing household income. However, the small-scale farmers are often challenged by pests and diseases among other constraints. The major diseases limiting snap bean production in Kenya include angular leaf spot (ALS) caused by *Pseudocercospora griseola*, bean rust, caused by *Uromyces appendiculatus*, anthracnose caused by *Colletotrichum lindemuthianum*, and bean common mosaic viruses (Kamiri et al., 2021). Farmers in Kenya use various methods to manage these diseases including the use of chemical sprays, which is unsustainable economically for the resource poor farmers. Therefore, deployment of host resistance is the best alternative strategy to manage these diseases. The objective of this study was to develop snap bean breeding lines with multiple disease resistance and evaluate their performance under field conditions in Kenya.

# MATERIALS AND METHODS

**Plant Materials:** A locally adapted snap bean variety Amy was used as the recurrent parent in a marker-assisted backcross breeding program. Two dry bean varieties were used as donor parents for anthracnose and ALS resistance, while a snap bean breeding line was the donor for rust resistance. Variety G2333 was the donor parent for anthracnose resistance genes ( $Co-4^2$  and Co-5) while Mexico 54 was the donor for ALS resistance gene (Phg-2). A locally developed snap bean breeding line (MU#13) was used as the source of rust resistance (Ur-?).

**Breeding Scheme:** The gene pyramiding program was accomplished through parallel backcrosses. The recurrent parent was backcrossed to each of the donor parents up to BC<sub>4</sub>. Thereafter, the four genes were combined through a four-way cross which were advanced through self-pollination to F<sub>4</sub>. In every generation, molecular markers were used for selection as follows: G796 (*Phg-2*), SH-18 (*Co-4*<sup>2</sup>) and SAS-13 (*Co-5*). For rust resistance, the lines were selected phenotypically.

**Field Evaluation:** The BC<sub>4</sub>F<sub>4</sub> population was subjected to preliminary yield trials where 16 lines were selected and evaluated in three locations in Kenya. Four snap bean genotypes (Amy, MU#13, Seagull and Source) were included as checks in the multi-location trials that were conducted between November 2021 and April 2022. Data was collected on total pod weight (g), pod length (cm), pod string (cm), percentage of extra-fine and fine pods (%EFF) and disease resistance. Data on pod parameters were subjected to analysis of variance and the means are presented in Table 1.

# **RESULTS AND DISCUSSION**

The four resistance genes were successfully pyramided in variety Amy through backcrossing to obtain breeding lines that were subjected to field evaluation. Most of the breeding lines were resistant to rust and ALS (82%), although the disease severity was low. Anthracnose was not recorded during the evaluation season. However, all tested genotypes were susceptible to bean common mosaic necrosis virus (BCMNV) in Embu. The BCMNV strain was spread from a disease

nursery that was near the experiments. Further, despite the successful introgression of resistance genes, the commercial cultivar 'Source' outperformed the breeding lines for yield, except for UoEm#1 (Table 1).

Visual inspection of the pods showed that the pod quality of the commercial cultivars was better than the breeding lines for some traits like pod color and texture. Reports have shown that the introgression of novel traits without disturbing pod quality is a main challenge affecting snap bean breeding and therefore, pod quality enhancement should be considered alongside other traits (Singh and Singh, 2015). Therefore, there is need to improve the breeding lines further before they are released for commercial production. This will enhance the marketability of the new varieties.

S/N	Genotype	Pod weight per plot (g)	Pod Length (cm)	Extra fine and fine pods (%)	Pod suture string (cm)
1	UoEm#1	2882.1ab	12.30b-c	97.92a-c	5.95a
2	UoEm#2	2584.2b-с	12.20b-f	97.48а-е	6.21a
3	UoEm#3	2401.9b-d	12.44b	95.41d-e	7.01a
4	UoEm#4	2317.8b-d	11.79e-i	99.03а-с	7.47a
5	UoEm#5	1632.5f-g	11.58h	80.19g	5.60a
6	UoEm#6	2352.8c-d	12.00bh	96.68b-e	7.27a
7	UoEm#7	1827.2 e-g	11.85c-i	99.9ab	7.10a
8	UoEm#8	1842.8d-g	12.29b-d	96.11c-e	6.48a
9	UoEm#9	2155.0c-f	12.31b-c	95.29e	5.59a
10	UoEm#10	2402.9b-d	11.76f-i	89.23f	5.37a
11	UoEm#11	2045.5c-g	11.70h-g	97.84a-d	5.93a
12	UoEm#12	1927.6e-g	11.97b-i	96.57b-е	6.14a
13	UoEm#13	1415.2g	11.50i	98.29а-с	7.97a
14	UoEm#14	1806.7d-g	11.71f-i	96.52b-е	7.88a
15	UoEm#15	1927.1e-g	11.78f-i	91.38f	6.22a
16	UoEm#16	1695.4e-g	11.82d-i	96.29с-е	6.41a
17	Amy	2441.5b-d	12.27b-d	99.91ab	6.66a
18	MU#13	2815.6a-b	13.03a	98.52а-с	8.47a
19	Seagull	2399.0b-d	12.15b-f	99.91ab	6.85a
20	Source	3302a	11.95b-i	99.77a	4.98
Mean		2208.74	12.02	96.11	6.58

**Table 1.** Means of pod parameters of snap bean breeding lines evaluated under field conditions in Kenya

<sup>a</sup> Means sharing the same letter are not significantly different at  $P \le 0.05$  according to Tukey's test.

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# THE GENOME OF MESOAMERICAN COMMON BEAN OAC REX – EVIDENCE OF INTROGRESSION OF DISEASE RESISTANCE INTO *PHASEOLUS VULGARIS* FROM *PHASEOLUS ACUTIFOLIUS*

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#### **INTRODUCTION**

Common bacterial blight (CBB) is a major seed-borne disease of dry bean crops throughout the world. It is caused by *Xanthomonas axonopodis* pv. phaseoli and *Xanthomonas citri* pv. fuscans The white navy bean variety OAC Rex was developed from germplasm derived from an interspecific cross with a wild relative *Phaseolus acutifolius* (tepary bean). The interspecific cross was made to introduce CBB resistance into cultivated beans (Michaels et al. 2006). The goal of this study was to characterize the genome sequence of OAC-Rex and examine the potential role of the introgression from *P. acutifolius* in its CBB resistance.

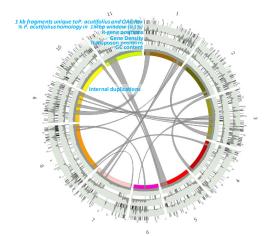
#### MATERIALS AND METHODS

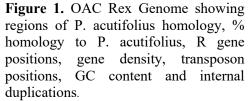
The complete genome sequence for OAC Rex was obtained by Illumina HiSeq sequencing to give 136x coverage and supplemented by 10x long-read PacBio<sup>™</sup> RSII data. The *de novo* assembly of the OAC-Rex genome was performed with RAY, SOAP2 *de novo* and ALLPATHS-LG. PacBio

RSII reads (10x) were added using PBJelly to create a V.1 assembly. Pseudochromosomes were built from a collection of 6,246 scaffolds and 28,743 contigs aligned with the SNP data from the BEANCap SNP chip. All 5361 SNPs mapped to the G19833 sequence were contained in one of the OAC-Rex contigs and 3809 were mapped to one of the OAC-Rex scaffolds used to create the 11 pseudochromosomes. The sequence collection was annotated using the MAKER pipeline after training with *Glycine max* and *P. vulgaris* (G19833) datasets, respectively. Comparisons of gene content between OAC-Rex, G19833 and *P. acutifolius* (PI440795) was conducted using OrthoMCL.

#### RESULTS

The pseudochromosome-level draft genome (Accession: PRJNA237957 ID: 237957) of OAC Rex comprises approximately 97% of the G19833 sequence and contains partial or complete representations for 96% of the





CEGMA conserved data set https://www.ncbi.nlm.nih.gov/nuccore/JADFUL0000000000. The annotation of OAC-Rex identified 29,435 genes over all 11 chromosomes, with a mean gene length

of 3,136bp, and a mean transcript length of 1224bp (407 amino acids). A comparison between

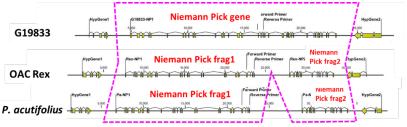
OAC-Rex and G19833 showed that they are largely syntenic over all 11 chromosomes.

A comparison of a contig-stage assembly for *P. acutifolius* accession PI440795 in the pedigree of OAC Rex (unpublished), with the OAC Rex

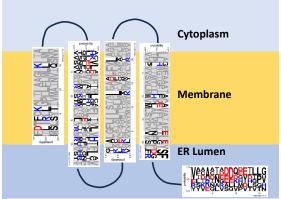
and G19833 genome sequences, showed that there are regions on

every chromosome that are shared between the P. acutifolius and OAC Rex but are missing from G19833. The regions of introgression from P. acutifolius in chromosome 8 include genes associated with disease resistance and a Niemann Pick-like sterol transporter.

A comparison of the OAC Rex sequence with the G19833 reference genome, which is susceptible to CBB, identified differences in gene content and structure in genomic regions that are associated with CBB resistance in OAC Rex, in particular on chromosome 8 near a marker (SU91) associated with CBB resistance. This region includes an introgression from *P. acutifolius* containing a Niemann Pick sterol transporter gene with a unique structure shared with *P. acutifolius*. In OAC Rex and *P. acutifolius* 



**Figure 2.** Nieman Pick gene structures on chromosome 8 (near the SU91 marker for CBB resistance in G19833 (susceptible), OAC Rex (resistant) and P. acutifolius (resistant).



**Figure 3.** Structural homology of the protein encoded by the Nieman Pick fragment 2 open reading frame with executor proteins from Xanthomonas-resistant rice and pepper (Ji et al.2022).

the Nieman Pick gene is broken into two open reading frames. The second open reading frame encodes a small transmembrane protein that has features of executor genes described previously in Xanthomonas-resistant rice and pepper genotypes (Ji et al. 2022).

#### CONCLUSION

The CBB resistance from *P. vulgaris* in OAC Rex may be associated with the introgression of a disrupted Nieman Pick gene that encodes a small transmembrane protein with executor protein characteristics.

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#### GENES IN DUE TIME FOR COMING STRESSORS

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Heat is coming, often associated with drought (IPCC 2023), forcing farmers, breeders and human societies to reconsider the water invoices of many crops. Some Old World pulses (i.e. moth bean, cowpea, or pigeon pea) have long endured such stresses, while the American Phaseolus, especially tepary and the Lima bean, have been challengers because of seed size (NRC 1979). The challenge seems to have been set early on because common bean was grown in France around 1508 (Camus 1894). Constraints set by market seed classes have made bean breeders reluctant to include wild species in their crossing programs, and failures to obtain anything beyond F1 have not helped either. Consequently, genebanks have not increased their collections with germplasm of little prospect for wide use, except some wild ancestral forms. Being largely unknown that alien germplasm is often reported as difficult to manage. In this regard, the Bermuda bean story demonstrates that a few tricks (e.g. plot within the right ecology, reclining pole) result in the production of thousands of seeds. But this apprehensive approach has often not helped much towards genetic progress. Fortunately, bean breeding technologies have changed much since the late 1990s (e.g. marker assisted selection, transformation, comparative mapping, and gene editing), and more changes are expected in years to come, and faster. It is thus the very logic for bean genebanks not to follow these changes but to anticipate them, and more so if the sources of genetic variation are becoming extinct (Parker et al. 2023).

Clade B has the five bean cultivated species and their ancestral forms (Porch et al. 2013). Three species, viz. common bean, scarlet runner and year-bean, are in the same section Phaseoli with seven species to date. That section has evolved in montane moist forests, with P. vulgaris spreading towards drier woodland savannahs. Thus, it may serve as source of multiple resistances to fungal diseases attacking leaves and pods. This is somewhat expected because several species (P. albescens, P. costaricensis, P. dumosus) of Phaseoli are large pluriannual polycarpic vines, often not producing seed in the first year, but just establishing themselves in cloud forest habitats. Their relatively large seeds (range of 100-seed weight: 15-30 g) help to that early growth in a competition for light. In that section, wild P. vulgaris abandoning perennialism, is perhaps the species with some tolerance to drought, but there are better sources. In relation to abiotic stresses, sources of tolerance might be where evolution has taken place for millennia under such stresses. Facing high temperatures and low moisture availability, several species (P. acutifolius, P. filiformis, P. microcarpus; range of 100-seed weight: 0.6-6 g) annual and monocarpic went for avoidance and became desert ephemerals. P. macvaughii is somewhat in a similar situation but modified by salinity. Early flowering (within 30 or less days after germination) and fast partitioning of photosynthesis products into pods and seeds became the rule at the expenses of additional late branching, the same happening in the root system. These species have smaller flowers as compared to the ones found in the *Phaseoli*, downplaying the role of large pollinators, but more importantly seem to have a better reproductive efficiency. P. costaricensis and P. microcarpus may have racemes with sixteen (or more) flowering nodes; there will be one or two pods at each node in the latter, not in P. costaricensis. In the Phaseoli, not all ovules develop into

seeds, namely the one proximal to the pedicel, while in *P. microcarpus* the ovule develops into seed (Freytag & Debouck 2002).

Facing drought, the species of the Coriacei section (linked to the Paniculati where the Lima bean is) distributed in the Sonoran and Chihuahuan Deserts went for another strategy: a waxy cuticle on all leaflets. Where solar radiation is intense at midday, many species across the genus have active pulvini and central variegation on all leaflets. P. leptophyllus, not found since its discovery in 1789, is unique in having narrow leaflets with revolute margins. Increased surface for photosynthesis is found in stipules of P. amblyosepalus or in primary bracts of P. macrolepis. Growth habit II has been obtained through breeding in P. vulgaris, but reduced branching exists naturally in P. parvulus. Roots vary from annual fibrous 45 cm long in some P. acutifolius to a pluriannual 1 cm spherical tuber in P. parvulus to a 1 m conical woody root in an 8-years P. rotundatus. Throughout the genus and its natural distribution (from Connecticut down to Córdoba, and from the Bermuda to the Galápagos), different 'building blocks' in relation to plant architecture, root phenes, epidermis, photosynthesis, partitioning, or plant duration are spread across the 81 species of the genus (Debouck 2021). As already developed for soybean (Xu et al. 2020) there are prospects of gene editing in traits controlled by a few genes, but in contrast to Glvcine there might be many more model species to get inspiration from in Phaseolus. Without incurring in difficult management problems, the representation of such 'building blocks' in genebanks should be improved. Breeders may continue to prioritize adaptation, thus be interested in the widespread species, but the majority of the 81 species of the genus are endemic (and more will come into this category), thus offering specific traits to particular environmental stressors. To provide genes in due time genebanks may find useful to spend some time with ecophysiologists!

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# USDA PHASEOLUS GERMPLASM COLLECTION, CURRENT AND FUTURE HAPPENINGS, COMMENTS AND COLLABORATIONS WELCOME!

## Sarah Dohle and Marilyn Warburton

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The USDA Phaseolus germplasm collection maintained in Pullman, WA contains over 17,750 bean accessions, including 56 taxa; primarily the 5 domesticated species of common bean (*P. vulgaris*), lima bean (*P. lunatus*), tepary bean (*P. acutifolius*), runner bean (*P. coccineus*) and year bean (*P. dumosus*), as well as 51 Crop Wild Relatives (CWR) (Table 1). Bean accessions originate from 110 countries. Approximately 75% of the collection is available to users and backed up off site.

Sarah Dohle started as the Phaseolus curator in August 2022 after a 2-year gap without a dedicated scientist in the position. The 2023 focus was on greenhouse regenerations for some of our most vulnerable germplasm and reestablishing field characterization methods, since all our regenerations are done in greenhouses. The collection is actively growing with domestic CWR collections of *P. polystachios* (A. Egan & L. Wallace 2022) from the southeastern United States and drought and heat tolerant relatives from New Mexico (D. Debouck, S. Dohle, R. Pratt M. Santaella, L. G. Santos & M. Urban 2023). We are participating in a 4-year SCRI funded collaboration project lead by UC Davis, "Development of Genomic Resources to Improve Lima Bean Breeding for Consumer Quality and Agronomic Traits", which will include genotyping and characterizing the available lima bean collection of >700 accessions, among other activities.

Between 2010 and 2020 we averaged 330 accessions regenerated annually (Figure 1). Ideally, we would be on a 20-year regeneration cycle to ensure seeds of high vigor for distribution. Key challenges we are addressing are limited greenhouse space, a significant portion of the collection being photoperiod sensitive, and perennial wild relatives that are not easily adaptable to cultivation or regeneration but contain potentially valuable diverse genetics.

Accessions generally remain on the available for distribution list if we have 300 or more viable seeds. A general distribution is 20-25 seeds depending on the species. Over a 20-year period, the most popular accessions were requested just under 100 times each, which would require 2,000 seeds to meet cooperators needs. The 50<sup>th</sup> most popular accession is requested just under 50 times, which would only need 1,000 seeds for distribution in 20 years. We are balancing meeting the needs of stakeholders' distribution requests with conserving the genetic diversity spanning all of the accessions because we cannot predict what will be needed in the future (Table 2, Figure 1).

Though the non-common bean *Phaseolus* species make up less than a quarter of the genebank collection, in recent years they account for larger than a quarter of the distribution requests, demonstrating interest from our researcher community in gaining genetics and knowledge about these less commercially utilized accessions. This is likely a combination of interest in other species, and the fact that the USDA genebank is one of the few sources of these diverse germplasm.

These non-common bean species tend to be more challenging than common beans to regenerate in our greenhouses but remain a top priority in our conservation efforts.

				%			
	Total	Total	Backup	Backed		%	
Taxon	PI	W6	NCGRP	Up	Available	Available	Total
P. vulgaris	11,349	2,423	11,931	87%	12,101	88%	13,772
P. coccineus	331	151	198	41%	32	7%	482
P. acutifolius	234	249	168	35%	208	43%	483
P. lunatus	1,028	1,246	684	30%	488	21%	2,274
P. dumosus	93	4	77	79%	53	55%	97
P. spp. and							
hybr.	92	124	65	30%	94	44%	216
P. 'other'	102	302	20	5%	64	16%	404
Total							
Collection	13,229	4,499	13,143	74%	13,040	74%	17,728

**Table 1.** USDA National Plant Germplasm Phaseolus Collection Status November 2023

Table 2. USDA National Plant Germplasm Phaseolus Collection Status November 2023

	<b>Phaseolus</b> Seed Packets Shipped to Cooperators Globally
Taxon	(01/01/2022-11/07/2023)
P. vulgaris	5,484
P. coccineus	169
P. acutifolius	935
P. lunatus	2,567
P. dumosus	108
P. spp. and hybr.	132
P. 'other'	150
Total	9,545

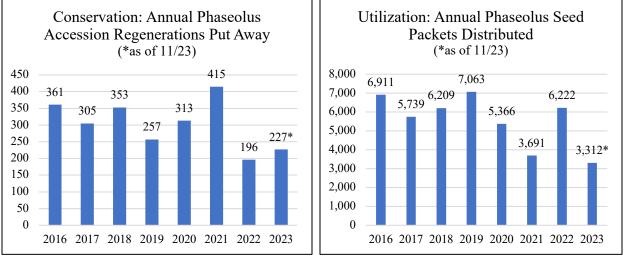


Figure 1. Annual conservation and utilization of Phaseolus collection

# CANDIDATE GENES AND MARKERS FOR RESISTANCE IN THE BCMV/BCMNV HOST-PATHOGEN INTERACTION IN COMMON BEAN

# Alvaro Soler-Garzón<sup>1</sup>, Timothy G. Porch<sup>2</sup>, Phillip E. McClean<sup>3</sup>, Valerie Geffroy<sup>4</sup>, and Phillip N. Miklas<sup>5</sup>

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**INTRODUCTION:** Bean common mosaic virus (BCMV) and Bean common mosaic necrosis virus (BCMNV) are related potyviruses that plague common bean worldwide. Drijfhout (1978) characterized host resistance conferred by the dominant 'I gene' and six recessive alleles across four loci: *bc-1*, *bc-1*<sup>2</sup>, *bc-2*, *bc-2*<sup>2</sup>, *bc-3*, and *bc-u* (helper gene). Our principal objective was to use new genomic tools to physically map and characterize the genes conditioning resistance to BCMV/BCMNV.

**MATERIALS AND METHODS:** *I* gene knockout mutants were identified by evaluation of 1,657 M3 lines from the BAT93 EMS mutant population (Porch et al., 2009) against the BCMNV NL-3 strain in greenhouse conditions. Recessive gene candidates were identified by GWAS in bean diversity panels inoculated with BCMV (US-6) and BCMNV (NL-8, NL-3) strains, along with large SNP datasets. Causal mutations were identified by sequencing candidate genes using Illumina and Pacbio technologies. Markers were developed for the causal mutations for marker-assisted selection.

**RESULTS AND DISCUSSION:** Our findings are summarized in Table 1, which includes Tmshift SNP and Indel markers for detecting resistance alleles. The identified alleles, their corresponding markers, and their genomic positions provide a foundation for further research and breeding efforts.

				•		
BCMV resistance alleles	ID marker	Chr.	Position (bp) G19833 v2.1	Allele Resistant	Allele Susceptible	Reference
I	Genel_x4	Pv02	Cluster NB-LRR	149 bp	-	unpublished
bc-1	S03_4203361	Pv03	4,203,361	Т	А	Soler-Garzón et al. (2021a)
<sup>a</sup> bc-2 <sup>[UI 111]</sup>	Pvvps4_del	Pv11	9,272,542 – 9,262,459	deletion of 10 kb	wild type	Soler-Garzón et al. (2021b)
<sup>a</sup> bc-2 <sup>[Robust]</sup>	Pvmit-2_C_del	Pv11	9,278,765	deletion of one base	С	Soler-Garzón et al. (2021b)
bc-3	PveIF4E <sup>1,3,4</sup> _PveIF4E <sup>2</sup>	Pv06	27,204,768	G	А	Hart and Griffiths (2013)
bc-u <sup>d</sup>	IND_05_36225873	Pv05	36,225,873	Insertion 84 bp	wild type	Soler-Garzón et al. (2023)
<i>bc-u<sup>r</sup></i> (Formerly <i>bc-4</i> )	Pvmit-1_T_G	Pv05	36,225,550	G	т	Soler-Garzón et al. (2021b; 2023)

**Table 1.** Tm-shift SNP and Indel markers for detecting BCMV resistance alleles.

<sup>a</sup>Different mutations, a 10 kb deletion and SNP, disrupt the protein encoded by *Bc-2*, but have similar effect, thus do not represent functional alleles.

Table 2 presents phenotypic reactions for *bc-u* 'helper alleles' combinations with *bc-1*, *bc-2*, and *I* resistance genes against NL-3 (Pathogroup PG-VI) and US-6 (PG-VII) strains, elucidating the complex interactions between resistance genes and virus pathogroups.

 		- (	,						
NL-3	(PG-VI)	<i>I</i> gene	No / gene	US-6 (PG-VII)		/ gene	No / gene		
bc-u <sup>r</sup>	bc-1	Top Necrosis	Mosaic	bc-u <sup>r</sup>	bc-1	No symptoms	Mosaic		
DC-U	bc-2	Top Necrosis	Mosaic	DC-U	bc-2	No symptoms	No symptoms		
bc-u <sup>d</sup>	bc-1	Vein Necrosis	Mild Mosaic	h a sid	bc-1	No symptoms	Mosaic		
DC-U <sup>a</sup>	bc-2	Local lesion	No symptoms	bc-u <sup>d</sup>	bc-2	No symptoms	Mosaic		

**Table 2.** Phenotypic reactions for *bc-u* 'helper alleles' combinations with *bc-1*, *bc-2* and *I* resistance genes against NL-3 (PG-VI) and US-6 (PG-VII) strains.

# CONCLUSIONS

- A specific TIR-NB-LRR (TNL) within a cluster of related TNL genes was identified as the *I* gene candidate. Mutations, including a SNP and a transposable element disruption, were found in this gene, affecting its translation (publication pending).
- Gene models encoding Vps4 AAA+ ATPase ESCRT proteins in Pv05 and Pv11, are candidates for the *bc-u* and *bc-2* resistance genes, respectively (Table 1).
- There are two *bc-u* alleles represented by different causal mutations within the same Vps4 candidate gene on Pv05: *bc-u*<sup>d</sup> interacts with *I*, *bc-1* and *bc-2*, whereas *bc-u*<sup>r</sup> only interacts with *bc-2* and only with BCMV strains (Table 2).
- There were two different causal mutations for the same Vps4 candidate gene on Pv11 for bc-2, a large deletion  $bc-2^{[UI 111]}$  and a bp deletion  $bc-2^{[Robust]}$ , but with exact same effect.
- The *bc-1* resistance gene on chromosome Pv03 is linked with two receptor-like kinases, but a specific candidate gene has not been identified.

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## POTENTIAL OF GENOMIC PREDICTION FOR WHITE MOLD IN DRY BEAN (PHASEOLUS VULGARIS L.)

## Molly Irvin<sup>1</sup>, Francisco E. Gomez<sup>1</sup>, and Qijian Song<sup>2</sup>

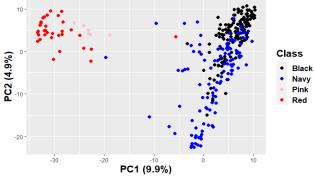
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**INTRODUCTION:** Michigan is the second largest producer of dry beans in the U.S. However, white mold, caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, is consistently ranked as the top yield limiting disease of dry bean production annually. To date there are no cultivars with high levels of resistance and progress to breed new cultivars has been hindered due to the quantitative inheritance of this trait and screening dependent on the presence of the pathogen under suitable environmental conditions. Due to the quantitative inheritance of this trait, methods such as marker-assisted selection (MAS), that aim to pyramid only a few target genes, limit progress towards developing more tolerant cultivars. A new alternative breeding strategy is using genomic prediction and selection, which utilizes genome-wide marker coverage to predict genotypic values for quantitative traits. Therefore, the purpose of this study is to evaluate a training population for genomic prediction to increase the accuracy of selection for white mold resistance in dry bean.

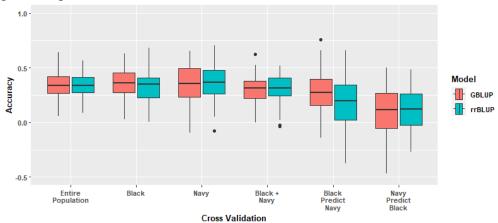
**MATERIALS AND METHODS:** A total of 300 advanced breeding lines from the three major market classes (black, navy, and small red) were used to evaluate the potential of genomic prediction models to screen for white mold resistance. All lines were grown under natural white mold infestation using overhead pivot irrigation in a disease nursery at Montcalm County, MI during the 2017-2023 growing seasons. Visual plot-wise disease severity was rated on a scale of 1 to 9, as described in Miklas et al. (2001)(3). Lines were genotyped using the Illumina Infinium BARCBean12k Bead chip in collaboration with the USDA-ARS, in Beltsville, Maryland. SNP quality filtering in TASSEL resulted in a total of 2,939 polymorphic markers. A principal component analysis (PCA) to evaluate population structure was also performed in TASSEL(1).Two genomic prediction models (rrBLUP and GBLUP) were implemented in the rrBLUP package in R and trained using phenotypic and genotypic data from the training sets using a 5 fold cross-validation scheme where 4 folds were used as the training set and 1 fold was used as the testing set (2). Best linear unbiased estimators (BLUEs) were predicted for individuals in the validation set and prediction accuracy was evaluated as the ratio between the observed and predicted genotypic values.

**RESULTS AND CONCLUSION:** The PCA analysis of the training population revealed a population structure with three distinct clusters (Figure 1). There is a significant overlap among black and navy beans which reflects the crossing scheme of the breeding program. Average prediction accuracy was moderate for every subset and model with a mean prediction accuracy ranging from 0.10 to 0.37 (Figure 2). For most subsets rrBLUP and GBLUP were not significantly different in prediction accuracy. These preliminary results indicate that genomic prediction has potential as a tool to assist screening efforts for white mold. Moving forward we will continue to increase/update the training population to further increase genomic prediction accuracy. Further evaluations of the accuracy of within/between market class predictions and the possibility of

genetic heterogeneity for white mold among the market classes are also interesting questions to explore in the future.



**Figure 1.** Principal component analysis for the entire population used as the training set for genomic prediction.



**Figure 2.** Comparison of genomic prediction accuracy for two models, rrBLUP and GBLUP, over training population cross validation subsets

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# GENE EXPRESSION ANALYSIS IN AMENDOIM CAVALO COMMON BEAN CULTIVAR CHALLENGED WITH *COLLETOTRICHUM LINDEMUTHIANUM*

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## **INTRODUCTION**

Anthracnose, caused by the fungus *Colletotrichum lindemuthianum*, poses a significant and widespread threat to the common bean crop. The use of plant genetic resistance has proven to be the most effective strategy for managing anthracnose disease incidence. The Amendoim Cavalo (AC) Andean cultivar has resistance against multiple races of *C. lindemuthianum*, which is conferred by the *Co-AC* gene. Fine mapping of this resistance gene to common bean chromosome Pv01 enabled the identification of *Phvul.001G244300*, *Phvul.001G244400*, and *Phvul.001G244500* candidate genes (Gilio et al., 2020). In this study, we aimed to assess the relative expression of Co-AC candidate genes, as well as other potential genes in the vicinity of this locus and known resistance genes, in the AC cultivar following inoculation with race 73 of C. lindemuthianum.

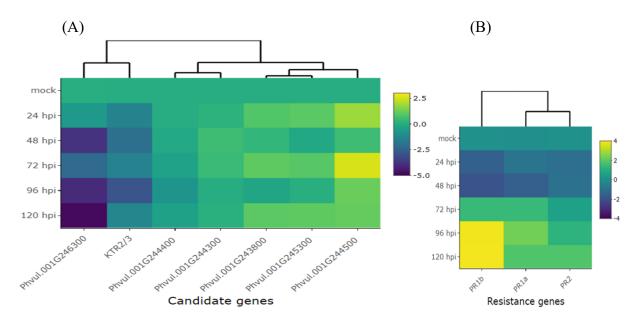
## MATERIAL AND METHODS

The AC cultivar and race 73 of C. lindemuthianum were used in our study. The plants were inoculated with a suspension containing  $2.0 \times 10^6$  conidiospores mL<sup>-1</sup>, at the v<sub>3</sub> development stage. Leaf samples of the first trifolium were collected 24, 48, 72, 96, and 120 hours post inoculation (hpi), and on mock. We use three biological replicates and three technical replicates. The total RNA was extracted, purified and the cDNA was synthetized. We evaluated the relative expression of the candidate genes for the Co-AC locus: Phvul.001G244300, Phvul.001G244400, and Phvul.001G244500 proposed by (Gilio et al. 2020), the candidate genes Phvul.001G246300 and Phvul.001G245300 identified by Gonçalves-Vidigal et al. (2020) and validated by Lovatto et al. (2023) for the CoPv01<sup>CDRK</sup> locus, the Phvul.001G243800 gene for Co-1<sup>2</sup> (Zuiderveen et al., 2016), KTR2/3 for Co-x (Richard et al., 2021) and the known resistance genes Phvul.003G109100 (PR1a), Phvul.006G196900 Phvul.009G256400 (PR1b), and (PR2).The genes Phvul.001G133200 (IDE) and Phvul.008G011000 (ACT) were used as reference genes, using the arithmetic mean of the Cq values. Relative expression was determined based on Cq values normalized with the reference genes using the  $2^{-\Delta \Delta CT}$  method (Livak and Schmittgen, 2001). The results are presented in the base 2 logarithmic scale of the fold change of relative gene expression. The calibrator condition was the mock. Data analysis were performed on the 'computational environment for statistical analysis R'. The heatmaps were built using the heatmaply R package.

## **RESULTS AND DISCUSSION**

Gene expression analysis revealed significantly higher expression levels of *Phvul.001G244500* with inoculation of race 73 of *C. lindemuthianum*. It was highly expressed in the AC cultivar at 24 and 72 hpi. Notably, *Phvul.001G244500* encodes a putative Basic Helix-Loop-Helix (bHLH) transcription factor, suggesting its involvement in the regulation of defense responses.

Furthermore, we observed a significant modulation of the expression of the defense related genes *PR1a*, *PR1b*, and *PR2*, especially *PR1b* at 96 and 120 hpi. Interestingly, the expression of *KTR2/3* and *Phvul.001G246300*, which were previously found to be highly expressed in the Jalo EEP558 (Mahiya-Farooq et al. 2019) and CDRK cultivars (Lovatto, et al., 2023) were downregulated in AC upon inoculation.



**Figure 1.** Heatmap of relative expression of the candidate genes to the *CoAC locus* (A) and other resistance genes (B) 0, 24, 48, 72, 96, and 120 hours post inoculation, and on mock in the AC cultivar inoculated with race 73 of *C. lindemuthianum*.

## CONCLUSIONS

Our study provides valuable insights into the genetic basis of resistance to *C. lindemuthianum* race 73 in the AC cultivar. These findings contribute to the development of improved strategies for breeding anthracnose-resistant common bean cultivars, thereby mitigating the impact of this devastating pathogen on crop yields and ensuring sustainable bean production.

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## **RESISTANCE TO BACTERIAL BROWN SPOT IN ADZUKI BEAN**

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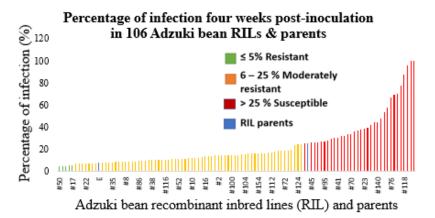
**INTRODUCTION:** Adzuki bean [*Vigna angularis* (Willd.) Ohwi & Ohashi] is the most profitable small-seeded bean grown in Ontario (Moran, 2016). It is cultivated in Canada primarily to be exported to Japan and generated over US\$32 million in foreign revenue for Canada in 2022. In that year, Canada ranked as the second-largest exporter of adzuki beans globally (Trend Economy, 2023). Bacterial brown spot caused by *Pseudomonas syringae* pv. *syringae* van Hall recently became a threat to the cultivation of adzuki beans in Ontario and can result in yield losses of up to 40 percent (King, 2019). *P. syringae* strains secrete virulence factors that target host defense mechanisms to circumvent host resistance (Xin *et al.*, 2018). Genetic resistance is the most effective way to manage this disease since other control measures (physical, chemical & cultural) are not effective. The challenge however is that there are no commercial adzuki varieties that are resistant to bacterial brown spot. This research addresses the hypothesis that genetic differences between the interactions between *P. syringae* pv. *syringae* and adzuki bean that result in resistance and susceptibility to bacterial brown spot can be identified and utilized to create molecular markers for breeding bacterial brown spot-resistant varieties.

MATERIALS AND METHODS: A strain of P. syringae pv. syringae (Pss27) was received from Agriculture and Agri-Food Canada, Harrow Research Centre. The identity of the strain was verified by amplification and sequencing of the 16S rRNA gene. A mapping population of 106 F<sub>6</sub> recombinant inbred lines (RIL) was developed from a cross between Erimo and a Chinese cultivar (CV) and screened for the response to Pseudomonas syringae pv. syringae inoculation under controlled conditions. Six-week-old plants were grown in two-gallon pots in a growth room at 20 - 24 °C and transferred to a misting tent for 48 hours at >95 % relative humidity. Pss27 was cultured on milk-tween agar for 24 hours at 28 °C. A liquid culture of Pss27 made by diluting agar culture in Ringer's solution to optical density >0.3 (approx.10<sup>8</sup> CFU/ml) was used for inoculation. Inoculation of the parents and RILs with Pss27 was achieved by wounding trifoliate leaves with a floral frog and applying a sponge soaked in the culture to the wounds. Weekly, visual disease rating on a 0-9 scale (Schoonhoven and Pastor-Corrales, 1987), was carried out for four weeks post-inoculation. The APS Access 2.0 software was used to digitally estimate the percentage of infection in digital images of inoculated leaves. The experiment was a randomized complete block design with four replicates. Long-read PacBio sequencing was carried out on DNA isolated from the RIL parents. The sequences were assembled and FASTGBS V2 was used to align short reads from genotyping by sequencing (GBS) of the RILs to call single nucleotide polymorphisms (SNPs) variants in the RILs.

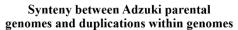
**RESULTS AND DISCUSSION:** From this research, a screening methodology for bacterial brown spot in adzuki was established. Results from inoculations show that the adzuki parents showed a disease differential response to Pss27 and the RILs showed segregation for resistance to bacterial brown spot. Erimo was the more resistant parent, and the CV was the susceptible parent.

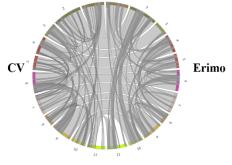
Assembly of long read sequence of the parental genomes using HiFiASM resulted in 534 Mb and 562 Mb contig sequences in Erimo and CV respectively.

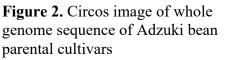
Filtering of bi-allelic sites in the GBS data of the RILs through the FASTGBS\_V2 pipeline yielded 14,614 SNPs. SNPs generated from GBS of the RIL population will be aligned with phenotypic data to detect QTL for resistance to bacterial brown spot. Virulence factors identified in *P. syringae* pv. *syringae* strains will be matched with host genes (candidate genes) in QTL regions to identify potential markers for resistance to bacterial brown spot.



**Figure 1**. Percentage of infection in Adzuki parents and RILs after one round of inoculation







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## IDENTIFICATION OF EFFECTOR BINDING ELEMENTS LOCALIZED IN PROMOTER REGIONS OF GENES OF *PHASEOLUS VULGARIS* L TARGETS OF *XANTHOMONAS* SPECIES TRANSCRIPTION ACTIVATOR-LIKE EFFECTORS

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#### **INTRODUCTION**

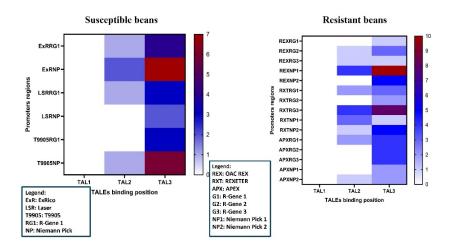
Common bacterial blight (CBB) disease is considered one of the most devastating common bean diseases worldwide. It is caused by *Xanthomonas phaseoli* pv. *phaseoli* and *Xanthomonas citri* pv. *fuscans*. These pathogens harbor effectors called Transcription Activator-like Effectors (TALEs), which are injected into host cells via a type III secretion system (T3SS). These effector proteins migrate to the nucleus and mimic eukaryotic transcription factors to modulate host cell gene expression, by recognizing and binding to specific promoter regions in the host genome called Effector Binding Elements (EBEs) and interacting with general transcription factors from the plant. Some common bean varieties have genes for CBB resistance and molecular markers associated with CBB resistance loci such as SAP6, BC420, or SU91. The most effective source of resistance is associated with SU91 on chromosome eight. A few genes near to this molecular marker have been identified, including R genes and a Nieman Pick gene, that may be related to resistance in the host (Perry et al. 2013).

#### **MATERIALS AND METHODS**

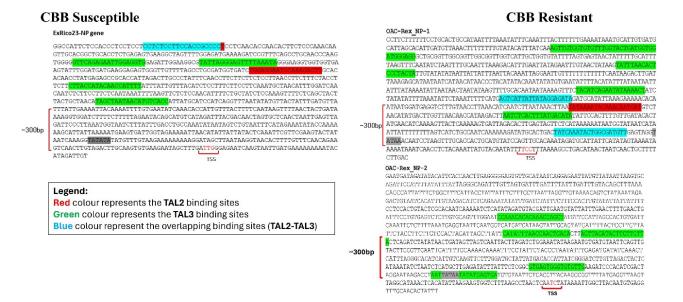
Six common bean genotypes whose genomes are totally sequenced (Perry et al, unpublished) where utilized to identify and characterize the EBEs for TALE proteins in the promoter regions of genes surrounding the SU91 molecular marker. Three are susceptible (ExRico, Laser, T9905) and three are resistant to CBB (OAC Rex, Rexeter and Apex). In addition, three different TALE sequences corresponding to *X. phaseoli* pv. *phaseoli* and *X. citri* pv. *fuscans* strains, respectively were used to localize the TALEs binding sites on promoter region of the studied genes. Bioinformatic tools such as CLC Genomic Workbench 22 (QIAGEN, Inc), FGENESH and TSSPlant (http://www.softberry.com/) allowed us to find the gene positions as well as the TATA boxes and transcription start sites (TSS) in the promoter regions of each analyzed gene on chromosome eight in order to find the positions of the DNA arrangements recognized by general plant transcription factors as well as by TALE proteins. Online platforms, such as TAL Effector Nucleotide Targeted 2.0 (https://tale-nt.cac.cornell.edu/) and PrediTALE (http://jstacs.de/index.php/PrediTALE), were used to identify and predict the DNA target preferences for TALE proteins.

#### **RESULTS AND DISCISSION**

In the 105 genes that surround the SU91 molecular marker, 94 genes contain binding sites for more than one TAL effector protein. In addition, the number of effector binding elements in the promoter regions differ between susceptible and resistant genotypes respectively (Figure 1). Furthermore, the positions of the TALE binding sites in the promoter regions of genes in resistant genotypes were closer to the transcription start sites compared to those sites in the genes in susceptible lines, meaning their expression might depend on the proximity of the EBEs to the TSS in the gene promoter region (Figure 2).



**Figure 1**. Number of TALE binding sites in the promoter regions of R genes and Niemann Pick genes in bean genotypes.



**Figure 2**. TALE binding site positions on the "ExRico" Niemann Pick promoter and "OAC-Rex" Niemann Pick promoter regions.

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## STUDY OF IMPROVED BEAN VARIETIES, IN MONOCULTURE AND INTERCROPPED WITH MAIZE, AND OF THE RHIZOBIA-BEAN SYMBIOTIC SYSTEM UNDER DIFFERENT AGRONOMIC TREATMENTS

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## INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is an annual legume species, predominantly autogamous, and constitutes an extremely important food since its dried grain represents the main source of vegetable protein in the human diet in many parts of the world. The general objective of this work was the study of improved varieties of common bean, under different cropping systems and different treatments and agronomic cultivation methodologies.

## MATERIAL AND METHODS

The agromorphological and qualitative study of four improved bean varieties (Andecha, Galaica, Montcau and Matterhorn, Figure 1) under different agronomic conditions included:

- 1. Soil amendments
  - With lime= 30% CaO + 7% MgO
  - No lime
- 2. Cropping systems
  - Monoculture
  - Intercropping with maize ('Tui' landrace, Spain)
- 3. Fertilization
  - Control= 0
  - Nitrogen= OSMOFORM NXT 22-5-11+2Mg+TE
  - Inoculation with *Rhizobium etli* CFN 42 (Figure 1)

The field trials were carried out in 2019 and 2020, according to a factorial design with two replications (Steel et al., 1997) in the experimental fields of the MBG-CSIC (Pontevedra, Spain, 20 masl, 42° 26' N, 08° 38' W). Twenty-four traits were evaluated in root, plant, leaf, pod and grain.

## **RESULTS AND DISCUSSION**

The crop biological cycle indicator trait (flowering) presented significant differences between varieties, treatments and years, as well as significant interactions. These results are expected due to the genetic differences between the varieties for growth habit (type IV: Andecha, Galaica and Montcau; type II: Matterhorn), very different biological cycles and the influence of the conditions of each growing season on the development of the plants.

Most of the grain traits have shown significant differences between treatments, varieties and years, as well as their interactions. There were not been significant differences in nitrogen and phosphorus content in dry leaves.

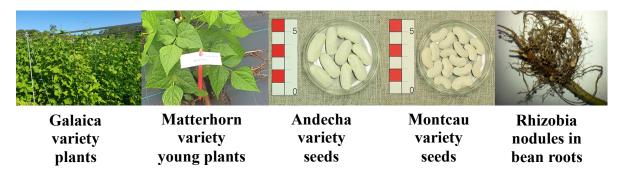


Figure 1. Common bean varieties studied and rhizobia nodules.

There have been significant differences between treatments in yield per plant and per hectare, which is a highly variable character over the years. Significant effects were also detected in their interactions. The different treatments present significant differences in all variables except those related to nodulation, which seems to indicate that this trait has not been affected by the limestone amendment of the soil, nor by the cropping system (monoculture/intercropping) (Drevon et al., 2015). These results should contribute to generating new knowledge about the performance of bean varieties under different cropping systems and agronomic conditions. This knowledge allows for innovation in bean cultivation, especially regarding improved varieties with high market value and the impact of the bean crop in the environment. Table 1 displays averages of some characteristics of the improved bean varieties studied.

VARIETY		TRAIT						
	First flower (d)	Yield (kg/ha)	Nodules/ plant	Grain size (g 100 grains <sup>-1</sup> )				
Andecha	65.1	1501	63.4	85.7				
Galaica	65.4	1303	44.0	86.8				
Montcau	64.4	1515	99.7	36.2				
Matterhorn	45.1	990	14.5	35.3				

 Table 1. Average main characteristics of the improved bean varieties studied.

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## NITROGEN EFFECTS ON FIXATION IN COMMON BEAN

## Yarmilla Reinprecht<sup>1</sup>, Lyndsay Schram<sup>1</sup>, Jamie Larsen<sup>2</sup>, Brett Hill<sup>3</sup>, Thomas H. Smith<sup>1</sup>, and K. Peter Pauls<sup>1</sup>

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## **INTRODUCTION**

Common bean is considered a poor nitrogen fixer and nitrogen fertilizer is frequently used to achieve high yields. However, this has a negative effect on nitrogen fixation. Our initial work demonstrated variation in inhibition of nitrogen fixation by addition of nitrogen fertilizer among genotypes from a Sanilac x Mist RIL mapping population (Reinprecht et al. 2020). In some genotypes, nitrogen fixation was less affected by added nitrogen fertilizer. In the current study, this investigation was extended to include some cultivated common bean varieties. The main objective of this work is to reduce nitrogen application in common bean production. The focus of the current study was to evaluate efficiency of nitrogen fixation under different nitrogen regimes in a set of diverse beans.

## MATERIALS AND METHODS

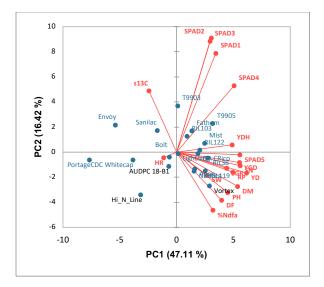
Twenty-one selected beans were evaluated under nine different nitrogen regimes in replicated field trials at University of Guelph Elora Research Station (ERS) on N-poor land over three years (2019 - 2021), using Split-Split-Plot Design (SSPD) with three replications in 4-row plots.

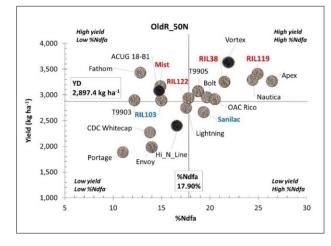
Rhizobium (R) included three levels: no Rhizobium (0R), commercially available combination of *Rhizobium leguminosarum* biovar viceae, *Rhizobium leguminosarum* biovar phaseoli and *Bradyrhizobium* sp. (York, PEI, Canada) used in the initial work (OldR), and Nodulator® (BASF, Canada) (NewR). Nitrogen (N) included three levels: no nitrogen (0N), 50 kg ha<sup>-1</sup> (50N), and 100 kg ha<sup>-1</sup> (100N). Genotypes (G) included 18 white and 3 black beans: nine genotypes from the 2016-2018 N study (*OldR\_100N / OldR\_0N ratios*), ten beans from the MDP navy bean panel (*based on %Ndfa, J Wilker's work*) and two extra black beans (*no %Ndfa data*).

Data were collected for conventional in-field measured traits including flowering (DF, days), maturity (DM, days), harvestability (HR, score 1-5), plant height (PH, cm), seed weight (SW, g), yield (YD, kg ha<sup>-1</sup>), SPAD (4-5x, SPAD values), carbon isotope discrimination ( $\sigma^{13}$ C), and percent nitrogen derived from atmosphere (%Ndfa), as a measure of nitrogen fixation. Additional traits were derived from the field measurements [reproductive period (RP = DM-DF, days), yield gain per day (YGD = YD/DM, kg ha<sup>-1</sup>), seed growth rate (SGR = YD/RP, kg ha<sup>-1</sup>), seed number (SN = YD/SW, seed number x 10<sup>6</sup> seeds ha<sup>-1</sup>), and yield per unit of height (YDH = YD/PH, kg ha<sup>-1</sup>).

## **RESULTS AND DISCUSSION**

Significant differences among genotypes were identified for all analyzed traits. There was a positive correlation between yield and %Ndfa. First two principal components (PC) explained 63.5% of the variability (Fig. 1). Averaged over three years, nitrogen significantly affected most of the analyzed traits, while the effect of rhizobia was marginal. High yielding bean genotypes with good nitrogen fixation (%Ndfa) were identified/ confirmed (Fig. 2).





**Figure 1.** Genotype-trait biplot analysis of selected bean genotypes evaluated at ERS over three years. Three black beans are indicated (black). Length and vector orientation indicate strength of positive/ negative correlation among traits.

**Figure 2**. Selection of high yielding beans with good nitrogen fixation (%Ndfa). Yield and %Ndfa of 20 bean genotypes under the OldR\_50N treatment. Plot is divided into four quadrants based on the yield and %Ndfa mean values (black lines). The best performing beans are in the top right quadrant (they are both high yielding and good nitrogen fixers).

#### CONCLUSIONS

The work confirmed the existence of variation in nitrogen fixation (%Ndfa) and good performance of lines RIL38 and RIL119. These two genotypes were high yielding and good nitrogen fixers under most of the applied nitrogen regimes and may be useful for tandem improvement of both traits. The reduction of nitrogen fertilizer in bean production would significantly improve the ecological footprint of the bean crop and would be a major advance in profitability of the common bean industry in Canada.

#### ACKNOWLEDGEMENTS

This work was financially supported by the Ontario Bean Growers, Natural Sciences and Engineering Research Council of Canada (NSERC), Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) and Canada First Excellence Fund.

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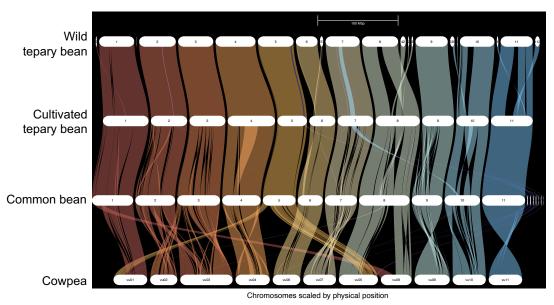
## **GENOME-ENABLED BREEDING ACROSS PHASEOLUS SPECIES**

# Yi-Wen Wang<sup>1,2</sup>, Joshua C. Wood<sup>1</sup>, John P. Hamilton<sup>1,5</sup>, Kathrine Mailloux<sup>1</sup>, Brieanne Vaillancourt<sup>1</sup>, Consuelo Estévez de Jensen<sup>3</sup>, Tim Porch<sup>4</sup>, C. Robin Buell<sup>1,2,5</sup>

<sup>1</sup>Center for Applied Genetic Technologies, University of Georgia, <sup>2</sup>Institute of Plant Breeding, Genetics, & Genomics, University of Georgia, <sup>3</sup>Department of Agro-Environmental Sciences, University of Puerto Rico-Mayagüez, <sup>4</sup>USDA-ARS, Mayagüez, PR, <sup>5</sup>Department of Crop & Soil Sciences, University of Georgia

**INTRODUCTION:** *Phaseolus vulgaris*, common or dry bean, is the most widely cultivated dry seed legume and an important source of plant protein for human consumption. Its sister species, tepary bean (*Phaseolus acutifolius*), which is native to the Sonoran Desert, is closely related to common bean and is also cultivated. Common bean is susceptible to several diseases and abiotic stresses for which resistance is present in tepary bean. In this project, we will transform common and tepary bean breeding by exploiting their close relationship through the development of a knowledgebase that permits rapid trait discovery and subsequent breeding between the two species.

**RESULTS AND DISCUSSION:** We constructed a pan-*Phaseolus* knowledgebase for rapid causal trait identification and seamless breeding between *Phaseolus* species. By using GENESPACE (Lovell et al., 2022), a comparative genome analysis tool, orthologs and syntelogs across legume genome assemblies were identified (Fig. 1).



**Figure 1.** Riparian plot of four legume genomes generated using GENESPACE: wild tepary bean, cultivated tepary bean, common bean, and cowpea.

To augment the knowledge of loci encoding agronomic traits, such as disease resistance, in tepary bean, we sequenced a 290 accession tepary diversity panel via whole genome shotgun sequencing. Separately, we identified six tepary accessions with key traits of interest (Table 1) and

assembled their genomes using Oxford Nanopore Technologies genomic long read sequencing data. All genome assemblies have at least 98% complete Benchmarking Single Copy Orthologs (BUSCOs; Table 2), indicating a high level of completeness.

TDP Accession		Biological	Country	Importance	
	No.	No.	status		
	TDP013	G40022	Cultivated	USA	Parent of RIL population
	TDP312	TARS-Tep 23	Cultivated	Puerto Rico,	Rust immunity, caused by Uromyces
				USA	appendiculatus
	TDP136	G40168	Wild	Mexico	Unique habit, only tepary accession
					with a terminal raceme (type 5)
	TDP154	G40177E1	Wild	USA	Large seed; high nodulation capacity;
					resistance to common bacterial blight
	TDP159	G40178	Wild	Mexico	Resistant to bean common mosaic
					necrosis virus (BCMNV) strain NL3
	TDP176	G40199	Wild	Mexico	Resistant to common bean weevil
					(Acanthoscelides obtectus)

**Table 1**. Tepary bean accessions used for whole genome sequencing and assembly.

Table 2. Tepar	y bean genome	assembly statistics.
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No.	Total	Number	Largest	N50	BUSCO
	length (bp)	of contigs	contig		
TDP013	619,577,581	1,488	27,457,760	6,315,316	C:99.5%[S:96.5%,D:3.0%],
					F:0.2%,M:0.3%,n:1614
TDP312	614,462,498	1,836	24,502,702	3,791,180	C:99.4%[S:96.5%,D:2.9%],
					F:0.3%,M:0.3%,n:1614
TDP136	608,568,985	2,281	10,280,135	1,786,457	C:98.9%[S:95.7%,D:3.2%],
					F:0.4%,M:0.7%,n:1614
TDP154	602,141,357	1,559	30,497,859	8,163,584	C:99.3%[S:96.3%,D:3.0%],
					F:0.4%,M:0.3%,n:1614
TDP159	720,374,745	4,628	24,884,061	2,360,081	C:99.2%[S:95.4%,D:3.8%],
					F:0.3%,M:0.5%,n:1614
TDP176	610,138,667	1,814	28,527,423	8,988,892	C:99.3%[S:96.1%,D:3.2%],
					F:0.3%,M:0.4%,n:1614

Further, to facilitate genome-enabled breeding of tepary bean, a Practical Haplotype Graph (PHG) (Bradbury et al, 2022) is being constructed to store and use with reduced representation genotyping data to impute haplotypes. The resources we generated in this study will be useful for identifying genes and quantitative trait loci for traits of interest in common and tepary bean.

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## DRONE IMAGERY PHENOTYPING USING MACHINE LEARNING APPROACHES TO ESTIMATE HARVEST MATURITY IN DRY BEANS

## Maria Roberta de Oliveira<sup>1</sup>, Juan M. Osorno<sup>1</sup>, Jose Figueroa-Cerna<sup>1</sup>, Sai Manogna<sup>2</sup>, Paulo Flores<sup>2</sup>

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### **INTRODUCTION**

Plant breeding programs aim to develop improved crop varieties combining many desirable traits of economic importance. Among these traits, measuring crop maturity is essential as it significantly impacts harvest management. While the conventional method for determining crop maturity involves visually assessing fields multiple times toward the end of the growing season (Volpato et al., 2021), this procedure is highly subjective and often demands a significant amount of time and costs. High-throughput data collection and computational analysis could be used to overcome these challenges (Dar et al., 2021). Therefore, this study aimed to investigate the potential of predicting harvest maturity dates for dry bean breeding genotypes using unmanned aerial systems (UAS) based data and computational methods.

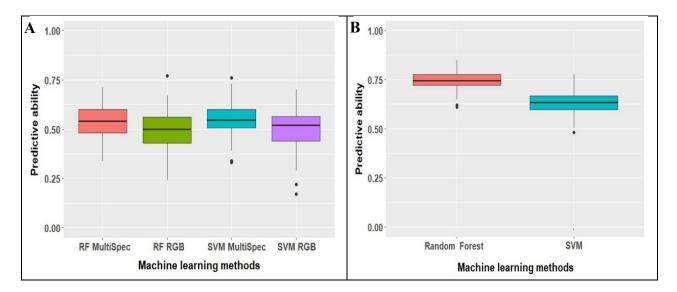
### MATERIALS AND METHODS

The study was carried out on field trials belonging to the dry bean breeding program at North Dakota State University throughout the 2022 and 2023 growing seasons. Evaluations were conducted on six market classes within advanced yield trials to estimate harvest maturity. These classes included pinto (PAYT), slow-darkening pinto (SDPAYT), great northern (GNAYT), navy (NAYT), black (BAYT), and red and pink (RPAYT) beans. Maturity assessment involved visually recording the number of days after planting required for a plot to exhibit 95% of pods with mature brown coloration. Simultaneously, aerial RGB and multispectral images were captured on a weekly basis, for four weeks in September using both Autel Evo II 6K and DJI Mavic 3M aircraft. Excess Green Index (ExGI) was calculated from the orthomosaic, and statistics (mean, median, and standard deviation) were generated for each plot. The study also evaluated the use of RGB versus multispectral images for determining the optimal UAS to be employed in the breeding program. Likewise, predictive ability was compared between the Random Forest and the Support Vector Machine models by combining data from 2022 and 2023. After selecting the best model, an empirical validation approach was used with the training set derived from 2022 data and the testing set from 2023 to estimate dry bean harvest maturity.

#### **RESULTS AND DISCUSSIONS**

Preliminary results from the 2022 season showed a strong correlation between the UAS data (ExGI) and days to maturity, with navy beans showing the highest value (r= 0.79). The correlation coefficient for other market classes ranged from 0.46 to 0.66. Results indicated that RF and SVM showed similar performance in predicting dry bean harvest maturity using data collected with either RGB or multispectral cameras in 2023. This suggests that from a cost-effective investment perspective, a simple UAS with RGB suffices due to its lower price, especially if focusing solely on maturity traits (Figure 1A). When combining data from 2022 and 2023, RF showed a slight better performance over SVM based on the observed predictive ability values, emphasizing the

importance of larger datasets for enhancing machine learning performance (Figure 1B). In the empirical validation approach for estimating dry bean maturity, higher prediction ability was observed in NAYT and RPAYT (r=0.60 and 0.70, respectively). This is likely due to the narrower maturity ranges found within these dry bean classes, which result from breeding programs favoring early-maturity lines through multiple selection cycles. Additional data will be collected during the 2024 growing season to enhance the predictive accuracy of the models. The ultimate goal of this research is to identify early-maturing lines or potentially eliminate late-maturity lines using image data, thus optimizing the pipeline efficiency.



**Figure 1.** A) Comparison of the prediction ability between RGB and multispectral cameras from data collected in 2023 using RF vs SVM models. B) Comparison between RF and SVM models using 2022 and 2023 combined data from RGB camera.

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## MAPPING OF QUANTITATIVE TRAIT LOCI LINKED TO MORPHO-AGRONOMIC TRAITS IN RILS DERIVED FROM CDRK×YOLANO COMMON BEAN (PHASEOLUS VULGARIS) POPULATION

## A.C. Calvi<sup>1</sup>, M.C. Gonçalves-Vidigal<sup>1</sup>, M. Vaz-Bisneta<sup>1</sup>, P.S. Vidigal Filho<sup>1</sup>, M.P. Caixeta<sup>1</sup>

<sup>1</sup>Departamento de Agronomia, Universidade Estadual de Maringá, Paraná, Brazil.

#### INTRODUCTION

The breeding of common bean (*Phaseolus vulgaris* L.) is essential for obtaining cultivars with high yield, plant architecture suitable for mechanized harvesting, appropriate growth cycle and grain type compatible with market demands. Marker-assisted selection using DNA markers is an important tool in plant breeding and linkage mapping is the most common approach to detect molecular markers linked to quantitative trait loci (QTLs). The objective of this study was to map QTLs related to morpho-agronomic traits in the CY population, consisting of 110 Recombinant Inbred Lines (RILs), derived from the cross between California Dark Red Kidney (CDRK)  $\times$  Yolano.

### MATERIALS AND METHODS

The population was phenotyped in field conditions over three agricultural years (2018, 2019 and 2020) for the following traits: number of days to flowering (NDF), plant height (PH), first pod height (FPH), number of pods per plant (NPP), seed weight (SW) and grain yield (GY). Analysis of deviance and G × E interaction, for each pair of years, and for all years (Comb) were performed using a linear mixed model of REML/BLUP in Selegen (Resende, 2016). The RILs and parental DNA samples were genotyped using the BARCBean6K\_3 Illumina Bead Chip (5,398 SNPs) (Song et al., 2015). The BeadChip was imaged using the Illumina BeadArray Reader, and automatic allele calling for each locus was performed using the Genome Studio software v2.0 (Illumina, San Diego, CA, USA). All allele calls were visually inspected and any errors in allele calling due to improper cluster identification were corrected resulting in 3.227 polymorphic SNPs. The QTL mapping was estimated using the software QTL IciMapping 4.2 (Meng et al., 2015) and QTLs were denominated according to Miklas and Porch (2018).

#### **RESULTS AND DISCUSSION**

A total of 30 QTLs were detected for six traits distributed across eight chromosomes, explaining up to 39.56% of the phenotypic variation. QTLs for NDF were found on chromosomes Pv01, Pv03, and Pv09. QTLs for PH were identified on chromosomes Pv01 and Pv02, while for FPH, they were identified on Pv06. QTLs for NPP were detected on Pv01, Pv06, Pv07, and Pv10, and the genetic control for SW were identified on Pv01, Pv06, and Pv09. Additionally, QTLs for GY were found on Pv02, Pv04, Pv07, and Pv09. The identification of QTLs linked to agronomic traits in the CY population is important for bean breeding programs. The molecular markers found to be linked to QTLs should be further validated for use in multi-trait marker-assisted selection.

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Trait	QTL	Agr. year	Chr.	Left marker	Right marker	LOD	PVE
	CY NDF1	2018	1	ss715648158	ss715648157	4.38	5.9
Number of	CY NDF1	2018	1	ss715644923	ss715647963	3.28	4.37
Days to	CY NDF3	2019	3	ss715642683	ss715646295	51.4	18.46
Flowering	CY NDF9	2020	9	ss715647262	ss715647274	7.31	0.8
	CY NDF1	Comb.	1	ss715639957	ss715639272	13.78	34.2
	сү PH2	2018	2	ss715647096	ss715647679	4.44	4.66
	сү PH2	2018	2	ss715646164	ss715646142	9.86	11.69
Plant Height	CY PH1	2019	1	ss715639957	ss715639272	3.56	14.25
8	CY PH1	2020	1	ss715646075	ss715650911	9.87	34.25
	сү PH1.2	Comb.	1	ss715648889	ss715639956	11.23	39.56
	сү FPH6	2018	6	ss715648487	ss715648483	56.42	20.09
	сү FPH6	2019	6	ss715644796	ss715639700	54.01	13.4
First Pod Height	сү FPH6	2019	6	ss715645110	ss715639610	56.7	14.31
8	сү FPH6	2019	6	ss715647252	ss715647260	16.82	1.49
	сү FPH6	Comb.	6	ss715647966	ss715645202	54.89	27.65
	CY NPP1	2018	1	ss715639531	ss715639532	81,27	14.08
Number of	CY NPP10	2018	10	ss715645509	ss715645511	65.88	6.89
pods per	CY NPP6	2020	6	ss715639202	ss715639205	51.4	16.41
plant	CY NPP10	2020	10	ss715645511	ss715645514	37.78	8.16
	CY NPP7	Comb.	7	ss715646352	ss715646353	11.33	6.11
	CY SW9	2019	9	ss715647184	ss715647187	3.14	8.76
Seed	SW6	2020	6	ss715639897	ss715647426	20.07	11.96
Weight	SW9	2020	9	ss715645712	ss715645701	14.34	7.67
	SW1	Comb.	1	ss715645252	ss715645251	34.79	12.09
	GY4	2019	4	ss715646780	ss715648124	12.4	4.7
	GY4	2019	4	ss715648126	ss715648129	19.31	8.68
Grain	GY2	2020	2	ss715647098	ss715647100	16.48	13.3
Yield	GY2	2020	2	ss715639669	ss715639559	8.44	5.59
	GY7	Comb.	7	ss715646351	ss715646352	20.91	14.98
	CY GY9	Comb.	9	ss715645712	ss715645701	13.33	7.91

 Table 1. Significant QTL found in single environments.

Agr. Year: agricultural year; Chr: Chromosome.

### UNCOVERING INFLUENCES ON SEEDLING ESTABLISHMENT

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**INTRODUCTION:** The *Phaseolus* family represents an increasingly significant economic, cultural, and dietary crop for most of the world <sup>1,2,3</sup>. Therefore, growing resilient *Phaseolus* species continues to be an important goal of both scientific and agricultural communities. While a multifaceted approach to crop improvement is necessary, a unique focus is that of seedling establishment. Seedling establishment marks the point at which a vulnerable young plant reaches an autotrophic subsistence – deriving its energy from photosynthesis rather than limited seed reserves. Despite extensive research on seedling establishment across various plant families, its integration into breeding approaches has historically received less focus. Understanding and utilizing establishment time provides a crop with a competitive advantage against many undesirable pressures while allowing growers to tailor the timing of the crop for maximal yield and growth potential<sup>4,5</sup>. The following work emphasizes differences in establishment time amongst three species within the *Phaseolus* family with the goal of providing both phenotypic and genotypic evidence for these differences. This has the potential to aid future breeding efforts.

#### MATERIALS AND METHODS:

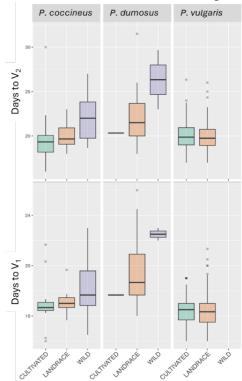
Plant material for this study was composed of accessions from *P. dumosus*, *P. coccineus*, and *P. vulgaris* (specifically the Andean Diversity Panel, Table 1). Seeds were scanned using an Epson V600 photo scanner and measured for size and

Table 1. Distribution of accessions by species and improvement

	P. vulgaris	P. coccineus	P. dumosus		
Cultivated	86	12	1		
Wild	0	13	2		
Landrace	88	21	52		
Total	174	46	55		

circularity using ImageJ software version 13.0.6. Two replicates of each line were planted at a depth of 2.5 to 3cm in perlite potting mix. Pots were arranged in a randomized block design and were grown in controlled greenhouse conditions at 24 °C and 57% humidity, given even application of water, and 16 hours of daylight provided by LED and HPS lighting. The following growth stages were collected daily by hand using the standardized dry bean growth staging guide from the Manitoba Pulse & Soybean Growers Association<sup>2</sup>: V<sub>E</sub> (emergence), V<sub>C</sub> (cotyledon stage), V<sub>1</sub> (first trifoliate), and V<sub>2</sub> (second trifoliate). This entire experimental procedure was conducted twice, observing plants until each reached the V<sub>2</sub> developmental stage. Taxonomic metadata was obtained from USDA-GRIN and subsequent statistical analysis was completed within the RStudio platform. A genome-wide association study (GWAS) was conducted using the GAPIT package in RStudio and version 2.1 of the *P. vulgaris* genome<sup>3</sup> to identify SNPs associated with seedling establishment.

**RESULTS AND DISCUSSION:** Preliminary analysis shows limited relationships between average seed size and time to establishment across the *Phaseolus* species accessions. There was no significant correlation between seed size and establishment time at the V<sub>1</sub> or V<sub>2</sub> stage in *P. vulgaris* (r = -0.05 and -0.09, respectively) and *P. dumosus* (r = +0.18 and -0.19, respectively). *P. coccineus* had a negative association between seed size and establishment time at r = -0.29 for V<sub>1</sub> and r = -0.40 for V<sub>2</sub>, but only the V<sub>2</sub> stage was significant (p= 0.01), indicating that larger seeds tend to establish faster at this stage.



Cultivation generally decreased establishment time across species with wild accessions being slower to reach each growth stage (Figure 1). The effect appears more pronounced during later stages (V1 and V2) rather than early in development. Genome wide association analysis of P. vulgaris using the Andean Diversity Panel, found preliminary significant SNPs at the V<sub>C</sub> and V<sub>1</sub> stages using the BLINK model and at the V<sub>C</sub> stage with the FarmCPU model. The SNPs at the beginning of chromosome 4 (S04 36912 and S04 1743734) had consistently high  $log_{10}(p)$  values for half of the growth stages and model combinations but was only significant at the V<sub>C</sub> stage. These findings require validation in the form of additional models and the inclusion of a larger dataset (Table 2). In conclusion, this work poses opportunity for future study of Phaseolus vulgaris accessions, geographic, and speciesspecific influences, and the validation of these exciting preliminary GWAS results using additional models.

**Figure 4.** Establishment time across species and cultivation status.

Table 2. Significant peaks from GWAS for establishment. SNPs are listed with chromosome (Chr) and position (Pos). Two models used, FarmCPU and BLINK.

SNP	Chr	Pos	p value	Model	<b>Growth Stage</b>
S03_50811858	3	50811858	6.171905e-08	FarmCPU	V <sub>C</sub>
S04_36912	4	36912	3.534143e-10	FarmCPU	V <sub>C</sub>
805_39027425	5	39027425	6.529082e-07	FarmCPU	Vc
807_30511085	7	30511085	5.651053e-08	FarmCPU	V <sub>c</sub>
S10_4644970	10	4644970	6.208979e-07	BLINK	V <sub>1</sub>
S10_41860750	10	41860750	2.394656e-09	BLINK	V <sub>1</sub>
S03_50811858	3	50811858	8.783544e-07	BLINK	V <sub>c</sub>
S04_1743734	4	1743734	4.344753e-10	BLINK	V <sub>c</sub>
S08_62366689	8	62366689	5.525742e-07	BLINK	V <sub>c</sub>

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### MARKET TRENDS OF NEW PULSE-BASED PRODUCTS LAUNCHED DURING 2012-2021 IN THE U.S. – DIVERSIFYING DEMANDS, CHALLENGES, AND OPPORTUNITIES

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**INTRODUCTION**: Pulses such as peas, chickpeas, beans, and lentils are high in protein, dietary fiber, vitamins, and minerals and provide various health benefits. Regardless, pulse consumption is low in the United States due to long cooking times and pulses not being in traditional Western diets. Milling whole pulses into flour for producing food products such as pasta, snacks, and baked goods may offer more familiar food choices to U.S. consumers and lead to an increase in pulse consumption. Indeed, pulse-based food products are increasingly becoming available in the market [1]. This study aimed to investigate pulse-based food products released in the past decade in the U.S. to characterize market trends.

**MATERIALS AND METHOS**: Bean consumption data was retrieved from USDA-ERS, per capita availability of dry beans, which is used as a proxy for per capita consumption (accessed on Sep 20, 2022). Mintel Global New Product Database (https://www.mintel.com/global-new-products-database) was used to identify food products newly launched in 2012–2021 in the U.S. (accessed on Sep 22, 2022). Selection criteria for product ingredients included whole pulses and their derivatives such as flours, fibers, starches, proteins, extracts, juice, and paste, excluding soybeans, sugar snap peas, peanuts, sword beans, alfalfa, and their derivatives.

**RESULTS AND DISCUSSION**: Dry bean consumption was stagnant from 1965 through 2015, with five-year averages ranging from 5.4–7.7 pounds per capita availability. However, 2015–2020 saw a slight increase in per capita availability with the five-year mean of 8.3 pounds. The high average was due to unusually high values in two years: 8.6 pounds in 2018 and 8.2 pounds in 2020. The high availability in 2020 may be due to the sudden increase of bean purchase after the outbreak of covid-19, which started in early 2020 [2].

Data search on Mintel Global New Product Database yielded 5,353 pulse-based food products launched between 2012–2021 in the U.S. The number of new products steadily increased over the decade, from 160 in 2012 to 662 in 2021. The market for pulse-based products has increased over time, and it is expected to grow further [3]. Figure 1A showed that food categories containing pulses have diversified over time. In 2012, Prepared dishes, Raw & canned, and Sauces & Spreads were the dominant categories, but the percentage of their use declined over time, and Bakery, Dairy, and Sweets categories increased their presence towards 2021. Snacks was the second most produced category along with Raw & Canned. Pea was used for Snacks (31%) and Other (37%) purposes including Dairy and Sweets (Figure 1B). Chickpea was used for Sauces & Spreads (34%), mainly hummus, followed by Snacks (21%). Prepared dishes accounted for 39% of Bean and 59% of lentil use, respectively. The results highlighted the wide variety of pulse-based food products and unique characteristics of food categories of each pulse type.

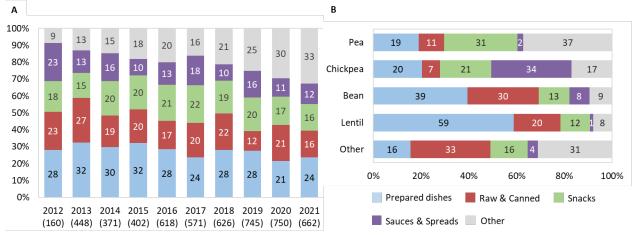


Figure 1. Food categories of newly released products during 2012–2021. (A) by year; (B) by pulse

Table 1. Claims used on  $\geq 15\%$  of all the newly released products.

C		% in
Group	Claim	5,353
		products
Suited for	Low/No/Reduced Allergen	57
	Gluten Free	53
	Kosher	45
	High/Added Fiber	17
"Natural"	GMO Free	45
	No Additives/Preservatives	27
	Organic	24
Environmental	Vegan/No Animal	32
Environmentai	Ingredients	32
	Environmentally Friendly	25
	Package	25
	Plant Based	15
Convenience	Microwaveable	30
	Ease of Use	17
Other	Social Media	24

Table 1 shows that allergenrelated, GMO-free claims were commonly used on the newly launched pulse-based products during 2012-2021. "Low/No/Reduced Allergen" claims were used on 57% of the 5,353 new products. In addition, "Gluten-Free" (53%), "Kosher" (45%), and "GMO Free" (45%) were considered to be the strengths manufactures that food emphasized.

Traditional challenges of pulsebased products include flatulence and "beany" taste and off-flavors [4]. There have been efforts to improve such traits via breeding and to optimize processing and product formulation methods via

food technology. Currently, pulse-flour-based products are associated with high prices and limited availability in store compared to conventional wheat-based products [5]; however, technological innovations for efficient and large-scale production and market expansion are expected.

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## FARMER PARTICIPATORY VARIETY SELECTION AND SEED DISSEMINATION OF HIGH YIELDING, FASTER COOKING AND IRON-RICH VARIETIES OF COMMON BEAN IN ZAMBIA

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## **INTRODUCTION**

The yellow beans are a major market class of common bean (*Phaseolus vulgaris*). In Zambia, yellow beans tend to fetch a higher price than other seed colors. One of the reasons for the strong consumer preference for yellow beans is because of its shorter cooking time than other market classes. Adoption of new bean varieties in Zambia remains low despite the availability of improved varieties. One of the reasons for low adoption include new varieties lacking traits considered important by the farmers such as seed type, seed color, and seed shape. One strategy that could potentially enhance variety adoption by farmers is participatory variety selection. The objective of this study was to conduct farmer participatory variety selection of high yielding, faster cooking and iron-rich varieties of common bean in Zambia.

## **MATERIALS AND METHODS**

The Yellow Bean Collection (YBC) of 255 genotypes consisting of landraces, breeding lines and varieties with variable shades of yellow seed colors from Africa, North America, South America, Europe, Central America, Caribbean, Middle East and East Asia (Sadohara et al., 2022). It was evaluated for agronomic performance in two field trials (2021 and 2022 growing seasons) at Malashi Research Station in Mpika, Zambia. The YBC was planted using a Randomized Complete Block Design with two replications. Each plot had two rows that were 4 m long. The plots were kept weed-free throughout the study. At harvest maturity, 20 farmers (10 women and 10 men) from Malashi village were invited for Farmer Participatory Variety Selection (FPVS). The farmers were asked to select the 10 best genotypes, then a consensus list of four genotypes that were consistently highest ranked in both growing seasons was developed. The four selected lines were evaluated for cooking time using the Mattson cooker at Cornell, USA and using an electric stove at the Zambia Collage of Agriculture in Mpika Zambia.

## **RESULTS AND DISCUSSION**

Of the 255 YBC genotypes that were evaluated for agronomic performance for two consecutive growing seasons, four genotypes (Ervilha, Y1609-1, Masindi and UGK93) (Table 1) were consistently ranked highest by the farmers. The average seed yield over the two growing seasons for the four selected genotypes ranged from 1,440 kg/ha (UGK93) to 2,050 kg/ha (Ervilha). The highest yielding genotype (Ervilha) among the four selected genotypes was also the highest yielding among the 255 YBC lines. Some of the traits or attributes that farmers considered in their selection of the four genotypes included productivity, seed size and seed color.

The four selected genotypes and five commercial checks were evaluated for cooking time in the laboratory (using a Mattson cooker) at Cornell University, USA and at Zambia College of Agriculture in Mpika, Zambia using an electric stove. The cooking time using Mattson cookers ranged from 66.7 minutes to 89 minutes. Cooking time using an electric stove followed a similar ranking to that of the Mattson cooker, with Ervilha having the fastest cooking time (130 minutes) while Mbereshi (commercial check) had the slowest cooking time (209 minutes).

## CONCLUSIONS

Four genotypes were selected from 255 genotypes using farmer participatory variety selection. One of the four selected lines (Ervilha) had the highest average seed yield and fastest cooking time.

**Table 1**. Seed yield and cooking time for the four genotypes that were selected using farmers participatory variety selection. The selected genotypes and commercial checks were evaluated for cooking time using Mattson cooker at Cornell University and an electric stove in Mpika, Zambia.

				<b>Cooking Time (Minutes)</b>		
Genotype	Seed Color	Origin	Yield (kg/ha)	Mattson Cooker	Electric Stove	
Selected Genotypes	(FPVS)					
Ervilha	Yellow (Manteca)	Angola	2,050	$66.7 \pm 1.5$ <sup>d</sup>	130	
Y1609-1	Yellow (Manteca)	USA	1,570	$73.0 \pm 1.0$ °	131	
Masindi	Yellow (Canario)	East Africa	1,529	$84.7\pm2.1$ $^{a}$	190	
UGK93	Yellow (Canario)	CIAT	1,440	$85.0 \pm 1.0$ <sup>a</sup>	182	
<b>Commercial Checks</b>	S					
Kilimanjaro	Yellow (Manteca)	Zambia	-	$82.0\pm2.6~^{\text{b}}$	137	
Zerengeti	Purple Speckled	Zambia	-	$78.3\pm2.5\ ^{\mathrm{b}}$	173	
Kabulangeti	Purple Speckled	Zambia	-	$82.0\pm2.0~^{\text{b}}$	132	
Lusaka	Yellow (Manteca)	Zambia	-	$87.3 \pm 1.5$ <sup>a</sup>	146	
Mbereshi (NUA45)	Red Mottled	CIAT	-	$89.0\pm3.0$ <sup>a</sup>	209	

FPVS = Farmer Participatory Variety Selection; CIAT = International Center for Tropical Agriculture

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## PHENOTYPING A GENETIC DIVERSITY PANEL OF MESOAMERICAN COMMON BEAN FOR DROUGHT TOLERANCE

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**INTRODUCTION:** Water deficit is one of the main factors that reduces yield of common bean (*Phaseolus vulgaris* L.) and the development of drought tolerant cultivars represents a strategy for food safety, since this legume is a staple food in the diet of Brazilian people and for population of some countries on the African and Asian continents. The aim of this study was to phenotype and subsequently identify genomic regions related to water deficit in a Brazilian Diversity Panel – BDP of Middle American common bean.

**MATERIAL AND METHODS:** The experiment was carried out under protected cultivation at the Experimental Station of IDR-Paraná, Londrina, Paraná State, Brazil, arranged in a randomized block design with two treatments: water deficit (WD) and well-watered (WW) in 2022 and 2023 autumn-winter harvest. In total, 146 genotypes (G) were phenotyped, including three control lines: BAT 477 (drought-tolerant), IPR Sabiá and BRS Pontal (drought-sensitive). The plants were submitted to WD from the R5 until the R7 stage, with a 30% of field capacity and the irrigation was monitored by 20 TDR (Time domain reflectometry) probes. The traits evaluated were: total number of pods per plant (NPP), mass of 100 grains [M100 (gm)], pod length [PL (cm)], pod thickness [PT (mm)], number of locules per pod (NLP), number of grains per pod (NGP), total grains mass per plant [GMP (gm)], and yield [YLD (kg ha<sup>-1</sup>)]. The heritability, principal component analysis, and the genetic diversity by Mahalanobis distance were calculated by Genes and R software.

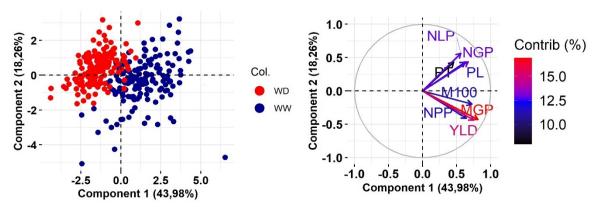
**RESULTS AND DISCUSSION**: ANOVA showed significant effects for genotypes (G), environments (E), and GxE for all the analyzed traits (Table 1).

Table 1. Mean square values of joint variance analysis for 146 bean genotypes grown in a
protected system, under two water conditions: well-watered (WW) and water deficit (WD) in 2022
and 2023 autumn-winter harvest in Londrina, Paraná State, Brazil.

				,		,			
Variation Source	GL	NPP <sup>/1</sup>	M100	PL	РТ	NLP	NGP	GMP	YLD
Genotype (G)	145	75.57**	42.74**	3.61**	1.05**	0.97**	1.08**	36.37**	1657772.53**
<b>Environment (E)</b>	3	5400.86**	4611.71**	42.21**	273.45**	8.29ns	49.18**	5298.87**	148623573.18**
G x E	435	67.24**	16.77**	2.66**	1.18**	0.57*	0.83**	32.25*	1631946.25**
Residue	580	22.84	11.98	0.89	0.75	0.48	0.67	26.76	773713.90
Average WW	-	15.57	28.54	10.03	7.72	7.06	5.92	16.53	3789.28
Average WD	-	9.86	23.12	9.39	7.56	6.85	5.51	10.26	2553.60
<b>Overall</b> Average	-	12.71	25.83	9.71	7.63	6.95	5.71	13.39	3171.43
CV (%)	-	37.57	13.40	9.71	11.31	10.00	14.37	38.62	27.73
Heritability (%)		69.77	71.96	75.35	28.92	49.84	37.71	26.40	53.32

<sup>71</sup>NPP: total number of pods per plant, M100:100 grains mass (gm), PL: pod length (cm), PT: pod thickness (mm), NLP: number of locules per pod, NGP: number of grains per pod, GMP: total grains mass per plant (gm) and YLD: yield (kg ha<sup>-1</sup>).

Values of heritability indicated a great contribution from genetic factors for NPP, M100, PL, PT e YLD, indicating a favorable situation for selection. For principal components analysis, MGP and YLD were the traits that contributed the most for the differentiation between the genotypes (Figure 1).



**Figure 1.** Principal component analysis of 146 bean genotypes grown in a protected system, under two water conditions: well-watered (WW) and water deficit (WD) in 2022 and 2023 in the autumn-winter harvest in Londrina, Paraná State, Brazil. NPP: total number of pods per plant, M100:100 grains mass (gm), PL: pod length (cm), PT: pod thickness (mm), NLP: number of locules per pod, NGP: number of grains per pod, GMP: total grains mass per plant (gm) and YLD: yield (kg ha<sup>-1</sup>).

For the WD condition, the genetic divergence analysis highlighted group one for containing the tolerant genotype BAT 477, besides 10 lines that presented the highest means for M100, PL, PT, NLP, NGP, MGP and YLD (Figure 2). All the genotypes were sequenced by genotyping-by-sequencing to identify SNPs. Afterwards genome-wide association studies for drought tolerance will be conducted.

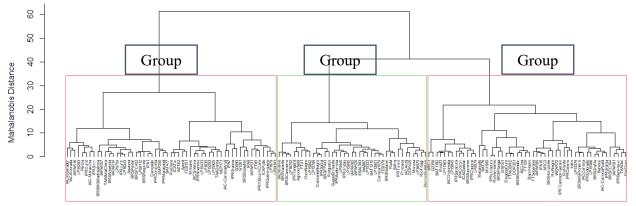


Figure 2. Mahalanobis distance of 146 bean genotypes grown in protected system, under water deficit (WD) condition in 2022 and 2023 autumn-winter harvest in Londrina, Paraná State, Brazil.

**CONCLUSIONS:** The results distinguished the genotypes and the promising lines under the WD condition for the common bean breeding program, and for testing in different cultivation regions.

**ACKNOWLEDGEMENTS:** This research was supported by Capes and Rural Development Institute of Paraná State– IAPAR-EMATER (IDR-Paraná) associated with State University of Londrina (UEL).

## DROUGHT TOLERANCE OF NEW ANDEAN BEAN LINES IN KENYA

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**INTRODUCTION:** Large seeded red mottled, red kidney and speckled sugar beans are the most important market classes in east, central and southern Africa (Kimani, 2004). However, these market classes, which belong to race Nueva Granada of the Andean genepool are susceptible to drought. Drought stress is serious threat to bean productivity in eastern Africa and is predicted to become even more severe by 2050, with large areas currently producing beans becoming unsuitable (Ojara et al, 2020). The decrease in climatic suitability of many bean production areas is due to increased temperature or more severe drought factors. Furthermore, predicted variation in temperature, greater evapo-transpiration combined with erratic and lower rainfall, will intensify the problem of drought stress especially for smallholder farmers who grow common beans under rainfed conditions. Breeding for drought nursery was established at the University of Nairobi in 2007. The objective of this study was to determine the performance of advanced lines selected from this nursery under drought stress conditions, and to select the most promising drought tolerant Andean dry bean genotypes of priority market classes.

**MATERIALS AND METHODS:** Study materials were 88 advanced Andean dry bean genotypes from a regional drought nursery established at the University of Nairobi in 2007. Most of these genotypes were classified as drought tolerant based on previous selection under intermittent and terminal drought in field sites since 2007. Of these, 28 were red mottled, 28 red kidney and 20 were speckled sugar. Twelve pinto and carioca lines were included for comparison. Two to five known drought tolerant varieties in each market class were included as checks. The study materials were evaluated on-farm in semi-arid Kirinyaga County (1<sup>0</sup>42'0 S, 37<sup>0</sup> 25' 0 E, 1720 m above sea level) and on-station at Kabete Field Station, University of Nairobi (1° 30'S, 30° 45'E and 1940 m above sea level) during the short (Sep-Dec) and long rain (April-Jul) seasons. Lines for each market class and appropriate checks were evaluated in separate trials. The experiments were laid out in a split-plot design with three replicates. Irrigation treatments (stress and no stress) were the main plots. Eighty-eight genotypes were the subplots. For the moisture stressed treatment, irrigation was withheld at flowering, but continued to maturity for the no-stress treatment. Yield data was subjected to analysis of variance using GENSTAT software. Least significant difference (LSD) test was used to compare the means yields of the genotypes.

**RESULTS AND DISCUSSION:** Analysis of variance showed significant season, location, stress level and genotypic differences (P<0.01) for duration to maturity, reproductive period, 100-seed mass and grain yield. Drought stress reduced 100-seed mass by 7.8%, 15.6%, 14.3 % and 20.6% for pinto carioca, speckled sugar, red kidney and red mottled lines, respectively. Drought also reduced number of pods by 25% to 33% across all the market classes. However, the magnitude of reduction varied with the genotypes. Drought stress significantly (P= <0.001) reduced grain yield of test genotypes (Table 1). However, the effects of drought stress varied with genotypes and market classes. Three red mottled (DRM 11-03, DRM 11-13 and DRM 11-17), four red kidneys (DRK 11-05, DRK 11-10, DRK 11-16 and DRK 11-18), and four speckled sugars (DSS 11-01, DSS 11-04, DSS 11-16, and DSS 11-17) were selected for good performance in drought stressed

and no stress conditions. The three red mottled lines had yield advantage of 20 to 48.6% over the local drought tolerant check under severe drought stress. The four red kidney lines showed yield advantage of 43.9 to 85% over the checks. The four speckled sugar lines had a yield advantage of 57.5 to 101.8% over the corresponding check varieties. However, the pinto and carioca lines had comparable yields to the local drought tolerant checks under severe drought stress conditions.

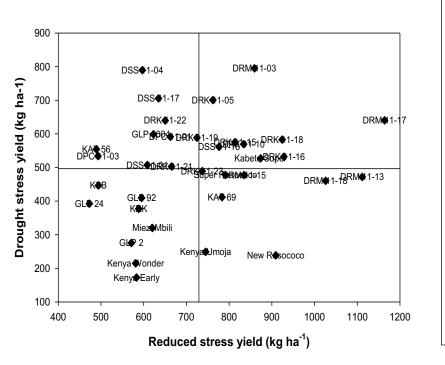


Figure 1. Comparisons of mean yield (kg ha<sup>-1</sup>) under severe drought stress (DS), and reduced drought stress (RS) of the top 20 Andean advanced bean lines and checks grown at Mwea and Kabete for two seasons. Vertical and horizontal lines represent trial mean yield under RS (x-axis) and DS (y-axis), respectively. Check varieties were: Red mottled: GLP 2, KAT 56, Kenya Umoja, New Rosecoco, Super Rosecoco; Red kidney: GLP 24, KAT 56, Kabete Super, Kenya red kidney; Specked sugar: Kenya Speckled sugar bean, Kenya Early, Miezi Mbili; Pinto and carioca: GLP 92, GLP 1004

**Table 1**. Grain yield (kg ha-<sup>1</sup>) of bean lines in moisture-stressed and no stress on-farm and on-station conditions in Kenya.

Market Class	No of lines	Season 1				Season 2					
		Kabete		Mwea		Kabete		Mwea			
		RS	DS	RS	DS	RS	DS	RS	DS		
Red mottled	23	1334.4	840.7	394.0	202.8	631.9	305.5	659.2	220.1		
Red kidney	23	1185.1	858.0	447.6	251.6	460.0	309.7	688.5	326.5		
Speckled sugar	17	1183.9	652.6	352.9	231.9	528.5	348.3	461.7	222.0		
Pinto and carioca		1099.1	698.6	407.9	282.5	630.2	399.5	517.8	279.8		

LSD<sub>0.05</sub> (treatments): 16.5; RS- moderate drought stress; DS =severe drought stress

Participatory selection showed that 62% of these drought tolerant genotypes had farmer and consumer preferred traits. These results indicate considerable progress in the development of large, seeded beans tolerant to drought combined with preferred consumer and producer grain types.

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## ANDEAN LINES SELECTED FOR HEAT TOLERANCE FROM BULK BREEDING PHASEOLUS IMPROVEMENT COOPERATIVE (PIC) POPULATIONS

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#### **INTRODUCTION**

The limited genetic diversity identified in Andean beans and their general sensitivity to heat and drought stress makes genetic improvement for abiotic stress tolerance a challenging and critical breeding goal. Heat tolerance has been achieved in breeding programs in locations with high ambient temperatures, e.g. 'Sacramento' light red kidney from Sacramento Valley Milling, Inc., (Ordbend, CA), or in landraces selected by farmers in hot climates, e.g. Indeterminate Jamaica Red (IJR) from western Punjab, India, and these lines have formed the backbone for breeding for heat tolerance in Puerto Rico resulting in the release of lines such as TARS-HT1 and -HT2, and PR9920-171. The goal of the Phaseolus Improvement Cooperative (PIC) was to create diverse bulk breeding populations that were generated from crosses between superior geographically diverse parents from the Andean Diversity Panel (ADP) (Cichy et al., 2016) that combine complementary traits for collaborative breeding. Through testing, selection and release in specific target environments, the goal was to increase the diversity of Andean beans available that combine biotic and abiotic stress tolerance in Africa and the Americas.

#### **MATERIALS AND METHODS**

Simple and three-way crosses were completed in Puerto Rico, South Africa and Washington State in 2011, 2012, and 2013 and bulked to the  $F_4$  generation. Single  $F_4$  plant selections were completed,  $F_5$  progeny rows were selected, and  $F_5$  or  $F_7$ -derived lines were tested in replicated trials in Puerto Rico, South Africa and Washington State. Superior lines were assembled in Puerto Rico for testing and selection under drought and heat stress. Lines identified with heat tolerance in average 33/23C trial conditions are presented here. Field trial data were adjusted for spatial heterogeneity using the SpATs R program (Rodríguez-Álvarez et al., 2018), and superior lines were selected based on the multi-year data (2019 to 2022). Simple means of the BLUPs for the four years tested for each trait were calculated. The lines were also evaluated with KASP markers using the Intertek platform generated from SNP markers linked to diseases resistance traits developed by the bean community ("Table of SNPs and INDELS converted to Tm-shift assays-2023"; http://www.bic.uprm.edu/?page\_id=91).

#### **RESULTS AND DISCUSSION**

Seven PIC lines selected from the multi-year replicated trials under high ambient temperature conditions in Puerto Rico were largely derived from populations with parents with moderate levels of heat tolerance, including: NY 105, TARS-HT1, AC Elk, Wallace 773-V98, and PR9920-171. Two of these parental lines were derived from IJR: TARS-HT1 and PR9920-171. The locations where the seven heat tolerant PIC lines were initially selected all represent high ambient temperature locations including Vaalharts, South Africa; Paterson, Washington; and Juana Diaz, Puerto Rico; and none of the locations with more ideal temperature regimes are represented. Thus, there appears to be a correspondence between performance under high ambient temperature stress

in one location with performance in another, although these locations may be quite distinct for soil type and other agro-climatic conditions. The characteristics of the heat stress in each location are also distinct: with Washington State characterized by high daytime and low night-time temperatures; Vaalharts, South Africa by high daytime and moderate night-time temperatures; and Juana Diaz, Puerto Rico by moderately high daytime and high night-time temperatures. The lines selected were earlier in maturity than IJR and had similar or higher yields and hundred seed weights (HSW, Table 1). As temperature conditions are consistent during the high temperature season in Puerto Rico, early maturity is not an escape mechanism from heat stress. The lines generally possess the *I*, *bc-1* combination for *Bean common mosaic virus resistance* and several have the *Co-1* or *Co-3* alleles for anthracnose resistance.

	DTM <sup>1</sup>	Yield	HSW			Location	
Line	(days)	$(\text{kg ha}^{-1})$	(g)	Type <sup>2</sup>	PIC <sup>3</sup>	4	Pedigree
4835-1HT	67.7	443	28.7	cream	63	SA	NY 105/KIJIVU
							NY 105/TARS-HT
16PR-48	65.8	660	31.0	LRK	65	PR	1
							NY 105/TARS-HT
WA-PR 0282	68.8	505	28.2	DRK	65	WA	1
				Purple			
				speckle			
5272-2HT	68.6	434	29.8	d	66	PR	AC Elk/KIJIVU
							Wallace 773-
4565-10HT	66.3	500	32.3	LRK	74	PR	V98/PR0637-2
16PR-76-4415-							PR9920-171/TARS-
1BB-6	63.6	744	31.2	LRK	76	PR	HT 1
				pink			PR9920-171/TARS-
PIC-076-V6-A	66.2	692	29.6	striped	76	SA	HT 1
				pink			
IJR (check)	82.3	432	25.6	striped			

**Table 1.** PIC lines selected for heat tolerance based on adjusted trait BLUPs from trialsconducted in Juana Diaz, Puerto Rico from 2019 to 2022.

<sup>1</sup>DTM - days to maturity, HSW - hundred seed weight; <sup>3</sup>Type: LRK- light red kidney, DRKdark red kidney; <sup>3</sup>PIC refers to PIC population; <sup>4</sup>Selection location: SA - Vaalharts, South Africa; PR - Juana Diaz, Puerto Rico; WA - Paterson, Washington State

## CONCLUSIONS

These lines represent an advance in heat tolerance, drought tolerance (data not shown) and broad adaptation in Andean common beans, and they are being considered for formal release.

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## GLOBAL WARMING EFFECTS ON NATURAL POPULATIONS OF THE DURANGO RACE COMMON BEAN COMPLEX

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**INTRODUCTION:** The state of Durango is located in the semi-arid highlands of northern México and is an important center for the genetic diversity and domestication of the common bean (*Phaseolus vulgaris* L.). The Durango Race Common Bean Population Complex is considered a gene reservoir for drought escape, plant recovery and stress tolerance that is used in breeding programs around the world. Global warming, land use changes, and ecosystem depletion for livestock feed have led to a loss of genetic diversity in the natural populations of common bean growing near of the city of Durango and other sites discovered in the state of Durango (GBIF, 2023). Global warming was categorized into different RCP (Representative Concentration Pathway) climate scenarios that show significant impacts on natural populations of common bean when considering different time periods (2030, 2050 and 2100) for the RCP emission scenarios (2.5, 4.5, 6.0 and 8.5) adopted by the IPCC (Intergovernmental Panel on Climate Change). The objective was to simulate the effects of global warming over current (2021) populations of common bean and future time periods (2041, 2061 and 2081) in the state of Durango, México.

**MATERIALS AND METHODS:** Worldwide databases were consulted in 2023 to obtain occurrence locations (GBIF, 2023) for *P. vulgaris* var. *aborigineus* and *P. vulgaris* var. *mexicanus* in Durango, México. Locations with missing fit data and those more than 30 arcseconds away from other locations were eliminated to avoid over-representation and redundancy. Global warming scenarios were estimated using the EC-Earth3-Veg model, which provides detailed estimates for vegetation dynamics, land use and atmospheric processes. Four time periods were considered, including 19 standard bioclimatic variables, 12 variables of monthly average minimum temperature (TMin<sub>*i*</sub>), 12 variables of monthly average maximum temperature (TMax<sub>*i*</sub>) and 12 variables of accumulated precipitation per month (PRC<sub>*i*</sub>), giving a total of 55 bioclimatic variables for each period, with a resolution of 30 arcseconds (Fick and Hijmans, 2017).

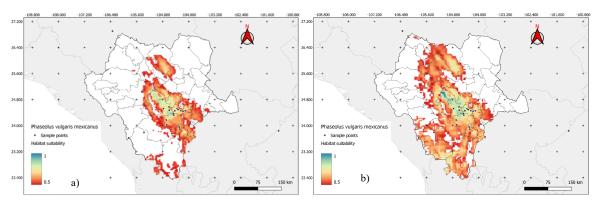
**RESULTS AND DISCUSSION:** High values for the area under the curve (AUC, > 0.99) were observed in the final models obtained for both species in all the evaluated scenarios, indicating that high and consistent predictive accuracy of the models was achieved. Important variables observed in the models were different for each plant species. For *P. vulgaris* var. *aborigineus*, temperature variation throughout the year (seasonality; BIO4) and rainfall frequency in April (PRC4) were crucial for the models used for studies on the adaptability of natural populations of this plant species (Table 1). Other variables that were less important for the permutation level were TMIN1 (mean minimum temperature in January) and rainfall frequency in August (PRC8) and December (PRC12). In *P. vulgaris* var. *mexicanus*, adaptability showed a dependence on several climatic variables with low permutational significance. One variable with higher importance was

the presence of areas with similar temperature values (BIO3, Isothermality) in Durango, but with decreasing values over the time periods. Depending on the period, other important variables were rainfall frequency in September (PRC9), March (PRC3), and April (PRC4), as well as the average value of the maximum temperature in April (TMax4). *P. vulgaris* var. *mexicanus* showed a higher potential for a broader adaptability in the state of Durango (Figure 1) and stronger interactions with different meteorological variables were also observed. The long-term effects of global warming favored the expansion of the ecological niche for wild populations of common bean in Durango, especially for *P. vulgaris* var. *mexicanus*.

Table 1. Permutational	significance	of agroclimatic	variables	related to	the effect	of global
warming on adaptability	of two comm	on bean species i	in four tim	e periods.		

Species	Variable	2021-40	2041-60	2061-80	2081-2100
P. vulgaris var. aborigineus	<sup>1</sup> BIO4	69.0	6.7	16.5	79.9
0	PRC4	22.2	85.1	70.8	14.8
	TMIN1	7.5	1.8	2.5	1.0
P. vulgaris var. mexicanus	BIO3	48.4	26.5	5.6	15.9
	PRC3	11.1	8.4	1.3	2.4
	TMAX4	9.8	5.9	2.6	19.2
	BIO4	1.2	15.2	7.3	2.4
	PRC9	6.5	9.2	61.1	6.5
	PRC4	1.5	4.0	4.0	16.9

<sup>1</sup>BIO4 = temperature seasonality, PRC3, 4, and 9 = rain frequency in March, April, and September; TMIN1 = min. mean temperature in January, TMAX4 = max. mean temperature in April and BIO3= isothermality.



**Figure 1**. Comparison of the predicted occurrence of *P. vulgaris* var. *mexicanus* in the state of Durango at two different time periods [a) 2041-2060; b) 2081-2100] and global warming scenarios.

**CONCLUSIONS:** Temp. and rain frequency before and at the start of the growing season and reproductive phase were related to long periods for biomass accumulation and seed production in natural populations of common bean related to the Durango Race Population Complex. *P. vulgaris* var. *mexicanus* showed a stronger interaction with different temperature patterns and rainfall frequency during the first months of the growing season and pod formation (September) to expand its adaptative range in the coming years. The expansion of the niche was related to the liberation of common bean populations from the high interspecific competition caused by global warming.

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# TEPARY BEAN (*PHASEOLUS ACUTIFOLIUS*) OUT-YIELDS COMMON BEAN (*P. VULGARIS*) IN SEMI-ARID ORGANIC CONDITIONS

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**INTRODUCTION:** Crop biodiversity offers an opportunity to counter worsening environmental limitations such as heat, drought, and low soil nutrition without the need for increased agricultural inputs. Here, we evaluated 27 diverse *Phaseolus* accessions across three species at two certified organic farms in the semi-arid California interior. Our results show highly significant yield differences between species and accessions, with tepary beans out-yielding common beans, and the one lima bean showing somewhat intermediate yield. These results will inform growers and breeders working in low-input arid environments.

Legumes are globally important staple crops, particularly in low-input and organic farming systems where they are valued for their nitrogen-fixing symbiosis with rhizobia, ability to break up pest and pathogen cycles in rotations, and long product shelf lives. Common bean (*Phaseolus vulgaris*) is the most consumed grain legume worldwide, but increasing global heat and aridity necessitate the investigation of alternative options with greater abiotic stress resilience. Potential alternatives include tepary bean (*P. acutifolius*), native to the Sonoran Desert (Moghaddam et al., 2021), and lima bean (*P. lunatus*), with an extremely broad adaptation (Parker and Gepts 2021). Currently, little is known about the yields of these crops in organically managed semi-arid environments. Here, we quantify the difference in yield between diverse *Phaseolus* accessions under these specific conditions.

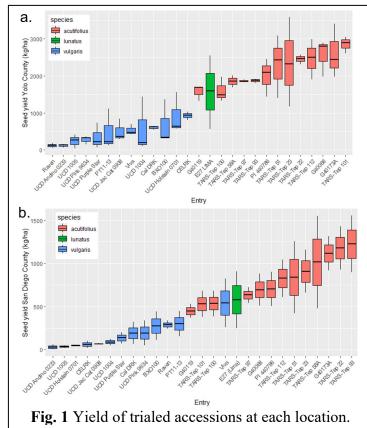
**MATERIALS AND METHODS:** Accessions of 13 tepary beans, 13 common beans, and one lima bean were planted in replicated complete blocks at two locations in 2022; at Rio del Rey Farm in San Diego County (33.277, -117.026), and the UC Davis Student Farm in Yolo County (38.542, -121.767). Germplasm included nine unreleased UC Davis common bean lines; nine tepary breeding lines from USDA-TARS (e.g., Porch et al. 2013, 2022, 2024); and other diverse materials from North American breeding programs or NPGS (Table 1). Plots consisted of 120 seeds planted into double rows, 6.1 m in length and 1.5 m in width (0.75 m per row). In Yolo County, 500 kg/ha of feather meal fertilizer was added before planting and trials were irrigated with approximately 1.8 cm of water/week until 85 days after planting. In San Diego County, the True Organic 10-5-2 fertilizer was used at planting (100 kg/ha) and True Organic 5-1-2 fertilizer was used at 40 days after planting (36 kg/ha), with 1.3 cm of irrigation/week. Weeds were manually removed. Trials were cut 106 days after planting, threshed, and cleaned seeds weighed.

**RESULTS AND DISCUSSION:** Highly significant differences were found among accessions ( $P < 2 * 10^{-16}$ , two-way anova), locations ( $P < 2 * 10^{-16}$ ), and accession\*location interaction ( $P < 2 * 10^{-3}$ ). All tepary beans ranked higher in yield in Yolo County than all common beans (Fig. 1), with 193% higher yields on average. In San Diego County, the common bean accession Viva ranked higher in yield than three of 13 tepary beans; all other common beans ranked lower in yield than all tepary beans (Fig. 1). Tepary beans averaged 372% higher yields than common bean at this

site. Based on multi-location data, the common bean accessions California Early Light Red Kidney (CELRK) and UCD Holstein 0701 did not differ significantly from the tepary bean landrace G40119; all other teparies significantly out-yielded all other common beans (Table 1). The one lima breeding line UCD E27 was somewhat intermediate in yield between the species, differing insignificantly from the seven lowest-yielding tepary bean lines, but significantly higher than all common beans. Teparies also reached harvest maturity sooner, averaging 80 days to harvest in Yolo County, at which stage no variety of common or lima bean had reached harvestable maturity.

These results showcase the value of tepary beans, including the new breeding materials, as a source of resistance to abiotic stresses imposed by semi-arid organic conditions. In contrast, common bean nearly collapsed in these environments. Our results highlight the value of tepary bean to the organic community. Nevertheless, tepary beans are known to lack diversity in seed color (e.g. absence of reds, purples, and various mottling patterns), lack large seed size, and are susceptible to bean common mosaic virus. Common bean and tepary bean can be hybridized, and interspecific hybridization could combine the beneficial characteristics from both species (Barrera et al. 2022). Future research could also involve testing the consumer preference between common bean and tepary bean to better understand the market potential of tepary beans for diverse consumers.

Entry	Species	Yield (kg/ha)*	Significance	
C 40172 A			groups**	
G40173A	acutifolius	2015	а	
TARS-Tep 22	acutifolius	1942	а	
TARS-Tep 101	acutifolius	1925	а	
G40068	acutifolius	1811	ab	
TARS-Tep 112	acutifolius	1810	ab	
TARS-Tep 23	acutifolius	1781	ab	
TARS-Tep 51	acutifolius	1721	abc	
TARS-Tep 93	acutifolius	1614	abed	
TARS-Tep 58A	acutifolius	1519	abed	
PI 440786	acutifolius	1481	abed	
TARS-Tep 97	acutifolius	1371	bed	
UCD E27 LIMA	lunatus	1176	cd	
TARS-Tep 100	acutifolius	1174	cd	
G40119	acutifolius	1119	de	
CELRK	vulgaris	581	ef	
UCD Holstein 0701	vulgaris	574	ef	
Viva	vulgaris	537	f	
BXO100	vulgaris	513	f	
Cal DRK	vulgaris	440	f	
PT11-13	vulgaris	411	f	
UCD 1004	vulgaris	382	f	
UCD Jac Cat 0908	vulgaris	327	f	
UCD Purple Star	vulgaris	259	f	
UCD Pink 9634	vulgaris	244	f	
Raven	vulgaris	187	f	
UCD 1005	vulgaris	156	f	
UCD Andino 0233	vulgaris	85	f	



\*Mean of all plots across both locations; \*\*Fisher's LSD

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## FIELD EVALUATION OF THE SYMBIOTIC AND AGRONOMIC EFFECTIVENESS OF PRESELECTED LOCAL RHIZOBIUM ISOLATES ON THREE BEAN (PHASEOLUS VULGARIS L.) VARIETIES

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## **INTRODUCTION**

Legumes such as common bean (*Phaseolus vulgaris* L.) have been introduced into cropping systems for sustainable soil management (Beebe, 2012). Consequently, the loss of fertility remains a major constraint to bean production because this legume is rarely fertilized, yet it is considered to be a poor nitrogen fixer in the absence of inoculation (Atse et al., 2023). To increase agricultural production, methods of natural soil fertilization by fallowing have been abandoned in favor of those based on the use of synthetic fertilizers and pesticides. These chemical inputs applied to the soil are costly and often inappropriate with harmful consequences to the environment and human health. To overcome this, this study was undertaken with the objective of seeking efficient local rhizobia to develop a bean inoculum formulation.

## MATERIALS AND METHODS

Twenty local rhizobium isolates (Figure 1) were tested on 3 bean varieties (SMR53, ROBA1 and Zabra), (Figure 2). A negative control (T0) and positive control (T+ : 50 kg of NPK/ha) were also used.



Figure 1. Two packages of rhizobium bacteria inoculum







SMR53ROBA1ZabraFigure 2. Seeds of three varieties used in the fertilizer tests.

## **RESULTS AND DISCUSSION**

The difference between the rhizobia isolates was highly significant as well as between the inoculated and non-inoculated controls for number of nodules. Indeed, the non-inoculated controls (fertilized and non-fertilized) had the lowest numbers of nodules independent of bean variety. Isolate 19 increased nodulation the most with 118.27 nodules formed in SM53, but the I18 isolate induced the highest yield among the local rhizobia tested with 3.18 t/ha. When using line ROBA1, the I2 isolate was the most infectious (118.93 nodules) and the yield was improved by isolates I4, I15 and I3. These bacteria induced higher yields than the synthetic fertilizer. They obtained yields of 3.19, 2.36 and 2.22 t/ha, respectively, compared to 1.02 t/ha for the fertilized control and 1.187 t/ha for the absolute control. Concerning the variety ZABRA, I20 induced the highest yield with 2.10 t/ha in contrast to I9 which induced the lowest yield.

<b>.</b> .	SMR	253	RO	BA1	ZABRA	
Isolates	Nodules number	Yield (t/ha)	Nodules number	Yield (t/ha)	Nodules number	Yield (t/ha)
I1	40.33±15.5	1.15±0.3	28.33±17.0	0.81±0.1	3.47±2.7	1.58±0,5
I2	$105.53 \pm 8.2$	$2.06 \pm 0.6$	$118.93 \pm 56.4$	$1.08 \pm 0.6$	43.93±20.6	$1.67\pm0,2$
I3	42.93±23.6	$1.58 \pm 0.0$	56.53±16.4	$2.22 \pm 0.2$	31.87±32.5	$1.53{\pm}0,0$
I4	50.80±17.4	$1.54{\pm}0.1$	22.80±22.9	3.19±0.6	41.60±38.7	$1.89{\pm}0,1$
15	52.47±38.6	$1.67 \pm 0.3$	59.33±59.6	$1.22 \pm 0.3$	22.20±18.2	$1.48\pm0,3$
I6	47.27±29.8	$2.15 \pm 0.0$	33.60±12.2	$2.18 \pm 0.4$	31.53±38.9	$1.65\pm0,4$
I7	35.20±10.9	2.11±0.1	35.13±5.5	$1.12 \pm 0.2$	32.97±9.1	$1.35\pm0,2$
I8	19.20±16.5	$1.88 \pm 0.6$	48.87±39.0	$2.02 \pm 0.3$	0.93±1.6	$1.48\pm0,4$
I9	118.27±87.1	$0.87 \pm 0.1$	45.07±30.8	$0.95 \pm 0.2$	26.20±16.8	$0.72{\pm}0,1$
I10	42.13±9.8	$1.98 \pm 0.4$	76.93±17.0	$1.10{\pm}0.3$	26.80±28.3	$2.03\pm0,2$
I11	41.93±6.5	$2.82 \pm 0.4$	34.07±23.9	$1.31 \pm 0.5$	25.40±18.2	$1.74{\pm}0,1$
I12	67.53±35.1	$1.93 \pm 0.1$	52.33±14.6	$1.96 \pm 0.5$	36.30±42.8	$1.75\pm0,5$
I13	75.27±41.0	$1.49{\pm}0.2$	67.67±36.9	$2.03 \pm 0.2$	18.20±14.2	$1.50\pm0,2$
I14	57.93±35.9	$1.55 \pm 0.2$	31.53±18.9	$1.00{\pm}0.1$	24.00±17.5	$1.63 \pm 0.5$
I15	35.87±10.1	$2.00\pm0.1$	20.00±16.5	$2.36 \pm 0.6$	15.40±16.6	$1.57\pm0,7$
I16	73.67±10.2	$1.51\pm0.4$	43.47±8.0	$0.64{\pm}0.0$	27.07±36.6	$1.46\pm0,2$
I17	84.53±37.2	3.27±0.1	38.13±11.7	$1.37 \pm 0.2$	43.60±44.3	$2.04{\pm}0,6$
I18	67.00±12.4	$1.22 \pm 0.2$	38.27±25.8	$0.98{\pm}0.2$	$17.47 \pm 20.6$	$1.36\pm0,4$
I19	32.45±9.3	$2.28 \pm 0.2$	50.60±17.7	1.16±0.3	24.87±18.2	$1,63\pm0,3$
I20	26.47±9.8	$2.18 \pm 0.2$	51.80±7.0	$2.07 \pm 0.4$	55.47±40.3	2,10±0,8
T0	16.67±2.5	1.77±0.5	20.20±6.8	1.18±0.3	10.93±12.2	1.50±0,5
T+	19.20±26.7	1.14±0.2	13.60±3.8	$1.02{\pm}0.3$	15.60±21.1	1.23±1,0
Means	52.43±22.46	40.15±5.30	44.05±22.29	32.97±6.80	26.17±23.18	1.45±0.34
Probabilities		0.0020	0.0042	0.0051	0.0390	0.0050
CV (%)	42.84	13.20	48.33	20.62	88.57	23.82

Table 1. Number of nodules and yield at varieties SMR53, ROBA and ZABRA



**Figure 3.** View of the experimental plot (plants at pod fill stage)



Figure 4. View of elementary plot

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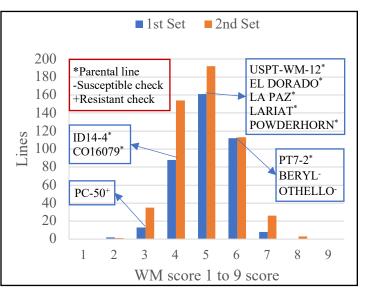
## ENHANCING WHITE MOLD RESISTANCE IN DRY BEAN THROUGH GENOMIC SELECTION

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**INTRODUCTION:** Dry bean growers from North Dakota and Minnesota ranked white mold (WM - *Sclerotinia sclerotiorum* Lib. de Bary) among the worst disease problems in dry bean production during the last 20 years (Knodel et al., 2023). Resistance to WM is inherited as a quantitative trait, making it complex to breed new germplasm and cultivars. However, the incorporation of genomic selection (GS) into early breeding phases has shown the potential to improve selection for quantitative traits in other crops (Ma et al., 2016).

**MATERIALS AND METHODS:** A Multiparent Advanced Generation Inter-Cross (WM-MAGIC) population (Escobar et al., 2022), and a set of 421 breeding lines were used in this study. For logistics issues, the WM-MAGIC population was divided in two sets (1<sup>st</sup> set with 384 lines and 2<sup>nd</sup> set with 524 lines). The disease reaction phenotype was collected under greenhouse conditions using the seedling straw method by Arkwazee and Myers (2017) (Figure 1). Phenotypic data from the first set of the WM-MAGIC population was obtained from a previous study by Escobar et al. (2022). Genotypic data was obtained via genotype-by-sequencing (GBS)

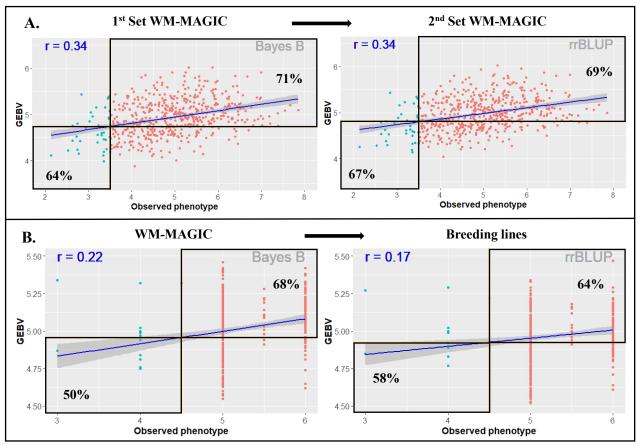


**Figure 1.** Phenotypic distribution of the WM-MAGIC population.

following the protocol described by Escobar et al. (2022). Sequencing reads were aligned to the common bean reference genome UI111 v1.1. Final HapMap was generated from SNPs with a minor allele frequency (MAF) > 0.01 and heterozygosity < 0.15. Genomic predictions (GP) models of ridge regression best linear unbiased prediction (rrBLUP) and Bayes B were evaluated. For GS purposes, the first set of the WM-MAGIC population was used as training population, and the second set and the set of breeding lines were used for validation. The predictive ability (r) was calculated as the Pearson correlation between the genetic estimated breeding values (GEBV) and the observed phenotypes.

**RESULTS AND DISCUSSION:** Empirical validation within the WM-MAGIC population shows a predictive ability of 0.34 for both models, while the predictive ability (r) using the whole WM-MAGIC to predict the set of breeding lines ranged from 0.17 to 0.22 (Figure 2). Promising results were observed when the GS was used for negative selection using a cutoff threshold in the GEVB

calculated by fitting a linear regression between the GEBV and the phenotype. Bayes B model detected 346 of 487 susceptible lines (71%) in the second set of the WM-MAGIC population, whereas for the breeding lines, 280 of 409 susceptible lines were detected (68%). Similar results were observed for rrBLUP model detecting 335 of 487 susceptible lines (69%) in the second set of the WM-MAGIC population and 260 of 409 susceptible lines (64%) in the breeding population (Figure 2). However, both models misclassified resistant/tolerant lines as susceptible (upper-left quadrant), with Bayes B misclassifying 13 resistant lines (36%) and rrBLUP 12 resistant lines (33%) in the second set of the WM-MAGIC population, six (50%) of the resistant/tolerant lines for Bayes B and five (42%) for rrBLUP were misclassified.



**Figure 2.** Efficiency of the genomic prediction in the selection of resistant/tolerant and susceptible genotypes. The cut off threshold for selecting genotypes was estimated by fitting a linear model. **A.** 1<sup>st</sup> set of the WM-MAGIC population was used to predict the 2nd set of the WM-MAGIC population. **B.** The entire WM-MAGIC population was used to predict the 421 breeding lines (observed phenotype was estimated with the model). For fitting the GP models a total of 55,570 SNPs were used.

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## SCREENING COMMON BEAN GERMPLASM FOR TOLERANCE TO SCLEROTINIA SCLEROTIORUM (WHITE MOLD)

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**INTRODUCTION:** *Sclerotinia sclerotiorum* (Lib.) De Bary (*Ss*) is a necrotrophic fungus and the causal agent of the white mold (WM) disease in common bean (*Phaseolus vulgaris L*.). In North America, because WM can have devastating effects on common bean yield, new common bean cultivars are needed with durable genetic resistance to WM. The first step towards this end-goal is to identify sources of WM tolerance. WM response was investigated in the inter-specific hybrids (ISHs) VAX 1-VAX 6 (Singh and Munoz 1999), three of their common parents (XAN 112, G40001, and ICA Pijao; CIAT), two of the common parents of the ISHs INB 35-50 (G40102 and G40119; CIAT), three tepary bean cultivars (TARS-Tep 22, -Tep 23, and -Tep 32; Porch et al. 2013), and eight black bean cultivars (AC Black Diamond, AC Harblack, Black Knight, Condor, F04-2801-4-5-1, F04-2801-4-6-6, Zorro, and UI 911; BeanCAP 2017).

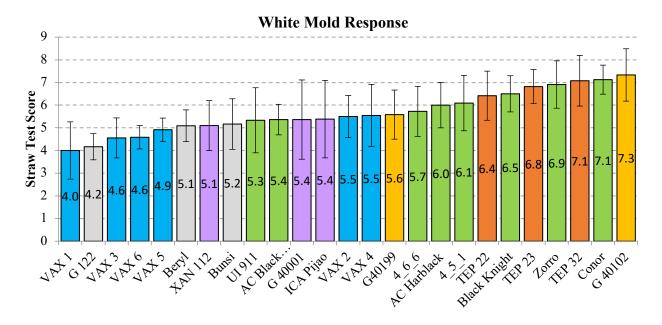
**MATERIALS AND METHODS:** Sclerotia of *Ss* were collected in November 2019 in Martintown, Ontario, Canada from soybean (*Glycine max*) and stored at 4 °C. Prior to inoculation, sclerotia were transferred to potato dextrose agar (PDA) plates and cultured at 25 °C, in the dark, for 72 hrs. In addition to the 21 lines selected for WM screening, G122, ICA Bunsi, and Beryl, were sown out as controls. Seeds were acquired from CIAT, MSU (Evan Wright, Rebecca Higgins), USDA-ARS (Phil Miklas, Timothy Porch), and AAFC-Canada. Using a randomized complete block design, 13 seeds were sown out per line, into five-inch pot and grown at 25 °C, 16 hr light, 8 hr dark in the greenhouse for 28 days before inoculation using the straw test method (Petzoldt and Dickson, 1996). Specifically, the main stem was cut, using scissors, one inch above the 4<sup>th</sup> node, after which the open end of a 200 µL plug pipette tip was pressed down into the outer edges of 72 hr old *Ss* mycelium grown on PDA plates, and placed on top of the cut stem. The inoculated plants were transferred into a polyethylene-enclosed cabinet ('mini-greenhouse') within the greenhouse and grown for seven days at >88 % relative humidity, which was maintained using a portable humidifier. After seven days, inoculated plants were scored based on the modified Petzoldt and Dickson scale (Terán et al., 2006).

**RESULTS AND DISCUSSION:** Starting with the controls, G122 had low intermediate tolerance to WM while both Beryl and ICA Bunsi had mid intermediate tolerance (grey bars in Fig 1.), matching previous studies (Singh et al., 2013; Higgins et al., 2018). Of interest was the low intermediate WM tolerance, similar to G122, observed in VAX 1, VAX 3, VAX 6 (matching similar scores in Abán et al. 2020), as well as in VAX 5 (blue bars in Fig. 1). In contrast, only mid intermediate tolerance was observed in VAX 2 and VAX 4 even though they share the same pedigree as VAX 1 and VAX 5 (Singh and Muñoz 1999). This could stem from differing levels of introgression of the common parents between VAX lines, as observed in the ISHs in Muñoz et al. 2004. Interestingly, three of the common VAX parents had mid-intermediate tolerance in XAN 112, ICA Pijao, and the *P. acutifolius* (tepary bean) G40001 (purple bars in Fig. 1), suggesting that

the increased WM tolerance in VAX 1, VAX 3, VAX 5, and VAX 6 could have arisen through pyramiding of WM tolerance from these common parents. To further track down this source of WM tolerance, the VAX common parent, A769 (XAN 87 x G1330 x Jules), will be screened next. Based on these findings, VAX 1, VAX 3, VAX 5, and VAX 6, and XAN 112 will be used to develop pre-breeding populations for improved WM tolerance in an adapted genetic background.

Three of the common parents of INB 35-50 had mid-intermediate tolerance; ICA Pijao, G40001, and the tepary bean line G40119, while the *P. parvifolius* parent G40102 was highly susceptible (orange bars in Fig. 1). Excluding those INB lines with the G40102 parent, increased WM tolerance relative to their common parents, might also be possible in these INB lines and as such these lines will be screened for WM response in the near future.

Of the 8 black bean and 3 tepary bean cultivars screened for WM response, three had midintermediate tolerance (4\_6\_6, AC Black Diamond, and UI 911; green bars in Fig. 1), which could be of interest for developing pre-breeding populations with improved WM tolerance. Of the remaining cultivars, five had high intermediate tolerance (4\_5\_1, AC Harblack, Black Knight, Tep 22, Tep 23, and Zorro), and two were susceptible (Conor and Tep 32; red and green bars in Fig. 1), indicating a lack of any WM tolerance that could be useful for breeding.



**Figure 1**. Straw test scores scored from 1 (resistant) to 9 (susceptible) seven days-post white mold inoculation. Error bars represent standard deviation. n = 8 - 13.

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## EVALUATION OF DRY BEAN BREEDING LINES FOR WHITE MOLD RESISTANCE IN THE GREENHOUSE AND FIELD USING MULTI-SITE SCREENING NURSERIES

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**INTRODUCTION:** In 2023, field and greenhouse evaluations were conducted to determine white mold disease tolerance in dry bean breeding lines from three breeding programs. Evaluations were conducted at sites located in five states (MI, NE, ND, OR, WA) and one province of Canada (Quebec). Collectively, these testing sites are representative of the major bean production environments of North America. Multi-site screening is essential for robust evaluation under varied environmental conditions and variable *Sclerotinia sclerotiorum* pathogen populations.

**MATERIALS AND METHODS:** Greenhouse evaluations were conducted at five locations with local isolates of *S. sclerotiorum* using the straw test method (Petzoldt & Dickson, 1996) that is intended to identify sources of physiological resistance in adapted and unadapted bean germplasm. Eighteen entries were evaluated, including G122 (partial resistance), Bunsi (mostly field resistance) and Beryl (susceptible) that were included as checks. Field tests were likewise conducted at five locations. However, yield data was collected from only three locations, while only two locations had sufficient disease pressure to collect meaningful disease severity data. As in years past, this illustrates the necessity of multiple sites for generating data despite weather or other natural complications in field trials. Disease severity in field trials were rated using the CIAT scale, with 1=most resistant to 9=most susceptible.

**RESULTS AND DISCUSSION:** Greenhouse results indicated that six entries were not significantly different than the resistant check G122 with scores ranging from 4.4 to 4.7 (Table 1). These were primarily pintos from Wisconsin, WMM-750 from North Dakota which was identified from a MAGIC population that combined multiple sources of resistance, as well as ND151660 LRK. These lines should be rescreened in 2024 to confirm their reaction and may be useful as parents in future breeding for white mold tolerance. The most susceptible entries were black beans from ND and MI, followed by navy and pintos from WI that were as susceptible to disease as Beryl. These data suggest that further attention should be given to incorporating physiological resistance into black and navy bean seed classes. Field trial data for white mold disease ratings were limited to MI and ND as environmental conditions at the other locations were not conducive for sufficient disease development to allow collection of meaningful data. ND151660 (4.7) and Ex 2146-P (5.4) did not significantly differ from resistant check G122 (4.8) for disease severity, but their yield potential was low to moderate (Table 2). Conversely, ND171707, WMM-556, and WMM-750 were the most susceptible to white mold in the field trial with high disease scores equivalent to susceptible check Beryl. However, they were also the highest yielding entries in the trial, underscoring the importance of selecting simultaneously for high yield potential under white mold disease pressure rather than focusing solely on a low disease rating.

Name	Source	WA	OR	MI	QC	ND	Mean*	Group**
ND151355	Osorno	8.8	5.4	8.7	9.0	8.3	8.2	А
B20591	Wright	8.0	4.8	6.8	8.5	5.5	7.1	В
B20536	Wright	7.8	4.3	6.2	8.6	5.3	7	В
LX1571.119	Kmiecik	6.8	6.7	5.6	7.1	7.2	6.8	BC
EX1804-N	Kmiecik	7.4	6.2	5.3	6.2	4.8	6.2	CD
LX1571.118	Kmiecik	6.8	7.2	4.7	6.1	4.8	6.2	CD
EX2149-P	Kmiecik	4.8	6.8	5.8	5.9	9.0	6.1	CD
Beryl	Check	5.7	5.8	7.9	5.9	5.2	6	DE
Bunsi	Check	6.5	4.6	4.7	5.7	4.6	5.5	EF
WMM-556	Osorno	3.8	3.8	5.9	6.4	7.0	5.3	FG
ND171707	Osorno	4.0	4.5	7.0	5.2	6.3	5.1	FGH
ND151660	Osorno	3.6	4.7	4.5	5.5	4.8	4.7	GHI
EX2146-P	Kmiecik	3.5	3.1	4.4	6.1	5.5	4.6	HI
EX2141-P	Kmiecik	3.5	3.8	3.6	5.7	5.0	4.6	HI
WMM-750	Osorno	3.7	4.3	4.5	5.3	4.8	4.6	HI
EX2143-P	Kmiecik	3.7	5.3	4.3	5.5	4.2	4.5	HI
EX2145-P	Kmiecik	3.4	2.9	4.0	5.6	6.0	4.4	Ι
G122	Check	3.0	3.6	4.0	4.9	5.5	4.2	Ι
Mean		5.2cd	4.9d	5.4bc	6.3a	5.8b	5.6	
*WM Score rate	d on Petzoldt	& Dick	son scal	e: 1-3 = res	sistant, 4-6	= intermedi	ate, 7-9 = sus	ceptible
**Means followe	d by the same	e letter n	ot signi	ficantly diff	ferent at p≤	0.05.		

Table 1. Straw test results for eighteen dry bean lines evaluated in five greenhouse locations.

**Table 2.** Yield and disease ratings for twelve dry bean lines evaluated in the field at three locations for yield and two locations for white mold disease severity.

Combined M	Iulti-State	Yield Su	nmary an	d Analysis		Multi-S	State	White Mold Sc	ores
Name	MI	ND	QC	Mean Yield*	Group	MI	ND	Mean WM**	Group
ND171707	3875.7	1867.3	3575.7	2983.5	А	7.7	7.3	7.5	А
WMM-556	3906.0	2477.3	2986.9	3060.0	А	8.7	6.4	7.4	А
BERYL	2853.7	1537.9	1721.2	1988.8	DE	6.7	8	7.4	А
WMM-750	3934.0	1807.4	3132.5	2844.1	AB	7.3	7.3	7.3	А
B20591	2924.3	2221.6	2042.4	2416.9	BCD	7.3	6.5	6.9	AB
ND151355	2151.0	1598.4	1500.2	1778.6	EF	7	6.8	6.9	AB
B20536	3099.3	2313.9	1983.0	2503.1	BC	8	5.6	6.8	AB
BUNSI	2420.0	2020.6	1396.4	2015.9	DE	7.3	5.8	6.6	AB
EX 2143-P	2416.3	1704.0	2317.0	2102.8	CDE	5	7.1	6	ABC
EX 2146-P	1876.3	1905.7	2329.5	2025.2	DE	4	6.9	5.4	BCD
G122	1381.3	1693.1	702.1	1303.4	FG	5	4.5	4.8	CD
ND151660	615.7	1953.5	945.7	1251.0	G	6.3	3	4.7	D
Mean	2621.1a	1925.0b	2112.1b	2193.4		6.4	6.3	6.3	
*Yield in pound	*Yield in pounds/acre, standardized @ 18% moisture.								
**Means follow	ved by the sa	me letter no	t significan	tly different at p	≤0.05. W	hite Mold	(WM)	rated on CIAT 1-9	Scale.

## MORPHOLOGICAL CHARACTERIZATION OF XANTHOMONAS SPP. ISOLATES FROM LEAVES IN COMMON BEAN

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#### **INTRODUCTION**

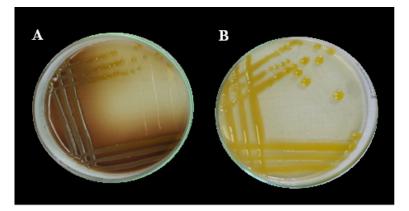
Common bacterial blight (CBB) is an important disease in common bean (*Phaseolus vulgaris* L.), caused by the bacteria *Xanthomonas citri* pv. *fuscans* (Xcf) and *Xanthomonas phaseoli* pv. *phaseoli* (Xpp). Xcf can be phenotypically differentiated from Xpp by the in vitro melanin production (Constantin et al., 2016). The use of resistant cultivars combined with management practices is the best way to control the disease in the field. The success of common bean breeding for CBB depends on knowledge of resistance sources in the host species and the genetic diversity of pathogen populations (Wendland et al., 2018). Therefore, morphological evaluation of different *Xanthomonas* spp. isolates allows for understanding the genetic diversity and evolution of the pathogen. Thus, this study aimed to morphologically evaluate isolates of Xcf and Xpp, collected in different seasons and years, from 2018 to 2023.

#### **MATERIALS AND METHODS**

In this study, 24 isolates were used. The isolates GUAXUPÉ, UFLA 02, UFLA 04, and UFLA 72 are from the collection of the Plant Bacteriology Laboratory of Departamento de Fitopatologia from Universidade Federal de Lavras (UFLA), including the reference isolate Xpp CFBP 6165 from the "Collection Français de Bactéries Phytopathogènes," preserved in herbarized common bean leaves. The other isolates (UFLA 01, UFLA 03, UFLA 05, UFLA 06, UFLA 07, UFLA 08, UFLA 09, UFLA 10, UFLA 11, UFLA 12, UFLA 13, UFLA 14, UFLA 15, UFLA 16, UFLA 17, UFLA 18, UFLA 19, UFLA 20 e UFLA 21) were obtained from leaves of symptomatic plants at the Centro de Desenvolvimento Científico e Tecnológico em Agropecuária da UFLA (CDCT) - Fazenda Muquém, from 2018 to 2023. The pathogen isolates were obtained using the parallel streak method (Romeiro, 2001), on culture medium 523 (Kado and Heskett, 1970). For the preserved isolates, the reisolation process was used. Three Petri dishes were used for each isolate. After isolation, each sample was properly identified and stored in a B.O.D. chamber at a temperature of 28°C. After growth on Petri dishes, pure colonies were transferred to test tubes and incubated at 28°C in a B.O.D. for 72 hours to observe brown pigment production. The assays were repeated twice.

#### **RESULTS AND DISCUSSION**

The *Xanthomonas* spp. isolates analyzed in this study exhibited standard cultural characteristics, such as yellow, convex, and shiny colonies with smooth and mucoid growth, consistent with previous studies on detection and characterization of *Xanthomonas* spp. isolates from common bean (Paiva et al., 2018) (Figure 1). Among the isolates studied, 67% showed brown pigment production and were therefore classified as Xcf (Figure 2). These results may explain the high number of symptomatic plants in the plant breeding experiments at UFLA, since the *fuscans* variety is more virulent (Fourie and Herselman, 2011). Further studies using genetic sequencing are needed for a detailed analysis of the diversity among these isolates.



**Figure 1.** Bacterial colonies of *Xanthomonas citri* pv. *fuscans* (A) and *Xanthomonas phaseoli* pv. *phaseoli* (B) on culture medium 523

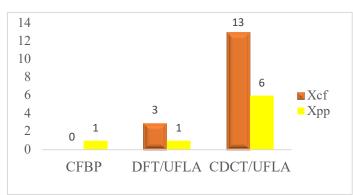


Figure 2. Classification and distribution of *Xanthomonas* spp. isolates from common bean

## ACKNOWLEDGEMENTS

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## REACTION OF *PHASEOLUS* SPP. GENOTYPES TO PRI21 *MACROPHOMINA PHASEOLINA* ISOLATE INOCULATED AT DIFFERENT PLANT GROWTH STAGES

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**INTRODUCTION:** Ashy stem blight caused by the fungus *Macrophomina phaseolina* (Tassi) Goidanich infects *Phaseolus* spp. genotypes in all growth stages (Beaver et al., 2020). The objectives of this research were to: (1) identify the response of *Phaseolus* spp. genotypes to *M. phaseolina* inoculated at vegetative and reproductive stages, and (2) determine the presence or absence of SMe1Em5.110 and SS18 molecular markers.

**MATERIALS AND METHODS:** Thirteen *Phaseolus* ssp. genotypes were planted in a randomized complete block design with four replications in greenhouses in Lajas and Isabela Research Substations. Plants were inoculated at the fourth internode and at the lateral branch close to the base of the main stem at flowering with the PRI21 *M. phaseolina* isolate by the cut-stem method (Viteri et al., 2024). The severity was scored at 42 days after the inoculation using a 1-9 scale (Viteri et al., 2024). The GoTaq® G2 Master Mix (Promega) was used to prepare the PCR solution with 15 µg/ml of DNA extracted from each genotype (QIAGEN). The primers for the sequence characterized amplified region (SCAR) markers SMe1Em5.110 and SS18, and their PCR and electrophoresis conditions were those described by Miklas et al. (2003) and Soule et al. (2011). These markers were used because Andean genotypes A 195, NY6020-4, 'PC 50', PRA154, and PRA155 possess them and had higher levels of resistance to *M. phaseolina*, and/or *Sclerotinia sclerotiorum* (Lib.) de Bary in previous studies (Viteri et al., 2015; Viteri et al., 2024).

**RESULTS AND DISCUSSION:** The response of each genotype to PRI21 isolate at both growth stages were presented separately because the variances from the data of the two environments were not homogeneous ( $\gamma^2 = 38.9$ ; P < 0.001). PI 462025 tepary bean and common beans BAT 477, SEA 5, and UPR-Mp-37, with the absence of both molecular markers, were intermediate (mean scores between 3.9 to 6.3) at both growth stages (Table 1). Thus, it would important to: (1) identify the genes or QTL (quantitative trait locus/loci) that conferred intermediate resistance to PRI21 in these genotypes, and/or (2) verify if the Phvul.003G175900 resistant gene, located on chromosome Pv03 and derived from BAT 477 (Viteri et al., 2022), is present in SEA 5 [pedigree: BAT 477/ 'San Cristobal 83'//'Guanajuato 31'/'Rio Tibagi' (Singh et al., 2001)], and UPR-Mp-37 (NY6020-4/A 195//SEA 5) (Viteri et al., 2024) breeding lines. 'Verano', with the absence of both SCAR markers, were susceptible at both growth stages in Lajas while NY6020-4, with the SS18 marker, was intermediate and susceptible at reproductive and vegetative stages, respectively in Isabela and Lajas (Table 1). The SS18 marker, linked with a QTL involved with internode length and located on chromosome Pv08 (Miklas el at., 2003), apparently provided intermediate resistance to PRI21 when the infection occurred at flowering. Andean common beans A 195, PC 50, PRA154, and PRA155, with the presence of SMe1Em5.110 and SS18 markers, and UPR-Mp-48 (BAT 477/NY6020-4//PRA154), with the SMe1Em5.110 marker, had mostly an intermediate reaction at both plant growth stages (Table 1). In contrast, UPR-Mp-22 (A 195/PC 50//PRA155), with SMe1Em5.110 and SS18 markers, were resistant (scores of 3.2) in Isabela at the vegetative and reproductive stages (Table 1). The SMe1Em5.110 marker, linked with the chalcone synthase protein *PvPR-2* (located on chromosome *Pv*02), was reported to provide resistance to necrotrophic fungi (Vasconcellos et al., 2017). Likewise, it would be useful to determine if the Phvul.003G175900 and Phvul.007G173900 (derived from PRA154) resistant genes located on chromosomes *Pv*03 (Viteri et al., 2022) and *Pv*07 (Viteri et al., 2024a), respectively, are present in UPR-Mp-22 and UPR-Mp-48 common beans. In general, at least two inoculations would be recommended to identify genotypes with durable resistance throughout the growing season.

	SCA	AR <sup>†</sup>				
Genotype	Mar	kers	Lajas	s, 2022	Isabe	la, 2023
	Sme <sup>‡</sup>	SS18¶	Vegetative	Reproductive	Vegetative	Reproductive
Tepary bean (Phas	seolus ac	cutifolius)				
PI 313488	-	-	$5.7^{\pm}$	3.6	6.5	5.9
PI 462025	-	-	5.4	3.9	4.3	4.4
Common bean (Pl	haseolus	vulgaris)				
A 195	+	+	5.5	3.8	5.1	3.4
BAT 477	-	-	5.6	4.4	6.1	6.0
NY6020-4	-	+	8.3	5.0	8.8	5.3
'PC 50'	+	+	4.4	4.3	4.3	5.8
PRA154	+	+	3.8	2.9	4.1	3.9
PRA155	+	+	5.5	3.4	5.1	3.6
SEA 5	-	-	5.3	4.5	6.3	5.1
UPR-Mp-22	+	+	3.5	4.2	3.2	3.2
UPR-Mp-37	-	-	4.6	5.3	4.9	6.3
UPR-Mp-48	+	-	4.4	4.1	3.0	4.4
'Verano'	-	-	8.6	5.1	8.3	8.0
Mean			5.4	4.2	5.4	5.0
LSD ( $P \le 0.05$ )			1.4	1.3	1.6	1.8

**Table 1.** Presence or absence of two molecular markers, and response of *Phaseolus* spp. genotypes to PRI21 *Macrophomina phaseolina* (Tassi) Goidanich isolate evaluated at 42 days after inoculation in two greenhouses at Lajas and Isabela Substations in 2022 and 2023.

<sup>†</sup>Sequence characterized amplified regions where + indicated presence and – absence. <sup>‡</sup>Abbreviation for SMe1Em5.110 linked with WM2.2 QTL derived from VA 19 (Soule at al., 2011). <sup>¶</sup>SCAR marker linked with the WM8.3 QTL and derived from NY6020-4 (Miklas et al., 2003). <sup>±</sup>Ashy stem blight disease severity was scored on a 1 to 9 scale, where 1 to 3.4= resistant, 3.5 to 6.4= intermediate, and 6.5 to 9= susceptible (Viteri et al., 2024).

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## RESPONSE OF SCARLET RUNNER BEAN AND COMMON BEAN GENOTYPES TO ASHY STEM BLIGHT

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**INTRODUCTION:** The necrotrophic fungus *Macrophomina phaseolina* (Tassi) Goidanich causes ashy stem blight in *Phaseolus* spp. genotypes (Viteri and Linares, 2017). Resistance (partial or complete) to this pathogen has been reported in common bean (*P. vulgaris* L.) (Viteri and Linares, 2022) and tepary bean (*P. acutifolius* A. Gray) (Miklas et al., 1998). However, the levels of resistance to *M. phaseolina* have not been evaluated in scarlet runner beans (*P. coccineus* L.). Our objective was to evaluate the reaction of 38 scarlet runner bean genotypes and six common bean breeding lines derived from *P. coccineus* to two *M. phaseolina* isolates.

**MATERIALS AND METHODS:** Forty-six scarlet runner and common beans were evaluated in a greenhouse at Isabela, Puerto Rico in September 2023. A randomized complete block design with three replications was used. One inoculation with the PRI21 *M. phaseolina* isolate was carried out at the fourth internode. A second inoculation was conducted (only in resistant plants), one week later, with the PRI23M isolate in a lateral branch by the cut-stem method (Viteri and Linares, 2022). The plants grew under favorable temperatures (> 28 °C) to promote a severe disease infection. The disease severity was evaluated at 14 and 42 days after inoculation (dai). A 1-9 scale was used to score the severity, and genotypes with values of 1 to 3.4 were considered resistant, 3.5 to 6.4 intermediate, and 6.5 to 9 susceptible (Viteri et al., 2023). Data were analyzed using SAS 9.4 PROC GLM (SAS Institute, 2021).

**RESULTS AND DISCUSSION:** There were significant differences ( $P \le 0.001$ ) between genotypes, time for evaluation, and genotypes x time for evaluation (Table 1). A higher disease severity was observed at 42 dai (mean score of 8.1) than 14 dai (6.3). However, 14 scarlet runner beans (e.g., PI 226594, PI 311202, PI 430174, and PI 438010; data not shown), and I9365-5, ICB-6, ICB-8, ICB-10, and TARS VCI-4B common bean breeding lines derived from P. coccineus were susceptible (mean scores > 6.5) to PRI21 *M. phaseolina* isolate at 14 dai (Table 2). These results were different from those reported by Miklas et al. (1999) where ICB-6, ICB-8, and ICB-10 were resistant to field epidemics of *M. phaseolina*. Differences may be associated with the methodology of screening (cut-stem method vs natural infection), pathogen virulence, and/or environment used (greenhouse vs field), among other causes reported in previous studies (Viteri and Linares, 2017; 2022a). Twenty-four scarlet runner beans (e.g., PI 311204, PI 417585, PI 438910, and PI 449381; data not shown) were also susceptible (scores > 6.8) to PRI21 isolate at 42 dai. For this reason, all these genotypes were not inoculated with the PRI23M isolate. In contrast, PI 183412 was the only resistant scarlet runner bean genotype identified in this screening, followed by PI 311214 and PI 325600 that were intermediate to PRI21 (Table 2). Furthermore, PI 311214 was intermediate (5.1) while PI 325600 was susceptible (6.8) to PRI23M isolate at 42 dai. Interestingly, PI 183412 also had a resistance response to PRI23M (3.4) and had significantly lower scores than common bean UPR-Mp-22 (5.4 and 4.8 for PRI21 and PRI23M isolates, respectively). Thus, the number and type of resistant gene (s) derived from PI 183412 need to be

identified through inheritance studies. Likewise, the resistant gene (s) may be introgressed from PI 183412 to PRA154, PRA155, UPR-Mp-22, UPR-M-34, UPR-Mp-37, and/or UPR-Mp-48 breeding lines that had partial resistance to *M. phaseolina* (Viteri and Linares, 2022; Viteri et al., 2023).

**Table 1.** Analysis of variance for ashy stem blight [caused by *Macrophomina phaseolina* (Tassi) Goidanich] severity for 46 *Phaseolus* spp. genotypes evaluated at Isabela in 2023.

Source	df	Mean squares <sup>†</sup>	
		Severity	
Replication	2	11.4*	
Genotype (G)	45	46.45***	
Time for evaluation (T)	1	841.75***	
GxT	45	8.87***	

<sup>†</sup>Significant at  $*P \le 0.05$  and  $***P \le 0.001$ .

**Table 2.** Mean ashy stem blight disease scores of *Phaseolus* spp. genotypes to PRI21 *Macrophomina phaseolina* (Tassi) isolate evaluated at 14 and 42 days after inoculation in the greenhouse at Isabela in 2023.

Genotype	Time of e	valuation	Mean	
	14 dai <sup>†</sup>	42 dai	_	
'Othello' (Susceptible check)	9.0 <sup>‡</sup>	9.0	9.0	
UPR-Mp-22 (Partial-resistant check)	3.6	5.4	4.5	
Phaseolus coccineus				
PI 183412	3.2	3.3	3.3	
PI 311214	3.8	5.9	4.9	
PI 325600	3.8	6.1	5.0	
Phaseolus vulgaris <sup>¶</sup>				
92BG-7	4.2	7.9	6.1	
19365-5	8.5	9.0	8.8	
ICB-6	9.0	9.0	9.0	
ICB-8	9.0	9.0	9.0	
ICB-10	7.4	8.7	8.1	
TARS VCI-4B	6.8	8.6	7.7	
Mean for 46 genotypes	6.3	8.1	7.2	
$LSD (P \le 0.05)$	1.6	1.3	1.0	

<sup>†</sup>dai, days after inoculation. <sup>‡</sup>The disease severity was scored on a 1 to 9 scale, where 1 to 3.4= resistant, 3.5 to 6.4= intermediate, and 6.5 to 9= susceptible. <sup>¶</sup>Interspecific breeding lines derived from *Phaseolus vulgaris* x *Phaseolus coccineus* crosses.

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#### PREVALENCE OF COMMON BEAN DISEASES AND ASSOCIATED DAMAGE IN THE MAIN PRODUCTION AREAS OF CÔTE D'IVOIRE

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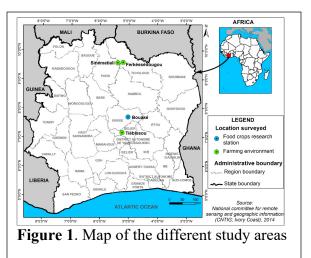
#### **INTRODUCTION**

The common bean is the main food and economically important legume within the Phaseolus genus (Missihoun *et al.*, 2017). It is a source of vitamins and proteins considered to be the 'meat of the poor' because it is cheaper than animal protein (IRAD, 2013). Despite its economic and nutritional importance, common bean, like other crops, faces many constraints including biotic constraints that affect its yield. This study was initiated by the Programme de recherche sur les Cultures Maraîchères et Protéagineuses (CMP) of the Centre National de Recherche Agronomique (CNRA) in order to assess the prevalence of diseases associated with common bean cultivation and the damage caused. Diseases associated with common bean (*Phaseolus vulgaris* L.) cultivation in Côte d'Ivoire are mainly due to crown and root rot, anthracnose and bean common mosaic virus. Prevalence rates varied according to zone, ranging on average from 8.43 to 40.5% for crown and root rots, from 10.03 to 32% for anthracnose, and from 7.5 to 32.5% for bean common mosaic. These diseases reduce bean production in areas that are both humid and hot, particularly in N'Guimbo, in the department of Tiébissou, where production losses are estimated at over 30%.

#### **MATERIALS AND METHODS**

The study was carried out at the Food Crops Research Station and in farmers' fields, notably in N'Gouimbo (Tiébissou) in central Côte d'Ivoire, and in Nangorigokaha (Sinématiali), Logokaha, Kamonnonkaha, Sandokaha and Laminevogo (Ferkessédougou) in northern Côte d'Ivoire (Figure

1). A diagnostic survey was conducted on the phytosanitary status of the common bean crop in various plots in these localities. The size of the plots visited varied from 0.2 to 1 ha. Disease prevalence was assessed on thirty (30) plants at a rate of 10 plants per replication, selected at random along the central and diagonal lines (Gadji et al., 2022). A line of 10 plants constituted a replicate. The data collected were subjected to a one-factor analysis of variance (ANOVA 1) using SPSS 22.0 software. The Duncan's test was performed, at the 5% threshold, when a significant difference was observed between the means of the prevalence rate.



#### **RESULTS AND DISCUSSION**

The results show that the locality of N'Gouimbo in the department of Tiébissou in the Centre of the country is an area with a high prevalence of crown and root rots (40.50%), anthracnose (32%) and common bean mosaic (28%) (Figure 2). Collar rot is caused by a complex of fungal agents consisting essentially of *Sclerotium* sp., *Pythum* sp., *Fusarium* sp. and *Rhizoctonia* sp. Production losses due to damage caused by these diseases in this locality are estimated at over 30%. This is not the case in the northern localities, where the prevalence rate of diseases ranged from 8.43 to 32.5%, with production losses of less than 20%.

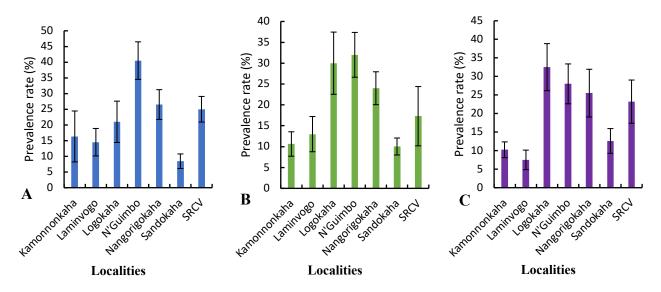


Figure 2. Mean prevalence rates of crown and root rots (A), anthracnose (B) and common bean mosaic (C) according to locality.

## CONCLUSIONS

Knowing the rates of disease prevalence in common bean crops and the damage caused means that the right decisions can be taken for plant health monitoring and better management of these diseases. Better disease control also involves the development of effective pest management methods that are environment and human health friendly.

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## RESPONSE OF DRY BEAN BREEDING LINES TO FUSARIUM AND RHIZOCTONIA ROOT ROT, CAUSED BY *FUSARIUM OXYSPORUM* AND *RHIZOCTONIA SOLANI*

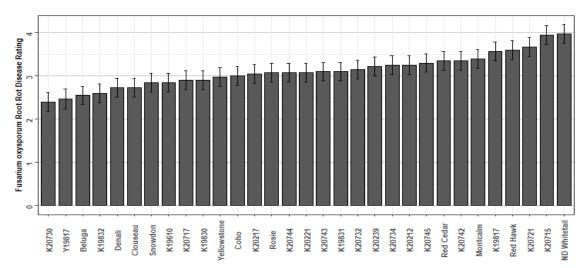
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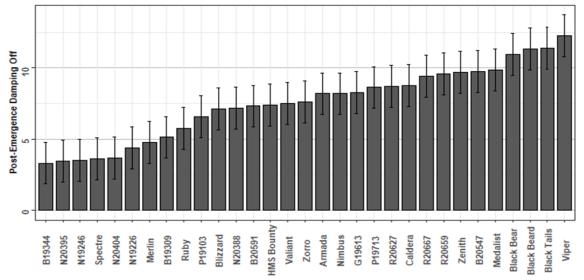
**INTRODUCTION:** Michigan is the second largest producer of dry beans in the U.S. However, root rots conferred by multiple soil borne fungi and oomycetes are the most yield limiting disease of large seeded Andean type beans and the second disease of small seeded Middle American types. In Michigan, the most common root rot pathogens belong to the *Fusarium* and *Rhizoctonia* species and root rots conferred by these pathogens cause up to 84% yield loss in susceptible dry bean cultivars through damage to root biomass, reduced vigor, and whole plant death (1). The primary objective of this two-year study was to screen advanced breeding lines from the Michigan State University dry bean breeding program as well as common commercial cultivars in Michigan for resistance to *Fusarium oxysporum* and *Rhizoctonia solani* conferred root rots.

**MATERIALS AND METHODS:** Two root rot disease trials were evaluated in the 2021-2022 field seasons. The *F. oxysporum* panel consisted of 20 advanced breeding lines and 11 commercial varieties from the Andean gene pool. The *R. solani* panel consisted of 15 advanced breeding lines and 16 commercial varieties from the Middle-American gene pool. The two trials were planted in a disease nursery in East, Lansing MI using a randomized-complete block design (RCBD) with four replicates. Entries were planted in four-row plots, 160 seeds per replicate (40 per row), where two rows contained non-inoculated plants, and two rows contained plants inoculated with *F. oxysporum* isolate F\_14-38 or *R. solani* AG2-2 isolate Rs\_14-17 colonized grain. *F. oxysporum* trials were scored for root rot disease severity on a 1-7 scale (2), one month after germination. *R. solani* trials were measured using the difference in stand between the first and last count per season (post-emergence damping off). All analysis for both trials was based off the inoculated treatment, with the non-inoculated treatment used as a confirmation of significant inoculum effect. A linear model was fit on line, trial, rep, and line by trial interaction where rep was set as random and all other variables as fixed. Disease severity for each line is reported as least squares means (LSmeans).

**RESULTS AND CONCLUSION:** In the *F. oxysporum* trial, multiple lines from the MSU program were identified among the most resistant lines, although a small difference between LSmeans for each line limits differentiation (Figure 1). White and light red kidneys as well as two recent yellow bean cultivars were among the most resistant. In the *R. solani* trial, differences between genotypes were more pronounced, with a larger range in post-emergence damping off (Figure 2). Viper, a commonly grown small red line in Michigan was the most susceptible line in the trial. B19344, recently released as the cultivar Black Pearl, was the most resistant. Overall, multiple MSU breeding lines were tolerant to root rot disease conferred by these two fungal pathogens demonstrating the previous efforts to improve root rot tolerance in the breeding program.



**Figure 1.** Results of the advanced Andean breeding line trial (2021-2022) challenged with *Fusarium oxysporum* using Least Squares means by genotype with standard error bars.



**Figure 2.** Results of the advanced Middle-American breeding line trial (2021-2022) challenged with *Rhizoctonia solani* using Least Squares means by genotype with standard error bars.

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## SEED YIELD AND REACTION TO WEB BLIGHT AND ANGULAR LEAF SPOT OF BLACK BEAN GENOTYPES IN VERACRUZ, MÉXICO

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**INTRODUCTION**: Angular leaf spot [*Phaeoisariopsis griseola* (Sacc.) Ferraris] is one of the main fungal diseases that commonly impact and affect dry bean crops in tropical and subtropical areas of the state of Veracruz, México (Tosquy et al., 2013). Less frequently, but also very important due to the damage it causes, is the incidence of the web blight [*Thanatephorus cucumeris* (Frank) Donk] (Tosquy *et al.*, 2012). These diseases can cause significant losses in seed yield, largely because most producers use landraces and some improved cultivars such as Negro Jamapa, which are susceptible to such diseases. The objective of this work was to identify black bean genotypes tolerant to web blight and angular spot and with higher seed yields than the Negro Jamapa cultivar, commonly used by local farmers in Veracruz, México.

**MATERIALS AND METHODS**: During the 2019-20 Fall-Winter (September-February) season, a field test was conducted under residual soil moisture conditions in two sites located in the municipality of Orizaba, in the "High Mountains" region of Veracruz, México. Eleven advanced tropical black bean breeding lines were evaluated, along with two improved cultivars Verdín and Negro Medellín, and Negro Jamapa was used as a check, in a randomized complete block experimental design with three replications and experimental plots that consisted of three rows 5 m long, 0.80 m apart. The reaction of bean genotypes to the natural incidence of web blight was recorded in Rincón Grande site, while that of angular leaf spot in Rincón Chico site. The general scale of 1 to 9 from CIAT (van Schoonhoven and Pastor-Corrales, 1987) was used to evaluate the reaction of bean germplasm to these fungal diseases. At the end of the maturation stage, plants were harvested and seed yield was estimated in kilograms per hectare at 14% humidity. Individual analyzes of variance of the quantified variables and combined analysis of seed yield were carried out. Statistical test based on the Least Significant Difference (LSD,  $\alpha = 0.05$ ) was used for separation of mean values. Correlation analyzes were also carried out at each evaluation site between web blight and angular leaf spot incidence and seed yield of bean genotypes.

**RESULTS AND DISCUSSION**: In the Rincón Grande site, web blight incidence was present at the beginning of the pod filling stage, which significantly reduced bean yield ( $r = -0.810^{**}$ ). Negro Jamapa was the most affected cultivar with plant damages statistically similar to those shown by the group of breeding lines derived from the cross Negro Citlali/XRAV-187-3, and higher than the rest of the genotypes. In contrast, three black breeding lines derived from the cross Jamapa Plus/XRAV-187-3, together with Papaloapan/SEN 46-3-2 and Papaloapan/SEN-46-2-6 breeding lines and cultivar Verdin showed resistance to web blight with average incidence values between 2.0 and 3.33 (Table 1). In turn, in spite of the fact that angular leaf spot was present in the Rincón Chico field test site, it did not significantly affect grain yield (r = -0.454 ns), mainly because its incidence was until the end of the pod filling stage. Negro Medellín cultivar presented the highest incidence value (mean = 5.33), which was statistically similar to that displayed by Negro Jamapa and the breeding lines Negro Papaloapan/SEN 46-3-2 and Negro Papaloapan/SEN 46-7-7, but significantly higher than the rest of the genotypes, which showed angular leaf spot mild symptoms

(Table 1). The genotypes identified for their low reaction to the incidence of both diseases included three breeding lines Jamapa Plus/XRAV-187-3-4-4, Jamapa Plus/XRAV-187-3-4-1 and Jamapa Plus/XRAV- 187-3-1-2, along with the improved cultivar Verdín; this group of genotypes were also the most productive with average seed yields greater than 2000 kg ha<sup>-1</sup>. In contrast, cultivars Negro Jamapa and Negro Medellín along with breeding line Negro Citlali/XRAV-187-3-1-5 obtained the lowest average seed yields due to significant damage mainly from web blight but in the case of Negro Medellín, from angular leaf spot (Table 1).

Genotype	Web blight <sup>1</sup> (scale 1 - 9)	Angular leaf spot <sup>2</sup> (scale 1 - 9)	Mean seed yield (kg ha <sup>-1</sup> )
Negro Papaloapan/SEN 46-2-6	3.33	2.67	1942.67 *
Negro Papaloapan/SEN 46-3-2	2.00	4.00 *	2083.33 *
Negro Papaloapan/SEN 46-7-7	4.33	4.00 *	1938.33 *
Negro Papaloapan/SEN 46-7-10	4.33	3.00	1810.50
Negro Papaloapan/SEN 46-7-12	4.33	2.00	1994.33 *
Negro Citlali/XRAV-187-3-1-5	5.67 *	2.67	1660.00
Negro Citlali/XRAV-187-3-1-6	5.33 *	3.00	1971.50 *
Negro Citlali/XRAV-187-3-1-8	4.67 *	2.67	1829.67
Jamapa Plus/XRAV-187-3-1-2	3.33	3.33	2107.83 *
Jamapa Plus/XRAV-187-3-4-1	2.00	2.00	2361.33 *
Jamapa Plus/XRAV-187-3-4-4	3.00	3.33	2406.33 *
Negro Medellín	4.33	5.33 *	1716.67
Negro Jamapa (regional check)	6.00 *	4.33 *	1435.00
Verdín	3.00	2.33	2169.00 *
Site average	3.97	3.19	1959.03
ANOVA	**	**	*
CV (%)	22.11	26.79	9.70
LSD (0.05)	1.476	1.435	518.91

**Table 1**. Reaction of black bean genotypes to web blight and angular leaf spot incidence in two locations: Rincón Grande and Rincón Chico, Orizaba, Veracruz, México.

<sup>1</sup> = Rincón Grande. <sup>2</sup> = Rincón Chico. Incidence values using the general CIAT scale (1 to 9) to evaluate the reaction of bean germplasm to fungal diseases. ANOVA \*P  $\leq 0.05$ . \*\*P  $\leq 0.01$ . \*Genotypes with statistically higher values according to the LSD (0.05) test.

**CONCLUSIONS:** Under the environmental conditions of the High Mountains region of Veracruz, México, the breeding lines Jamapa Plus/XRAV-187-3-4-4, Jamapa Plus/XRAV-187-3-4-1 and Jamapa Plus/XRAV-187 -3-1-2 and cultivar Verdín showed resistance to web blight and angular spot, and obtained significantly higher seed yield than the regional check cultivar Negro Jamapa.

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## WHITE MOLD RESISTANCE SCREENING OF A LIMA BEAN DIVERSITY PANEL

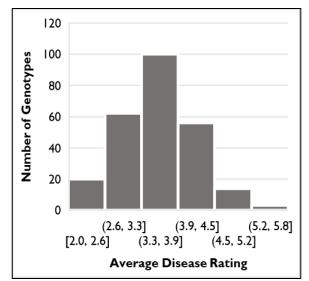
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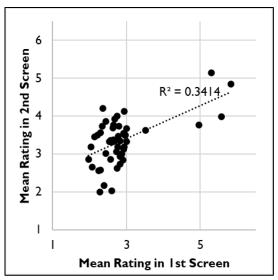
**INTRODUCTION:** White mold, caused by *Sclerotinia sclerotiorum*, causes yield and quality loss in baby lima bean (*Phaseolus lunatus*) grown in Delaware, Maryland, New York and Wisconsin. Use of fungicides to control white mold in lima bean is costly and requires multiple applications, because the crop has an extended flowering period. In a screen of thirty-eight USDA Plant Introductions conducted in 2014-2015, five PIs with physiological resistance were identified using the cut stem method (Selleck *et al.*, 2016), suggesting the possibility of breeding white mold resistant lima bean cultivars. The aim of the current study was to confirm the existence of white mold resistance/tolerance in lima bean and identify highly resistant lines to use in the University of Delaware lima bean breeding program.

**MATERIALS AND METHODS**: The Delaware lima bean diversity panel includes 255 lines originating from 19 countries. Half of the panel is of US origin. The panel includes Andean and Mesoamerican lines and some panel members are photoperiod sensitive. In April-May of 2022, the seedling straw test (Arkwazee & Myers, 2017) was used to screen all 255 lines for physiological resistance to white mold. Use of this method, rather than the standard straw test, facilitated screening of the photoperiod sensitive and indeterminate lines. Seedlings were grown singly in 5 cm square pots and inoculated 16 days after planting with a *S. sclerotiorum* isolate collected in Delaware. Disease reaction was rated 4 days after inoculation according to the 1-9 rating scale of Arkwazee & Myers (2017). The initial screening of all lines was conducted in three rounds with 5 seeds of each line sown per round. The arrangement of the lines in the greenhouse was randomized in each of the three rounds. In July 2022, fifty of the panel members were screened in a second round of screening where 16 seeds of each line were sown and arranged in a randomized complete block design with four blocks and four plants per block.

**RESULTS AND DISCUSSION:** Sample sizes were unequal due to differences in germination, so least squares mean disease ratings for genotypes were calculated based on a mixed model. LS-mean ratings ranged from 2.0 to 5.8 and were normally distributed (Figure 1). The LS-mean disease ratings for the standard cultivars C-elite Select and Cypress were 4.0. The 45 most resistant lines, which had average ratings ranging from 2.0 to 3.0, and five check varieties with average ratings of 3.5 to 5.8, were included in the second screening. There was correlation ( $R^2=0.34$ ) between the LS-mean rating for the first round of screening and the second round of screening (Figure 2). The seven most resistant lines and the three most susceptible lines in the trial are listed in Table 1. Some of the most resistant genotypes were wild accessions. However, the cultivar Jackson Wonder was also very resistant. Some Andean types were resistant lines are photoperiod sensitive. Three Mesoamerican genotypes of US origin were the most susceptible genotypes identified.



**Figure 1.** Distribution of LS-mean white mold disease ratings for the screen of 255 diverse lima bean genotypes.



**Figure 2.** Correlation between LS-mean disease ratings by genotype for the 50 genotypes screened in two experiments.

Table 1. Name, Gene Pool, Domestication Status, Origen, LS-Mean Disease Rating and Sample
Size for the Most Resistant and Susceptible Genotypes Identified

Name	Gene	Status	Origin	Experime	ent 1	Experime	ent 2
	Pool			Rating	n	Rating	n
PI 535343	Meso	wild	Mexico	2.29	13	2.00	15
Jackson Wonder	Meso	cultivar	USA	2.40	15	2.17	13
PI 535341	Meso	wild	Mexico	2.60	15	2.03	2
PI 451925	Meso	wild	Guatemala	2.07	15	2.67	15
PI 433928	Meso	landrace	Mexico	2.24	5	2.57	14
PI 362772	Meso	landrace	Brazil	1.97	13	2.87	15
PI 257363	Meso	landrace	Colombia	2.29	11	2.58	14
PI 260411	And	landrace	Peru	2.74	14	2.63	16
PI 201478	Meso	landrace	Mexico	2.58	10	2.87	15
PI 355841	And	landrace	Ecuador	2.83	12	2.74	7
G27539	Meso	landrace	DR	2.90	12	2.85	14
PI 260408	And	landrace	Peru	2.83	13	2.94	13
Improved Kingston	Meso	cultivar	USA	5.57	8	3.99	15
PI 347804	Meso	landrace	USA	5.30	14	5.15	14
Nemagreen	Meso	cultivar	USA	5.83	13	4.86	15

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### GENETIC DIVERSITY IN WILD GERMPLASM COLLECTION OF COMMON BEANS FROM MÉXICO

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**INTRODUCTION:** The conservation and utilization of genetic diversity is recommended for the most important plant species used to feed people around the world, such as the common bean (*Phaseolus vulgaris* L.). In México, the common bean is also an essential crop for the economy, nutrition and human health. The genetic diversity of the common bean includes wild germplasm that grows naturally in different municipalities of Durango and other states of México, from which intermediate forms (regressive or weedy), landrace and improved cultivars have been derived. Several common bean collection tours have been conducted throughout México (Debouck, 1979; Cárdenas *et al.*, 1996; Wallander *et al.*, 2022) and the genetic diversity collected has been stored in gene banks in Colombia (CIAT, 2023), the USA (USDA-ARS-GRIN) and México (Cárdenas *et al.*, 1996). The aim was to start systematic studies of the genetic diversity of wild bean accessions from México.

**MATERIALS AND METHODS:** The online database of the CIAT (International Center for Tropical Agriculture) bean collection was consulted (https://genebank.ciat.cgiar.org/genebank/language.do?collection=bean). The data were filtered for missing passport data (mainly missing georeferenced collection points) and for some accessions the elevation of the collection point from the online tool Google Earth® was used. Each accession was classified by collection area and seed color using the images contained in the database. The data were analyzed using principal component analysis (PCA) and then cluster graphs were created using the UPMGA (Unweighted Pair Group Method with Arithmetic Mean), both using R statistical software (Ver. 2023.09.1+494).

**RESULTS AND DISCUSSION**: Intermediate values were recorded for the relative importance of the individual components in explaining the observed variance (Table 1), so that ten components were required to obtain values close to 100%. Some seed colors proved to be important in defining states with high levels of genetic diversity in wild bean populations, including shiny black, brown, pinto, black-pinto, borroso or rebocero (black-pinto with gray background), bayo (beige), alubia (small white) and ojo de cabra (black-striped goat's eye). The Mexican states with the highest diversity of wild populations of the common bean are located in the east of the central highlands of México (Bajío or lowland region), led by Jalisco (Figure 1), followed by Morelos, Guerrero and Michoacán. In the semi-arid highlands of northern México, the state of Durango showed a high genetic diversity of wild populations of common bean. The results seem to be influenced by the intensity of sampling in the individual states and regions. Therefore, sampling (collection tours) need to be increased, especially in the underrepresented states (Puebla, Hidalgo and Querétaro), to improve the collection, conservation and use of the genetic diversity of common bean.

**CONCLUSIONS:** States that have high diversity in common bean (Jalisco, Morelos, Guerrero, Michoacán and Durango), as well as states where few collection tours were conducted and where wild *Phaseolus* populations are more likely to be present, must be considered to improve their representation in gene banks. The study and utilization of genetic diversity is an important resource for solving problems in the commercial production of common bean and problems caused by the direct or indirect effects of global warming.

<b>Table 1</b> . Significance of the components obtained in the study of genetic diversity in wild common
bean germplasm from México.

Principal Component	Eigenvalue	Proportion of the Variance Explained (%)	Cumulative Variance (%)
<sup>1</sup> PC1	6.5	38.4	38.4
PC2	2.5	14.7	53.1
PC3	2.2	12.8	65.9
PC4	1.9	11.0	76.9
•			•
PC10	0.1	0.5	99.8

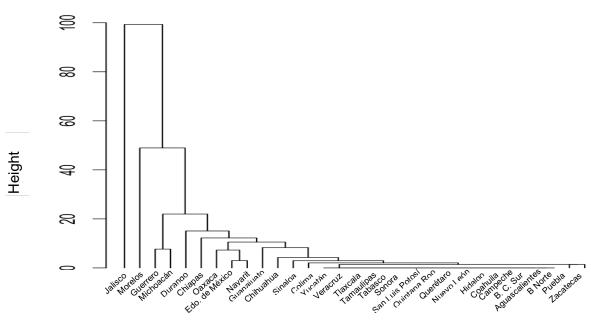


Figure 1. Dendrogram showing the degree of genetic diversity in the germplasm accessions of wild beans from different states of México.

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## GENETIC DIVERSITY OBSERVED IN THE DURANGO RACE POPULATION COMPLEX OF COMMON BEAN

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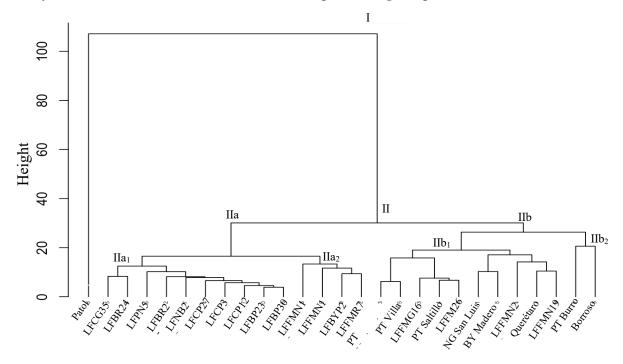
**INTRODUCTION:** Durango is considered one of the most important centers for the genetic diversity and domestication of common bean (*Phaseolus vulgaris* L.). The objective was to analyze the genetic diversity present in the Durango Race Population Complex by using morphoagronomic traits evaluated under field conditions.

**MATERIALS AND METHODS:** Seeds from individual plant were collected at random from wild and weedy common bean populations growing naturally near the archaeological zone "La Ferrería" in Durango, México. Landrace varieties were also obtained in the "Los Llanos" growing area. Information on the location of germplasm collection was registered, especially for wild and intermediate forms and landrace varieties. A group of 27 genetic common bean accessions [wild (11), weedy (7), landraces (4) and improved varieties (4)] were used. A variety of runner bean (known as patol or ayocote) was also used as a reference control as it belongs to a different plant species (*Phaseolus coccineus* L.). The accessions and varieties were sown under field conditions in Durango (INIFAP-CEVAG, Durango, México) to perform the morpho-agronomic characterization. Sowing took place on July 10<sup>th</sup>, 2023, and the agronomic management recommended in Durango was applied. The experimental plot consisted of a 5 m long row with a spacing of 0.81 m and 80 plants per row. Stakes were placed near each plant to help the vines climb in sections of giant reed (*Arundo donax*) held vertically 2 m above the soil surface.

Common bean accessions were characterized using 48 morpho-agronomic traits included in the UPOV, (2012) and SNICS, (2017) test guides. The data were analyzed using descriptive statistics (frequencies and averages) and, for the study of genetic diversity, using principal component analysis (PCA) and cluster analysis. The cluster analysis included a dendrogram created by calculating the Euclidean distances between the cultivated varieties and the weedy and wild accessions. The UPGMA (Unweighted Pair Grouping with Arithmetic Averages) method was used, and the cluster diagram was created using the R ver. 2023.12.0+369 computer package.

**RESULTS AND DISCUSSION:** The PCA showed a low proportion of variance explained by the individual components, so that 26 components were required to reach 100%. The main component with the highest variance explained was seed size and the average width of seeds and pods. The highest difference (height) was observed for the runner bean, which belongs to a different plant species (Figure 1). Low genetic diversity was found in accessions with small and typically wild seeds, with two subgroups including accessions with bayo (cream-beige), brown, black and black striped seeds (IIa<sub>1</sub>). Subgroup IIa<sub>2</sub> included accessions with small beige seeds (LFBYP) and flor de mayo, including the black (LFFMN) and pink (LFFMR) variegated coloration of the seed coat. Group IIb consisted of two subgroups, including landraces and improved varieties with pintocolored seeds and intermediate (regressive) forms with large flor de mayo (pink) seeds. A high

degree of differentiation in this subgroup was also observed in landraces (Negro San Luis) and improved varieties (Río Grande and Bayo Madero), as well as in regressive accessions with flor de mayo seeds. Another subgroup (IIb<sub>2</sub>) comprised two landrace varieties with a high degree of segregation, known as Pinto Burro and Borroso (Rebocero), which probably originated from interracial crosses. The use of morpho-agronomic traits allowed the evaluation of the genetic diversity of common bean wild and weedy accessions, as well as varieties related to the Durango race. Wild and weedy common bean populations showed persistence in Durango, where high genetic diversity related to its genetic and geographic origin was observed. The cultivated forms of common bean (landrace and improved varieties) belonging to the Durango race are descended from wild populations of common bean with small seeds showing the following colors: beige, brown, bayo rata (gray-striped beige) and pinto (brown and black striped). The presence of shiny black and flor de mayo (black and pink colored) seeds has been detected, which are predominant in the Jalisco race but are now also present in the Durango race. Some wild accessions and most weedy accessions resembled cultivated varieties grown in Durango, Chihuahua, and Zacatecas.



**Figure 1**. Dendrogram based on morpho-agronomic traits evaluated in common bean accessions, landrace and improved cultivars grown in Durango, Chihuahua, and Zacatecas.

**CONCLUSIONS:** Significant genetic diversity was observed and morpho-agronomic traits are considered an important tool for common bean characterization and genetic diversity studies due to difficulties observed with molecular markers.

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#### POTENTIAL OF NEW SITES FOR WILD POPULATIONS OF COMMON BEAN DETERMINED BY ECOLOGICAL NICHE MODELING

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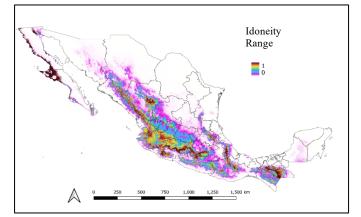
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**INTRODUCTION:** Several sites for plant observation and seed collection of wild and weedy (regressive or intermediate forms) populations of common bean (*Phaseolus vulgaris* L.) have been recorded in México (Debouck, 1979; Cárdenas *et al.*, 1996). Several collection tours have been carried out in the main distribution areas of *P. vulgaris* in the states with travel possibilities, such as Jalisco, Morelos, Guerrero, Oaxaca and Michoacán. Other states such as Durango, Chihuahua, and Baja California Norte seem to be underrepresented in the gene banks, depending on the possibilities of occurrence of wild populations of common bean. Niche modeling is an important tool for creating predictive models to determine specific areas to sample for *P. vulgaris* plant species. The use of predictive models increases the efficiency of collection tours and improves the use of genetic diversity in common bean breeding programs. The objective was to create niche models useful for identifying new areas for scouting and seed collection of new wild and weedy populations of common bean in México.

MATERIALS AND METHODS: The online database of the CIAT (Centro Internacional de Agricultura Tropical-International Center for Tropical Agriculture) for wild and weed populations of the P. vulgaris collection was consulted (CIAT, 2023). Using the "dismo" library (Hijmans et al., 2017) in the statistical software R (version 4.3.1, R core team, 2023), we checked the geographical projections of each dataset and eliminated duplicate records. In addition, we checked the coordinates by visual inspection and assessed the sampling bias by subsampling the geographic datasets. In total, we obtained 225 geographic datasets, 193 for wild and 32 for weedy populations of P. vulgaris. Climate information was obtained from 19 recent climate layers and elevation data available in the WorldClim database 2.1 (Fick and Hijmans, 2017). These layers contain climatic averages of weather conditions recorded from 1970-2000 with a spatial resolution of 30 arcseconds. For the selection of environmental variables, 1,000 background points were added to the polygon distribution area of P. vulgaris. The information of the 19 environmental variables was added to these points. With the information generated, a principal component analysis was performed using the "PRCOMP" function in the R Project program to reduce the dimensionality of the set of climate variables and to avoid collinearity errors. Following this process, a total of six variables were selected, using the variables in component 1 (PC1) and 2 (PC2) as criteria. The variables selected from CP1 were the annual precipitation (BIO12), the precipitation of the wettest quarter (BIO16) and the precipitation of the warmest quarter (BIO18), while for CP2 the variables annual mean temperature (BIO1), isothermality (BIO3) and the minimum temperature of the coldest month (BIO6) were considered. Prior to maximum entropy modeling, model calibration was performed using the "ENMeval" library (Muscarella et al., 2017) in the R Project program. The substrate pH and edaphology variables (CONABIO, 2023) were also considered for this

calibration, in addition to the bioclimatic variables. The information obtained from the calibrated model was projected onto Mexico, taking into account the environmental variables described above.

**RESULTS AND DISCUSSION:** The PCA showed a high proportion of variance explained by components 1 (PC1 = 66%) and 2 (PC2 = 25%), so that only five components were required to reach values close to 100%. PC1 comprised the variables BIO12 (annual precipitation), BIO16 (precipitation of wettest quarter) and BIO18 (precipitation of the warmest quarter). PC2 comprised the climatic variables BIO1 (annual mean temperature), BIO3 (isothermality) and BIO6 (minimum temperature of coldest month). In addition, the Jacknife values for the AUC (area under the curve) of P. vulgaris revealed a high significance for other environmental variables such as altitude and soil pH. The results showed that eight important climatic and soil variables have an impact on the adaptability of wild and weedy populations of *P. vulgaris*. Rainfall frequency throughout the year, and especially in the wettest and warmest quarters, were variables related to plant survival and productivity that altered the distribution of P. vulgaris populations throughout the Mexican territory. Similar results were observed for some variables related to the similarity of temperature patterns (isothermality) throughout the year and in different locations. Other variables apparently unrelated to the adaptability of *P. vulgaris*, such as BIO6, could be related to the survival of seeds, insects, and plant pathogens during the cold season. Insect pests and plant pathogens overwinter, and survival depends on the common bean plants and low temperatures (>4 °C), soil pH and



moisture, and specific tolerance to these environmental factors (Kang et al., 2009; Sharma et al., 2015; Harvey et al., 2020). In Jalisco and other states in the 'El Bajío' region, volcanic axis and southern México, a better suitability of common bean populations has been observed (Figure 1). Novel areas were discovered in Durango, in the Sierra Madre Occidental Mountain System, and in the west coast of Baja California Norte.

**Figure 1**. Idoneity of environments for presence and potential distribution of wild and weedy populations of *P. vulgaris* in México. 1) high fitness and 0) low suitability.

**CONCLUSIONS:** Novel areas showing high idoneity for potential presence of wild and weedy populations of *P. vulgaris* were identified in Puebla, Hidalgo, Querétaro Durango, and Baja California Norte. Representativeness of some areas showing idoneity for presence of *P. vulgaris* also need to be analyzed and considered for future collecting tours.

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# EFFECT OF SOURCES AND DOSES OF NITROGEN ON THE CONTENT OF BEAN NITROGEN

## Luiz H.C. Almeida<sup>1</sup>, Paula P. S. Almeida<sup>1</sup>, Eli C. Oliveira<sup>2</sup>

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**INTRODUCTION:** Nitrogen (N) is a pivotal nutrient for common bean (*Phaseolus vulgaris* L.), significantly influencing its growth and productivity. Deficiency in nitrogen can lead to stunted growth, evidenced by pale green to yellow coloration symptoms, primarily observed in older leaves due to the central role of nitrogen in the structure of chlorophyll molecules. This study aims to delve into the intricate interplay between different nitrogen sources and application doses.

MATERIALS AND METHODS: The work was developed in an experimental plot in Guaíra-PR (24'15 "S, 54'10" W 585masl), in a red dystroferric latosol soil. The results of soil chemical analysis were  $H^++Al^{+3} = 3.05 \text{ cmol}_c \text{ dm}^{-3}$ ; organic matter = 3.50 g dm<sup>-3</sup>; K+ = 0.43 cmol\_c dm<sup>-3</sup>; P mehlich = 5.8 cmol<sub>c</sub> dm<sup>-3</sup>; Mg<sup>+2</sup> = 3.6 cmol<sub>c</sub> dm<sup>-3</sup>; Ca<sup>+2</sup> = 5.85 cmol<sub>c</sub> dm<sup>-3</sup> e Al<sup>+3</sup> = 0.00 cmol<sub>c</sub> dm<sup>-3</sup> <sup>3</sup>. A completely randomized design with 14 treatments and four replications was used. Each experimental plot consisted of an area of 24 m<sup>2</sup> (4m x 6m) with 9 rows spaced at 0.50m and a population density of 18 plants m<sup>-1</sup>. In the experiment, four nitrogen sources were used: Urea (N1), ammonium sulfate (N2), nitrocalcium (N3) and ammonium nitrate (N4) in three different doses (O, 45 and 90 kg ha<sup>-1</sup> of N) of fertilization. In the sowing fertilization, 60 kg ha<sup>-1</sup> of P<sub>2</sub>0<sub>5</sub>, 40 kg ha<sup>-1</sup> <sup>1</sup> of K<sub>2</sub>0 and 20 kg ha<sup>-1</sup> of the source used for side-dressing nitrogen fertilization were applied. One last treatment consisted of no nitrogen fertilization (N5). Seeds of common bean cv. IPR Sabiá were sowed on April 4th 2023, emerging eleven days later. Coverage was performed 20 days after emergence (DAE). In the experiment, biweekly irrigation was used, complementing the amount of rain up to 25 mm. In the last stage, chlorophyll-a, chlorophyll-b and total chlorophyll (a + b)were determined using the spectrophotometric method by N-N-dimethylformamide (DMF) extraction.

**RESULTS AND DISCUSSION:** The effects of nitrogen source application influenced the nitrogen content in leaves and seeds of common bean. This is probably due to the high organic matter content determined in the soil analysis, which was 2.9%. The carbon content is considered very high above 1.4%, and the irrigation system to which the experiment was subjected, supplying all water needs, as it has been noted that nutrient response is closely related to soil water availability (Férnandez-Luqueño et al., 2010). This is because nitrogen is absorbed at a higher rate by mass flow (Malavolta et al., 1997). Among the nitrogen sources, urea stood out negatively due to ammonia volatilization, which can be accelerated under high-temperature and alkaline pH conditions (Dempsey, 2015). The diverse nitrogen doses elicited discernible disparities solely in the nitrogen percentage present within the seeds, with the application of 90 kg N ha<sup>-1</sup> showcasing pronounced efficacy.

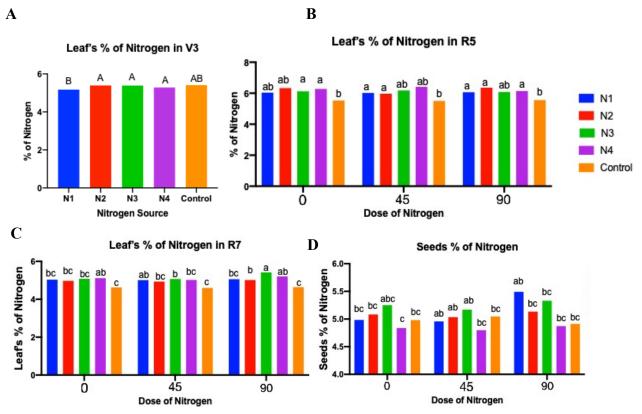


Figure 1. A) Leaf % of Nitrogen in V3, B) Leaf % Nitrogen in R5, C) ) Leaf % Nitrogen in R7, D) Seeds % of Nitrogen.

**CONCLUSIONS:** Different nitrogen sources and doses directly influence nitrogen content in common bean.

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#### MICROBIAL ACTIVITY OF SOIL GROWN WITH COMMON BEAN AND APPLICATION OF PROBIOTICS ASSOCIATED WITH INOCULATION OF MICROORGANISMS

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**INTRODUCTION:** The soil is a complex and dynamic ecosystem with all the transformations that occur in it due to the different microbial populations while their different chemical reactions can be altered whenever this ecosystem suffers interference. The supply of different types of products, which may have probiotic effects on microorganisms, can result in a qualitative and quantitative change in soil constitution. Different types of management may mean different substrate availability that will ultimately determine the establishment of the different microbial groups. Therefore, the objective of this work was to evaluate the response of two probiotics applied in soil cultivated with common bean, together with the inoculation of microorganisms with plant growth promotion effects.

**MATERIALS AND METHODS:** Samples of 100 g of soil were incubated with 70% of their water retention capacity in hermetically sealed glass tubes, containing two beakers with water and NaOH, in the dark for 7 days. These soil samples received the inoculation of the microorganisms and the solution of the coded probiotic product 1 to 800 ml<sup>L-1</sup> and the coded product 2 100 ml<sup>L-1</sup>. After 7 days of incubation, respiratory activity was performed with the technique of Grisi et al. (1978). Ten grams were removed from the soil to perform the microorganisms count by the dilution technique serialized by the technique of Paul & Clark (1989). The soil was then dried at room temperature for 7 days. After drying, microbial biomass carbon analyses were performed according to Sparling (1992) and dehydrogenase activity according to Kumar et al. (2013). The microorganisms inoculated in the soil cultivated with the variety of beans IPR TANGARÁ were: *Azospirillum brasilense, Bacillus amyloliquefaciens, B. subtilis, Bradyrhizobium japonicum, Rhizobium, Beauveria bassiana, Metharizium anisoplie* and *Trichoderma harzianum*.

**RESULTS AND DISCUSSION:** Probiotics 1 and 2 increased the respiratory activity of virtually all treatments with microorganisms. These results strongly suggest that processes such as nutrient cycling, nutrient availability and energy flow in the soil were improved with the application of the products (Figure 1).

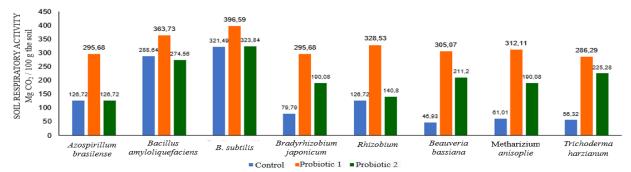
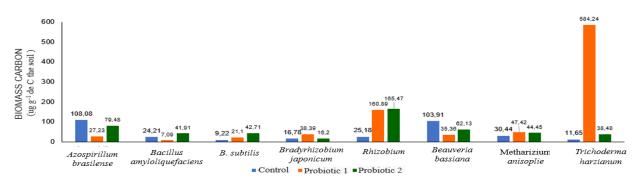


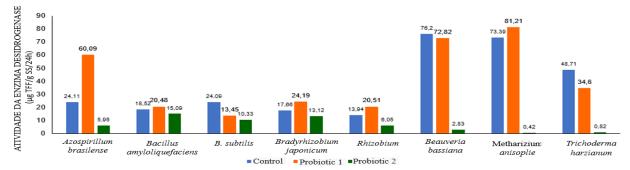
Figure 1. Values of soil respiratory activity with application of different microorganisms and probiotics 1 and 2.

Regarding microbial carbon biomass, a parameter directly proportional to bacterial population density, it had a deleterious effect on microorganisms and products, specifically, *A. brasilense* and probiotic 1; *B. subtilis*. However, there was an increase in microbial biomass with the products, respectively, *B. amyloliquefaciens* and probiotic 2; *B. subtilis* and *Trichoderma harzianum* for both products (Figure 2).



**Figure 2.** Carbon values of soil microbial biomass with application of different microorganisms and probiotics 1 and 2.

The activity of the enzyme dehydrogenase, which is a biotic enzyme, not exogenous, and its activity is restricted to microbial cellular respiration, it is also related to microbial population density. The reduced values for the activity of this enzyme with microorganisms and products were *A. brasilense*, Rhizobium and B. *bassiana* for the probiotic 2. The values that were increased by the application of the product were: *A. brasilense* and *Rhizobium* for probiotic 1 (Figure 3).



**Figure 3.** Activity of the soil dehydrogenase enzyme with application of different microorganisms and probiotics 1 and 2

**CONCLUSIONS:** Probiotics 1 and 2 at concentrations of 80 and 100 ml<sup>L-1</sup> showed an ability to increase soil microbiological activity by improving the parameters that are dependent on it. However, these products need to be better studied in the pod bean crop.

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## SEED TREATMENT WITH PHYTOHORMONES AND MICRONUTRIENTS ON COMMON BEAN (*PHASEOLUS VULGARIS*)

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<sup>1</sup>State University of Londrina - UEL, Brazil

#### **INTRODUCTION**

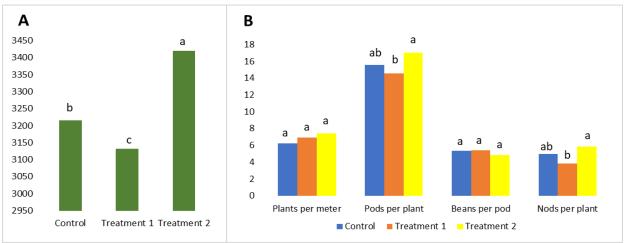
The common bean (*Phaseolus vulgaris*) plays a vital role in global food security, serving as a fundamental source of nutrients. However, to address environmental challenges and ensure sustainable production, it is crucial to optimize the initial establishment of the crop. In this context, seed treatment with substances such as Cobalt+molybdenum and Cobalt+ molybdenum+ gibberellic acid+4-indol-3ylbutyric acid, emerges as a promising strategy, aiming to improve biological nitrogen fixation, consequently the nitrogen supply to the plant and productivity. This study aimed to assess the effects of these treatments on the early stages of common bean development, contributing to the understanding of innovative practices in the management of this important crop.

#### **MATERIALS AND METHODS**

The experiment was conducted in the city of Salmourão-SP, Brazil, from May to July 2022. Comprising three treatments, each replicated four times, the experimental design was a randomized complete block. Each plot consisted of six rows, each 5 meters in length, with a row spacing of 0.45 m. The seeding density was adjusted to achieve 12-13 plants m<sup>-1</sup> after emergence. Sowing took place on 25/5/2022, utilizing the IAC Carioca Eté cultivar. Seed treatments included: Control (no application), Treatment 1 (Cobalt+molybdenum+gibberellic acid+4-indol-3ylbutyric acid) and Treatment 2 (Cobalt and molybdenum). Initial fertilization in the sowing furrows was calculated based on soil analysis, following the recommendations of Pauletti and Motta (2019). A specific formulation of 180 kg ha<sup>-1</sup>, comprising the 8-28-16 formula, was applied. Topdressing nitrogen fertilization, using urea, was carried out 15 days after plant emergence, aiming to provide additional nutrients to support vegetative growth during critical stages of the cultivation cycle. Weed control, 25 days after emergence, involved the application of the herbicide fluazifop-p-butyl + fomesafen (200 + 250 g ha<sup>-1</sup> of a.i.). The remaining cultural practices adhered to the generally recommended practices for bean cultivation in the region, accompanied by supplemental irrigation to meet the crop's water requirements. Evaluations conducted at the R6 stage (first flower) included nodulation count per plant, with plants uprooted and assessed within a 1-meter linear segment per plot. Other parameters assessed included plants per meter, pod count per plant, grain count per pod and yield, conducted at the point of physiological maturity adjusted to 13%.

#### **RESULTS AND DISCUSSION**

When analyzing the grain yield of beans in kg ha<sup>-1</sup> (Figure 1.A), there was a significant difference between treatments, with treatment 2 (Cobalt and Molybdenum) surpassing the control (no application), a result that corroborates findings by Avozani (2023), demonstrating that the addition of these micronutrients directly influences biological nitrogen fixation and consequently final productivity. The number of nodules per plant and the number of pods per plant also differed significantly (Figure 1.B), indicating that the addition of cobalt and molybdenum increased nodulation, and with a higher available nitrogen content for the plant, there was also an increase in the number of pods (KUSDRA, 2003). There was no significant difference between treatments when analyzing plant height and number of grains per pod (Figure 1.B) as found by Avozani (2023). When phytohormones were used in combination with cobalt and molybdenum in treatment 1, the results were inferior to the control without application, which disagrees with findings by Abrantes et al. (2011).



\* Equal letters do not differ from each other, according to Tukey test at 5% significance level.

**Figure 1. A-** Yield of beans in Kg ha<sup>-1</sup>. **B-** Plants per meter, Pods per plant, Beans per pod and Nods per plant.

## CONCLUSIONS

The application of Cobalt and Molybdenum significantly increased grain yield and nodulation in beans, supporting their role in enhancing biological nitrogen fixation and overall productivity.

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## SLUDGE COMPOST ON SNAP BEAN (PHASEOLUS VULGARIS) CULTIVATION

## Luiz H.C. Almeida<sup>1</sup>, Paula P.S. Almeida<sup>1</sup>, Maria de Fátima Guimarães<sup>1</sup>

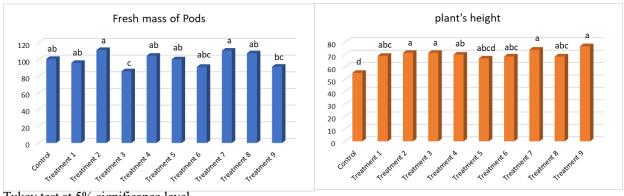
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**INTRODUCTION:** The growing demand for sustainable and innovative agricultural practices highlights the need to explore alternative sources of nutrients to promote healthy crop growth. In this context, green beans (*Phaseolus vulgaris*) stand out as a crop of economic and nutritional importance. The potential to enhance its performance through the strategic use of organic fertilizers, such as sewage sludge compost, offers an intriguing opportunity to boost agricultural efficiency. The aim of this study was to investigate the impacts of sewage sludge compost on the morphological characteristics, productivity, and nutritional content of green beans.

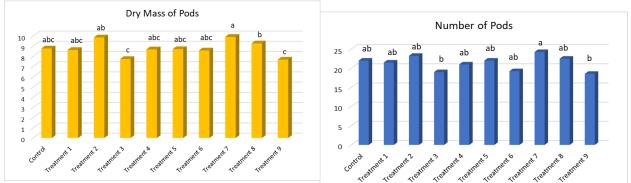
MATERIALS AND METHODS: The experiment was conducted in a greenhouse in Londrina-PR, Brazil. The pots were filled with soil previously sieved (4 mm), from the surface layer of a Eutroferric Red Latosol, with clayey texture. Each pot received 4.0 liters of soil. The soil analyzed had: pH=6.6;  $H^{++}Al^{+3} = 2.50 \text{ cmol}_c \text{ dm}^{-3}$ ; organic matter = 8.23 g dm<sup>-3</sup>;  $K^+ = 0 \text{ cmol}_c \text{ dm}^{-3}$ ; P mehlich = 21.2 mg dm<sup>-3</sup>; Mg<sup>+2</sup> = 1.44 cmolc dm<sup>-3</sup>; Ca<sup>+2</sup> = 3.29 cmol<sub>c</sub> dm<sup>-3</sup>; and Al<sup>+3</sup> = 0.08 cmol<sub>c</sub> dm<sup>-3</sup>. The contents of Sewage sludge compost was:  $OM = 170 \text{ g dm}^{-3}$ ;  $N = 80 \text{ g Kg}^{-1}$ ; P = 16.32 g $Kg^{-1}$ ;  $K = 15.23 \text{ g Kg}^{-1}$ ;  $Ca = 9.98 \text{ g Kg}^{-1}$ ;  $Mg = 3.22 \text{ g Kg}^{-1}$  and  $S = 6.42 \text{ g Kg}^{-1}$ . The treatments consisted of: Control: Mineral fertilizer (600 kg ha<sup>-1</sup> of 04-14-08), treatment 1: 2 t ha<sup>-1</sup> Sewage sludge compost + 75% of mineral fertilizer, treatment 2: 2 t ha<sup>-1</sup> Sewage sludge compost + 50% of mineral fertilizer, treatment 3: 2 t ha<sup>-1</sup> Sewage sludge compost + 25% of mineral fertilizer, treatment 4: 4 t ha<sup>-1</sup> Sewage sludge compost + 75% of mineral fertilizer, treatment 5: 4 t ha<sup>-1</sup> Sewage sludge compost + 50% of mineral fertilizer, treatment 6: 4 t  $ha^{-1}$  Sewage sludge compost + 25% of mineral fertilizer, treatment 7: 8 t ha<sup>-1</sup> Sewage sludge compost + 75% of mineral fertilizer, treatment 8: 8 t ha<sup>-1</sup> Sewage sludge compost + 50% of mineral fertilizer, and treatment 9: 8 t ha<sup>-1</sup> Sewage sludge compost + 25% of mineral fertilizer. The sewage sludge compost, in the amounts corresponding to each treatment, was applied and incorporated into the soil of the pots. Next, the mineral fertilizer was applied locally, being homogenized in the layer up to 10 cm deep in the pots. All pots were adequately moistened to reach a moisture equivalent to 70% of the maximum water holding capacity. Over a period of 30 days, the pots remained at rest, receiving only irrigation to replace evaporated water. After this period, on 04/25/2023, the green bean seeds were sown, distributing four seeds per pot. Between 5 and 10 days after the emergence of the seedlings, thinning was carried out, leaving only two plants per pot. Twenty days after emergence, topdressing was performed, applying 0.5 g of urea per pot. At the end of the experimental period (48 days), the aboveground part of the plants was collected, properly identified, and packaged in plastic bags. All collected material was sent to the laboratory, where the pods were separated, counted and weighed, in addition to measuring the stem diameter at the collar region and the length of the plants. After obtaining these measurements, the corresponding materials had the content of macronutrients in the dry matter quantified, and a soil sample was collected for analysis.

**RESULTS AND DISCUSSION:** The average results of the plant characteristics revealed that the application of sewage sludge compost at a rate of 8 t ha<sup>-1</sup>, associated with 75% of mineral fertilization, resulted in the highest numbers of pods per plant, dry mass of pods per plant, fresh

mass of pods per plant, and plant height when compared to the control, which received only mineral fertilizer. Additionally, a significant increase in fresh pod mass was observed as the dose of sewage sludge combined with mineral fertilizer increased. These findings are consistent with previous studies, such as that of Oliveira et al. (2023), which highlight the benefits of using sewage sludge as fertilizer in agriculture. The combination of sewage sludge with mineral fertilization can promote a substantial increase in plant productivity, as observed in this study. Furthermore, a dose-response effect was observed, where higher doses of sewage sludge resulted in corresponding increments in fresh pod mass, corroborating the results of Vieira (2002).



Tukey test at 5% significance level. **Figure 1.** Fresh mass of pods per plants and plant's height average



\* Equal letters do not differ from each other, according to Tukey test at 5% significance level. **Figure 2.** Number of pods per plant and dry mass of pods per plant in grams

**CONCLUSIONS**: Sewage sludge combined with mineral fertilizer increased snap bean productivity and can be used as a potential source of nutrients for the crop.

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## INDIRECT SELECTION ON ROOT ARCHITECTURE IN THE MSU PINTO DRY BEAN BREEDING PROGRAM

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**INTRODUCTION:** Dry beans can be grouped into four major growth habits based on determinacy and shoot architecture<sup>1,2</sup>. Type 1 beans are determinate and upright, while Type 2, 3, and 4 are indeterminate. Type 2 is upright; type 3 is upright with prostrate growth; and type 4 presents a climbing growth pattern<sup>2</sup>. Many dry bean breeding programs in the United States, including Michigan State University, focused on type 2 growth habit (shoot architecture) to enhance yield and reduce disease spread<sup>3</sup>. Shoot and root development are closely related mechanistically and affecting one can cause associated changes in the other<sup>4–7</sup>. We hypothesize that when breeding programs selected for type 2 growth habit, root architecture was indirectly selected upon. Using cultivars from the MSU Pinto Dry Bean Breeding program, we aim to identify if breeding for shoot architecture indirectly selected upon root architecture in dry beans.

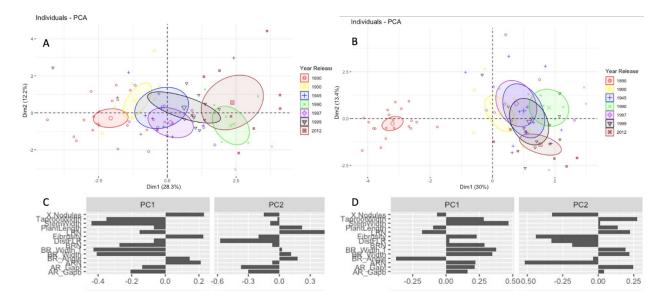
**MATERIALS AND METHODS:** Six cultivars from the MSU pinto breeding program and the two wild accessions were grown and assessed at MSU's Montcalm research farm in 2020 and in 2021. Cultivars were chosen because they were the first cultivar released to reach a targeted growth habit (Table 1). Roots were collected at the early flowering stage via "shovelomics,<sup>8</sup>" photographed, and measured with ImageJ. Traits measured include stem diameter, taproot diameter, basal root diameter, percent nodulation, fibrosity, distance between the basal roots and first lateral root, and adventitious root number. Analysis of root architecture traits were all completed in R (4.3.1). Due to large effects of year in the model, year was evaluated separately for all analyses.

Genotype	<b>Growth Habit</b>	Year Released <sup>9</sup>
PI 535428	Type 4 vining	N/A
W6 24135	Type 4 vining	N/A
Common pinto	Type 4	Landrace
UI-111	Type 4	1944
Sierra	Type 3	1989
Maverick	Type 3	1997
Kodiak	Type 3	1997
Eldorado	Type 2	2012

 Table 1. Characteristics of selected cultivars

**RESULTS AND DISCUSSION:** In 2020 and in 2021, the earliest and most recently released pinto cultivars are separated using principal component analysis (PCA). In 2020, dimension 1 accounts for 28.3% of the variation and dimension 2 accounts for 12.2%. The genotypes are primarily separated across dimension 1 by stem diameter, taproot diameter, and basal root diameter. In 2021, dimension 1 accounts for 30% of the variation while dimension 2 accounts for 13.4%. In 2021, the wild beans were strongly separated from the cultivated beans in the first

principal component, namely by stem width and basal root angle. Amongst the cultivated genotypes, components in dimension 2, such as percent nodules and fibrosity were strongly associated with year released. Given that the genotypes separate accordingly with year released, these results support the hypothesis that root architecture traits were indirectly selected upon. Evaluation of genetic regulation on root traits is needed to confirm indirection selection. Future work will incorporate leaf and seed mineral nutrition to determine if changes in root architecture have affected root foraging and nutrient uptake.



**Figure 1**. Principal component analyses. (A) PCA of pinto root architecture by year of release in 2020. (B) PCA of pinto root architecture by year of release in 2021. (C) Eigenvector graph of PC1 and PC2 in 2020. (D) Eigenvector graph of PC1 and PC2 in 2021.

**CONCLUSIONS:** This study encompasses how breeding for shoot architecture historically has influenced root architecture by indirectly selecting upon it. This data will help support future research in root architecture and future breeding programs for improving the quality of dry beans.

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### RESPONSE OF THE TEPARY DIVERSITY PANEL TO COMBINED ASIAN BEAN FLOWER THRIP AND LEAFHOPPER PRESSURE

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#### **INTRODUCTION**

The Asian bean flower thrip, *Megalurothrips usitatus* (Bagnall) (Thysanoptera: Thripidae), is a new pest reported recently in Florida, the Caribbean and Central America (Rodríguez-Arrieta et al., 2023; Cabrera Asencio and Estévez de Jensen, 2023) that is causing extensive damage to common bean reproductive and vegetative tissue, resulting in significant crop loss. Given its recent arrival to the region, there is limited information regarding the response in common bean, resistance mechanisms, and genetics of host resistance, as well as chemical, biological and cultural control measures. The endemic leafhopper pest, *Empoasca* spp., is a significant constraint to common bean production in the same regions and in North America. Tepary bean (*Phaseolus acutifolius*) has served as a valuable source of disease resistance in common bean, as well as bruchid resistance, and the genetics of several traits have been reported. While *Empoasca* spp. resistance has been identified in tepary bean, response to the Asian bean thrip is lacking.

#### **MATERIALS AND METHODS**

A total of 313 entries, including cultivated, improved, weedy, and wild accessions, and checks, from the tepary diversity panel (TDP) were planted on March 10, 2023 in Isabela, Puerto Rico in a randomized complete block design with two replications. The plots were 1.5m in length with 0.76m row spacing. The seed were treated with fungicide, the plots were fertilized at planting, but no pesticides were applied during the duration of the experiment. Leaf curl (LC) data were collected visually on the 1-9 CIAT scale, while yield component data was collected after harvest. The presence of both *Empoasca* spp. and the Asian bean thrips resulted from natural infestation and was monitored throughout the experiment with high population densities noted from before flowering to harvest. The data were adjusted for spatial heterogeneity using the SpATs R program and BLUPs are presented.

#### **RESULTS AND DISCUSSION**

High *Emposca* spp. and Asian bean thrips pressure resulted in reduced leaf size, meristem dieback (data not shown) and leaf curl response in some accessions of the TDP. The average leaf curl reading (5.1) indicates moderate susceptibility, while the average yield in the trial was low, 209 kg/ha, with a relatively low average hundred seed weight of 7.8 g because of the inclusion of wild accessions and insect pressure. The average days to maturity in the trial was 69, while extended maturity in some tepary bean accessions was noted as a result of intermittent rain post-flower. A subset of the TDP was identified with potential tolerance to both *Empoasca* spp. and Asian bean thrips based on adjusted yield (Table 1). These entries represent improved (TARS-Tep 23, TARS-Tep 54, TARS-Tep 100, 21IS-8799-6), cultivated landrace (G40151, G40177A1, G40159, PI 549447, G40016), weedy (G40173B, G40177A2), and wild (G40192, PI 653254) accessions that yielded above 500 kg/ha and were superior to the checks, G40001 and Sacaton white. The lack of correlation between leaf curl and yield (data not shown) could indicate independent mechanisms

of vegetative and reproductive resistance to these two pests or it could be due to interactions given the complexity of this combined insect pest evaluation.

2023.						
				DTM <sup>2</sup>	Yield	HSW <sup>3</sup>
Line	TDP	Туре	LC <sup>1</sup>	(days)	(kg/ha)	(g)
G40177A2	149	weedy	5.3	63.9	998	10.2
21IS-8799-6	NA	improved	5.9	66.8	926	7.5
G40151	121	cultivated	4.7	74.8	663	11.8
TARS-Tep 23	312	improved	6.3	66.6	650	15.7
G40177A1	148	cultivated	5.2	63.8	594	13.3
G40159	128	cultivated	7.7	77.9	586	11.0
G40192	168	wild	5.5	61.8	558	5.2
TARS-Tep 100	437	improved	6.3	66.5	533	15.6
PI 549447	301	cultivated	6.2	68.5	528	11.6
G40016	373	cultivated	7.6	65.1	527	15.5
G40173B	402	weedy	5.6	64.2	513	9.0
TARS-Tep 54	419	19 improved	6.2	66.0	505	10.1
PI 653254	333	wild	4.1	59.2	501	5.7
		cultivated				
G40001	1	check	4.6	70.0	117	11.9
		cultivated				
Sacaton white	407	check	6.5	68.2	281	8.7
Overall Mean			5.1	69.0	207	7.8

**Table 1**. Adjusted trait BLUPs for superior accessions for yield from the Tepary Diversity Panel (TDP) under concurrent *Empoasca* spp. and Asian bean thrip pressure in Isabela, Puerto Rico in 2023.

<sup>1</sup>LC – leaf curl (1 to 9; 1 resistant and 9 susceptible); <sup>2</sup>DTM - days to maturity, <sup>3</sup>HSW - hundred seed weight.

## CONCLUSIONS

These results from a single trial with combined *Empoasca* spp. and Asian bean thrip pressure show a number of lines with potential tolerance to concurrent infestation, while additional trials are needed to confirm these results. Given the presence of both pests in the region, targeted location or controlled greenhouse evaluations may be needed to study the specific response to the Asian bean thrip.

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## ASSESSMENT OF DAMAGE CAUSED BY PESTS TO THREE VARIETIES OF DRY BEAN IN THE CENTRE REGION OF COTE D'TVOIRE

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**INTRODUCTION**: To revive dry bean cultivation, which is tending to disappear in Côte d'Ivoire, the National Center for Agronomic Research (CNRA) and PABRA has undertaken to select efficient dry bean varieties with high yields and tolerant to biotic and abiotic factors. To do this, 35 accessions collected or introduced were cultivated. Of these accessions, three were selected using a participatory approach. Pests pose a serious threat to this crop (Ossey *et al.*, 2021). It is therefore necessary to know their behavior towards attacks by these pests. We propose to evaluate the damage caused by different groups of pests to the three varieties in order to determine which one is less attacked by pests.

**MATERIAL AND METHODS**: The plant material consists of three varieties of dry bean (Figure 1). Observations focused on three groups of pests (defoliators, sucking insects and borers). Commonly observed insects were captured and identified using a microscope and identification keys. Leaf damage was assessed on 10 plants of each accession by calculating the percentage of damaged leaves. Foliar damage was scored using a visual scale of Karel & Rweyemamu (1985) of 0 to 5, where 0 = no defoliation, 1 = 1-5% defoliation, 2 = 6-25% defoliation, 3 = 26-50% defoliation, 4 = 51-75% defoliation, and 5 = 76-100% defoliation. Regarding damage to the pods, thirty (30) plants of each variety were inspected and the pods infested or attacked by borers and sucking insects were counted. Three repetitions were made. Thus the percentage of pods infested or attacked was calculated by the ratio of the number of pods attacked to the total number of pods multiplied by one hundred.







Roba1 (HARI35/GHA19SMR53 (HARI25/GHA19)Zabra (HARI36/GHA19)Figure 1. Seeds of three (3) dry bean varieties proposed for evaluation

**RESULTS AND DISCUSSION:** The lowest percentage of damaged leaves per plant and the lowest foliar damage score induced by defoliators were recorded in Roba1 (Hari 35/GHA19). SMR53 (Hari 25 / GHA 19) and Zabra (Hari 36 / GUI 20) presented the highest percentages of damaged leaves per plant and the highest severity indices. Regarding damage to the pods for these two varieties, the percentages of pods attacked by sucking insects and pod borers were greater than

10%. The percentages of pods attacked in Roba1 (Hari35 / GHA19) were less than 5%. The pods of this variety were less attacked by sucking insects and pod borers (Table 2).

Pests group	Main insect pests
Defoliators	<i>Ootheca mutabilis</i> (Chrysomelidae), <i>Medythia quarterna</i> (Chrysomelidae)
Sucking insects	Riptortus dentipes (Alydidae), Coptosoma cribraria (Plataspidae), Cletus sp (Coreidae)
Borers	Maruca testulalis (Pyralidae), Euchrysops malathana (Lycaenidae)

 Table 1. Main insect pests encountered according to pest groups.





Figure 2. Damage caused by different groups of pests to the three dry bean varieties, including sucking insects (a) and borers (b) on bean pods

**a:** abortion of seeds in the pod; **b:** perforation of the pod

	Percentages of	Foliar damage	Pod attack rate (%)		
Variétés	damaged leaves per plant	scale induced by defoliators	Borers	Sucking	
SMR53 (Hari 25 / GHA 19)	81.54±7.51 <sup>a</sup>	$1.673 \pm 0.08^{a}$	10.20±1.88 <sup>ab</sup>	$10.48 \pm 0.48^{a}$	
Zabra (Hari 36 / GUI 20)	$78.71 \pm 6.39^{a}$	1.503±0.24ª	10.54±2.14ª	$10.65{\pm}1.37^{a}$	
Roba1 (Hari 35 / GHA19)	41.46±7.73 <sup>b</sup>	$0.84{\pm}0.11^{b}$	$4.56 \pm 0.77^{b}$	3.01±1.12 <sup>b</sup>	
F	9.5415	7.1827	3.86278	16.7636	
Р	0.013	0.025	0.008	0.003	
Df	2	2	2	2	

Table 2. Damage to leaves and rate of attack on pods caused by pests on different varieties

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## COMPARATIVE STUDY OF THE EFFECTS OF GROWING GREEN BEANS (PHASEOLUS VULGARIS L.) UNDER NETS AND WITHOUT NETS AND PHYTOSANITARY TREATMENTS ON DEVELOPMENT AND YIELD PARAMETERS

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**INTRODUCTION:** Thanks to the implementation of a policy aimed at ensuring food security, agriculture has diversified with the promotion of market garden crops (PNIA, 2017). These include green beans (*Phaseolus vulgaris*), which play an important role in the human diet as a source of vitamins (A, B and C), proteins, fiber and minerals. Like other legumes, green beans can also be used to improve soil fertility by fixing atmospheric nitrogen. However, the damage caused by pests and diseases to green bean production has led some growers to switch to other crops, or to make intensive use of chemical pesticides (Program PIP, 2011). Faced with this situation, it is important to propose effective cultivation methods for good production. The aim of this study is to assess the effectiveness of anti-insect nets and biopesticides on growth, vegetative development and yield parameters.

**MATERIALS AND METHODS:** The plant material consisted of accession HARIV01 and accession HARIV02. The trial was conducted in a greenhouse with insect netting, and four phytosanitary treatments (T0: control, T1: chemical treatment, T2: biopesticides, T3: single net, T4: single net + biopesticides) were applied to each variety. Measurements were taken on vegetative vigour at flowering, fruit set, yield and ratio.



Figure 1. Green bean accession seeds : (a) HARIV01, (b) HARIV02



Figure 2. Insect nets used for the trials **RESULTS AND DISCUSSION:** The table shows the vegetative vigour index at flowering stage (VVI), fruit set, yield and ratio of two green bean varieties. Treatment T1 (control) gave the highest VVI with HARIV01, while T3 (single net) with HARIV02 gave the highest. The fruit set values obtained were not statistically different for the bean varieties studied, regardless of the treatment applied. Concerning the yield, T1 (chemical treatment) gave the lowest value for HARIV01, in opposite to T3 (single net) that gave the highest value when using the HARIV02 accession. The ratio values were not statistically different for the two bean accessions studied. In short, treatment T3 performed better than the other treatments for HARIV01 and HARIV02.

Varieties	Treatments	VVI	Fruit Set rate (%)	Yield (t/ha)	Ratio
HARIV01	TO	-0.14±0.11a	85.00±19.14 <sup>a</sup>	$7.15 \pm 5.87^{b}$	$0.55{\pm}0.23^{a}$
HARIV01	T1	$0.04{\pm}0.24c$	$100.00{\pm}0.00^{a}$	$4.06 \pm 1.36^{a}$	$0.58{\pm}0.19^{a}$
HARIV01	T2	-0.01±0.33b	$85.00{\pm}19.14^{a}$	7.85±1.15 <sup>bc</sup>	$0.50{\pm}0.13^{a}$
HARIV01	T3	-0.12±0.19a	$60.00{\pm}28.28^{a}$	$14.43 \pm 3.84^{d}$	$0.51{\pm}0.08^{a}$
HARIV01	T4	-0.11±0.24a	$85.00{\pm}30.00^{a}$	10.24±3.63 <sup>cd</sup>	$0.59{\pm}0.02^{a}$
HARIV02	Τ0	0.19±0.13d	55.00±19.15 <sup>a</sup>	8.11±3.84°	$0.70{\pm}0.05^{a}$
HARIV02	T1	$0.08 \pm 0.05$ cd	73.33±30.55 <sup>a</sup>	$4.47{\pm}0.97^{ab}$	$0.53{\pm}0.15^{a}$
HARIV02	T2	$0.04{\pm}0.10c$	$80.00{\pm}16.33^{a}$	$7.10{\pm}1.84^{b}$	$0.71 \pm 0.22^{a}$
HARIV02	T3	0.32±0.13e	$60.00{\pm}28.28^{a}$	21.24±10.37 <sup>e</sup>	$0.68 \pm 0.16^{a}$
HARIV02	T4	0.16±0.12d	$60.00{\pm}0.00^{a}$	21.16±6.90 <sup>de</sup>	$0.73{\pm}0.03^{a}$
Averages		0.04	74.36	10.58	0.61
Probabilitie s		0.0209	0.0820	0.0000	0.2161
s CV (%)		-	32.03	15.77	24.86

**Table 1.** Index of vegetative vigor at flowering stage, setting rate, yield and ratio in two green bean accessions.

In the same column, values followed by the same letter show no significant difference at the 5% threshold (Fischer test).

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## IDENTIFICATION OF GENOMIC REGIONS ASSOCIATED WITH GRAIN YIELD IN COMMON BEAN

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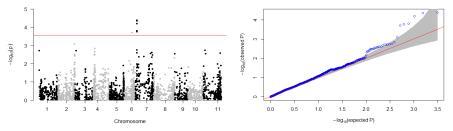
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**INTRODUCTION:** Addressing malnutrition and promoting food security requires strategies to increase the yield of highly nutritious crops such as common bean. Siddiq and Uebersax (2022) emphasize the crucial role of common bean in providing essential nutrients. Although grain yield has seen a consistent increase (Vandermark et al., 2015), the use of new breeding tools offers an exciting opportunity to accelerate this progress. However, a significant challenge is genetic architecture of grain yield in common bean. This is a complex quantitative trait influenced by numerous minor genes (Izquierdo et al., 2023). To navigate this complexity, researchers have defined various production components for focused analysis. Additionally, Numerous genomewide association studies (GWAS) have been conducted to discover the genetic architecture of grain yield (Izquierdo et al., 2023). This study aimed to identify and characterize genomic regions linked with grain yield in common bean.

MATERIALS AND METHODS: In this study, a panel consisting of 121 common bean lines from the Germplasm Bank of the Universidade Federal de Lavras (UFLA, Lavras, Brazil) was evaluated. The experiment used a randomized complete block design with two replications. The grain yield (YD) was measured in grams per plot and then converted into kilograms per hectare. After obtaining the adjusted average of the scores for each block, the Best Unbiased Linear Estimator (BLUE) was applied using the R software (R Core Team, version 4.0.4). Genomic DNA was extracted from 20 mg of leaf tissue from individual plants using a modified CTAB (hexadecyltrimethylammonium bromide) extraction protocol (Doyle and Doyle, 1987) and genotyped using the 5,398 SNPs BARC Illumina chip assay at the USDA-ARS Soybean Genetics and Improvement Laboratory in Beltsville, MD. Principal component analysis (Price et al., 2006) was used to estimate population structure. Population-relatedness was calculated using the GEMMA algorithm for centered-relatedness. Two models were tested using GEMMA within each phenotypic distribution. The MM (mixed model) includes population structure and relationship (2PCA + kinship matrix), and the EMMA (efficient mixed-model analysis) model only accounted for relatedness. The model with the lower mean square deviation (Mamidi et al., 2011) was used for further analysis, in this case MM for YD. Candidate genes were inferred using Jbrowse Phytozome v13.0 (Goodstein et al., 2012) and Phaseolus vulgaris genome v1.1 UI111 the common bean reference genome (Middle American). A genomic region was delimited considering a 200-Kb window centered for each SNP.

**RESULTS AND DISCUSSION:** There was a significant difference in YD, with a significance level of 5%. The coefficient of variation was 24%, while the average YD reached 3,359 kg ha<sup>-1</sup>. Five significant SNPs were identified on chromosome Pv07, along with one on Pv06. Pv07 stood out for its potential impact on YD. A specific region (4063954-4103683bp) harbored five influential SNPs (ss715646471, ss715646470, ss715646473, ss715646472, ss715646466) and

contained 23 kinase proteins, including four Inositol-tetraksphosphate 1-kinases, 18 Cysteine-rich receptor-like protein kinases, and a Serine/threonine protein kinase (MAPK). These proteins are crucial for plant defense against pathogens, highlighting a previously unknown link between disease resistance and high yield (Richard et al., 2021). This connection is further supported by the high disease incidence observed in the experiment. Pv06 revealed different significant genes, including methyltransferases and Cation/H(+) antiporter proteins previously associated with increased grain yield in saline environments (Song et al., 2018). These findings suggest that focusing on the Pv07 genomic region could be a promising strategy for breeding higher-yielding crops. Further investigation is needed to confirm and exploit this potential.



**Figure 1**. Manhattan plot and its respective quantile-quantile plot of grain yield (YD) data for UFLA common bean diversity panel genotyped with 3117 SNPs. Markers red-colored passed the cutoff value of 0.05.

**ACKNOWLEDGEMENTS:** CNPQ, CAPES, and FAPEMIG for financial support and by Northarvest Bean Growers Association and USDA Pulse Crop Health Initiative.

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## MEASURING CANOPY HEIGHT IN COMMON BEAN MICROPLOTS USING UAV DIGITAL PHENOTYPING METHODS

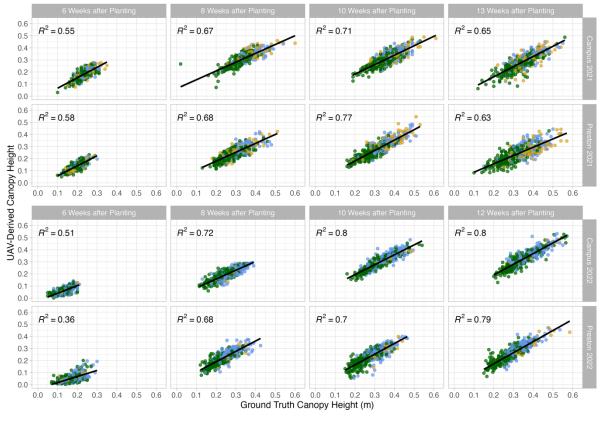
## Quinn Sturby<sup>1</sup>, Ana Vargas<sup>1</sup>, Hai Ying Yuan<sup>1,2</sup>, Kirstin Bett<sup>1</sup>

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**INTRODUCTION:** Canopy height, area, and volume are informative phenotypic traits of interest in breeding programs and are used as indirect measurements of crop growth rates, biomass, stress tolerance, growth habit, and yield (Borra-Serrano et al., 2020; Luo et al., 2021; Madec et al., 2017). Researchers most commonly measure these traits manually, which is labor intensive, time consuming, subjective, prone to human error, and not practical when studying large areas or many plots requiring repetitive measurements (Luo et al., 2021; Madec et al., 2017). High throughput digital phenotyping using unoccupied aerial vehicles (UAVs) allows for efficient, less labour intensive, non-destructive, multi-temporal, and precise phenotyping (Morota et al., 2022). Compared to traditional phenotyping, UAV digital phenotyping methods better enable crop improvement and genetic gain by increasing the accuracy and efficiency of selection and the identification of candidate genes related to complex traits through quantitative trait loci (QTL) analyses (Cobb et al., 2013; Morota et al., 2022). The goal of this study is to validate the use of UAV-derived data for further phenotypic and genetic analyses of canopy characteristics in a common bean (*Phaseolus vulgaris*) population.

**MATERIALS AND METHODS:** Experimental field trials used in this study were grown at two locations, Campus and Preston, in Saskatoon, Saskatchewan, Canada, in 2021 and 2022. This study consisted of a bi-parental recombinant inbred line (RIL) population derived from a cross between CDC WM-2 (Bett et al. 2013) and Higuera-E. CDC WM-2 is a slow darkening pinto bean with type II indeterminate growth habit and Higuera-E is a yellow bean with type I determinate growth habit. The field trials consisted of both parental genotypes and 154 RILs that were grown in onemeter square microplots with three rows and replicated 3 times in a randomized complete block design. Manual canopy height measurements were taken at all site-years using a barcoded meter stick and a scanner at 6, 8, 10, and 13 weeks after planting in 2021 and at 6, 8, 10, and 12 weeks after planting in 2022. UAV flights were performed weekly for all site-years starting in the middle of June after emergence and ending when most plots were reaching maturity in the beginning of September. A DJI Matrice 600 equipped with a RedEdge multispectral sensor was used for data collection and flown at an elevation of 15 meters. Pix4D Mapper was used to process the images and create orthomosaics. QGIS was used to segment the plots and extract canopy height data from the previously created orthomosaics using similar methods to what was described by Parker et al. (2020). The normalized difference vegetation index (NDVI) was used to create thresholds to separate plant material from the soil. The 95<sup>th</sup> percentile for canopy height was used as the measurement for each plot. Correlations were calculated between the UAV-derived and manually collected ground truth canopy height measurements for the weeks where both measurements taken.

**RESULTS AND DISCUSSION:** Correlations ( $\mathbb{R}^2$ ) ranged between 0.36 and 0.8 for all site-years. The strongest correlations occurred at 10 weeks after planting for both site-years in 2021 and at 10 and 12 weeks after planting for Campus and Preston respectively in 2022 (Figure 1). The lower  $\mathbb{R}^2$  values prior to 10 and 12 weeks after planting could be due to a lack of canopy closure at that time leading to more of the lower canopy being visible to the UAV from directly above. Similarly, senescence during maturity could contribute to the lower R<sup>2</sup> values after 10 weeks after planting due to more variability in the canopy being visible to the UAV from above. These findings show that there is potential in using UAVs to measure canopy characteristics and use UAV-derived data in further phenotypic and genetic analyses, but further methods could be implemented to increase the accuracy of measurements taken at the beginning and end of the growing season.



Canopy Height Correlations by Plot

Growth Type • Type I • Type II • Type III

**Figure 1.** Relationships between manually collected ground truth and UAV-derived canopy height measurements at 6, 8, 10, and 13 weeks after planting for both locations in 2021 and 6, 8, 10, and 12 weeks after planting for both locations in 2022.

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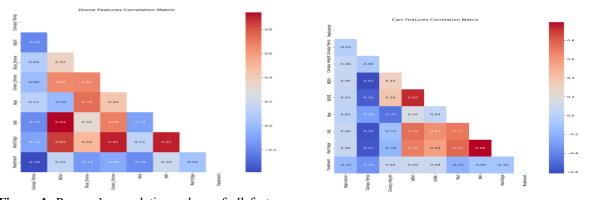
### SYNERGISTIC APPROACH FOR DROUGHT/NON-DROUGHT CLASSIFICATION OF BEANS: HARNESSING THE POTENTIAL OF MULTISOURCE DATASETS USING MACHINE LEARNING

## Muhammad Usman<sup>1</sup>, Xin Zhang<sup>1\*</sup>, Suraj A. Yadav<sup>1</sup>, Timothy Porch<sup>2</sup>

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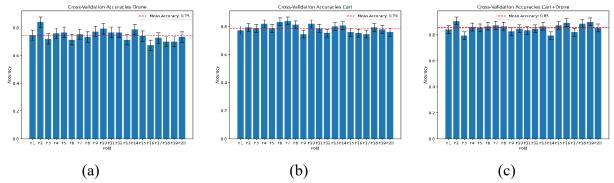
**INTRODUCTION:** Drought stress significantly impacts crop growth and development, ultimately affecting crop yield. Under drought stress, plants experience water deficiency, during which their physiological processes, such as photosynthesis, nutrient uptake, and metabolism, are negatively affected. Early detection and understanding of drought stress will enable growers to implement effective water conservation measures, adjust irrigation strategies, and breed drought-resistant cultivars. This study investigated the effectiveness of multisource datasets acquired using an uncrewed aerial system (UAS) and a ground-based proximal sensing cart to classify the drought and non-drought conditions for bean crops.

**MATERIALS AND METHODS:** A multispectral imaging sensor was equipped with a UAS for drone-based data acquisition in Juana Diaz, Puerto Rico. Furthermore, a Crop Circle multispectral canopy sensor, an ultrasonic sensor, and an Apogee infrared thermometer sensor were deployed on a ground-based proximal sensing cart. A total of 68 features were extracted from the collected datasets, including photosynthesis data using a MultispeQ and manually measured agronomy data, to classify drought/non-drought conditions using machine learning techniques [1]. Canopy temperature and height, and multispectral data are crucial indicators for identifying drought stress, while alterations in spectral reflectance patterns are key markers of drought stress. The study focused on multisource datasets collected through drone-based remote sensing and ground cartbased proximal sensing, while missing values were handled by adopting a "mode imputation" method [2]. Moreover, correlation analysis for drone and cart features corresponding to the treatments was completed to identify robust predictive parameters. The multisource datasets were imbalanced with 35,056 data points of drought class versus 5,628 data points of non-drought class. Therefore, 5,000 data points were randomly selected from each class. A support vector machine (SVM) classifier was then used for the drought/non-drought classification analysis in this report.



**Figure 1**. Pearson's correlation values of all features from (a) drone-based remote sensing and (b) ground cart-based proximal sensing for the drought/non-stress conditions.

**RESULTS AND DISCUSSION:** The Pearson's correlation results are shown in Figure 1, and the threshold for selecting robust predictive features was set to the absolute value of 0.2 to identify more important components contributing to the drought/non-drought classification. The SVM classifier was then trained with the selected robust features under three conditions: drone dataset only, cart dataset only, and combined datasets. The full dataset was split into 70% of training set and 30% of testing set, respectively. As shown in Figure 2, test results revealed that the SVM classifier performed better with the combined multisource datasets (mean accuracy of 0.85 from 20-fold cross-validation) compared to individual source of data (mean accuracies of 0.75 with drone dataset and 0.79 with cart dataset from 20-fold cross-validation). In addition, Figure 2 showed the test accuracies from K-fold (K = 1, 2, ..., 20) cross-validation, providing a clear view of the classifier's consistency in drought/non-drought class prediction. Error bars reflected the standard deviations in each fold of cross-validation. The dashed red line in each subfigure denoted the mean accuracy across all folds, demonstrating a benchmark for overall performance assessment of the SVM classifier. Based on these results, we aim to explore integrating additional multisource datasets from the MultispeQ and agronomy measurements, anticipating further accuracy improvement in classifying the drought and non-drought conditions.



**Figure 2.** K-fold (K = 1, 2, ..., 20) cross-validation classification accuracies (i.e., drought or non-drought class) using a support vector machine (SVM) classifier with the (a) drone dataset only, (b) cart dataset only, and (c) drone and cart combined datasets.

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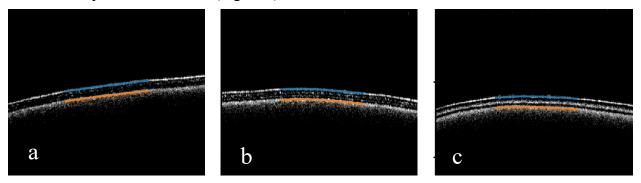
## USING OCT TO MEASURE SEED COAT THICKNESS IN COMMON BEAN

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**INTRODUCTION**: The seed coat is an important factor in germination and imbibition, adaptability to the environment, cooking time and nutrition (Chitwood et al, 2021). The thickness of a bean seed coat can determine its demand due to its effect on these consumer and producer categories and is, therefore, an important factor to investigate. Mesoamerican lines typically have lighter seed weights and smaller seeds and Andean lines are more likely to have heavier weights and larger seeds (Beaver et al., 2015). How these differences play out with respect to seedcoat thickness and how this might impact other characteristics of the beans is not well known. We tested the hypothesis that large-seeded Andean varieties have thicker seed coats compared to the smaller seeded Mesoamerican varieties.

**MATERIALS AND METHODS**: Twenty-two different varieties of common bean were chosen at random, 16 Mesoamerican types from the races Durango and Mesoamerica, and 6 Andean types. The seeds were selected from a short season bean diversity panel at the University of Saskatchewan, and grown and harvested from field plots in the 2021 growing season in Saskatoon. Seed coat thickness was estimated non-destructively using optical coherence tomography (OCT). This method has already been shown to work for lentil seed coats (Nguyen et al. 2021). Ten scans from ten different seeds of each variety were taken using a OQ LabScope 2.0 System (Lumedica Systems, Durham, NC, USA). The mean, median, standard deviation were extracted from each image by calculating the distance between the curves of the outer and inner layer of the seed coat (Figure 1, Nguyen et al. 2021). The mean seed coat thickness for each variety was calculated and distributions plotted in R studio (Figure 2).

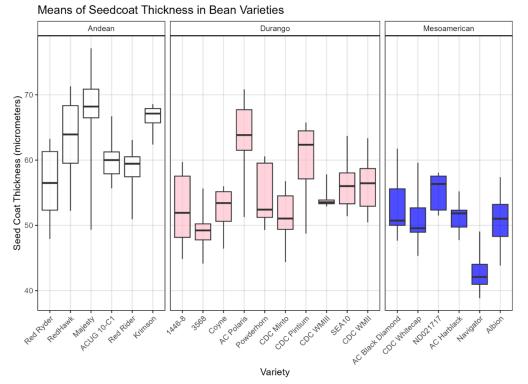


**Figure 1**. OCT-generated images of seed coats of a) ACUT 10-C1, b) ND021717#5, c) Red Rider. Top line of each image represents the outer layer of the seed coat and the bottom is the layer between the seed coat and the cotyledon.

**RESULTS AND DISCUSSION**: The results of this study show that the Andean beans typically have a greater mean seedcoat thickness (62 micrometers) than those of Mesoamerican lines (54 micrometers). The race Durango types were slightly thicker (55 micrometers) than the race Mesoamerican types (51micrometers). One interesting and unexpected occurrence throughout this study was the presence of a third line in the middle of the inner and outer layers of the seed coat

of some varieties. This extra line was more prominent in the cranberry class but also occasionally found in some individual seeds of different varieties across the Andean and Mesoamerican types. Cranberry beans are typically late maturing in the Saskatoon environment so this third line may be an indication of a not-quite fully mature seed coat.

The results from this study supported the hypothesis that Andean beans tend to have thicker seed coats relative to the Mesoamerican lines. OCT is a viable method for calculating seedcoat thickness in the common bean with a little adaption of the procedure for measuring lentils.



**Figure 2**. Mean and range of seed coat thickness from 10 seeds of each variety of common bean measured using OCT.

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## LONGITUDINAL ASSESSMENT OF VARIABILITY COMPREHENSIVE ANALYSIS (YEAR 3 OF 3)

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**INTRODUCTION:** Black beans are one of the most important dry bean market classes in North America. Year after year, there continues to be strong demand both domestically and internationally for high-quality Michigan grown beans. Many of these beans go to the canning market. However, there are significant and unique challenges to dry bean quality after thermal processing for the black bean market class. Black bean color is derived from anthocyanins present in the seed coat; however, anthocyanins can readily leach into the water or brine during processing. This can leave the end-product a light shade of brown or red rather than the deep black color that is desirable. A second measurable trait that is important to processing quality is the overall appearance of the bean (water uptake, splits, and texture). As the industry advances and looks to continue meeting the trend of increased demand, it is essential that we understand processing quality of newly adopted varieties in this market class and how they compare to current market standards. Identification and validation of unique quality characteristics in commercial black beans is essential for the success of the entire dry bean supply chain.

**MATERIALS AND METHODS:** Six black bean varieties were selected for testing: Adams, Black Beard, Nimbus, Spectre, Zenith, and Zorro. Varieties were chosen to represent both commercial standards for processing quality, as well as new varieties that commercial dry bean growers are beginning to adopt based on improved agronomic traits. All six varieties were planted at two separate locations in Michigan in 2020, 2021, and 2022. Dry beans were seeded at 105,000 seeds per acre in a plot size of 6.6'x 24'. This plot size consists of 4-row plots at 20-inch row spacing. Trial design was a randomized complete block design with four replications at all locations. Since differing county locations were used between year for the remainder of this report locations will be referred to as Eastern (Huron and Tuscola) and Western (Bay County). Standard agronomic practices were followed to ensure optimal growing conditions until harvest. Seed was direct harvested utilizing a Wintersteiger Quantum combine in September of all years. Moisture adjusted yield was taken on cleaned seed from each plot and used to calculate yield per acre in pounds at 18% moisture.

Dry bean samples were then canned using a standard research protocol developed at MSU (Wang et. al. 2021). Three field replicates from each variety were used to create individual canning replications for each variety within a location. Hydration coefficients (HC) were calculated as the ratio of sample weight after blanching to the weight of the original dry seed. After processing, cans were allowed to rest and equilibrate for approximately four weeks prior to opening. Upon opening, bean samples were visually evaluated for overall appearance (splits, clumps and color) on a 5-point scale as follows: 5= excellent appearance, 4= very good appearance, 3= average appearance, 2= poor appearance, and 1= unacceptable appearance. Color was visually scored separately on a 5-point scale with 5 = most black and 1= least black canned seed color. A colorimeter, Hunter Labscan XE, was also used for extracting color parameters from drained black bean samples. For this analysis, three measurements of color were considered: L\*, a\* and b\*. In food research, color is frequently represented using the L\*a\*b\* color space to match human perception (Sangwine

2000). L\* is the lightness component that goes from 0 (black) to 100 (white), and parameters, a\* (from green to red), and b\* (from blue to yellow) are the two chromatic components, varying from -120 to +120. The weight of the entire canned sample after draining and rinsing off the brine was also recorded. The ratio of this value to the soaked weight of the sample was determined and is referred to as the washed drained coefficient (WDC). Texture was measured by placing 100 g of each rinsed and drained canned sample into a texture analyzer (model TA-XT, Texture Technologies, Hamilton, MA) with a shear-compression cell attachment. Values are reported as kg per 100 g. The ideal texture readings for black beans are between 55 and 65 kg with higher values indicating firmer beans. Statistical analysis of yield and phenotypic data was conducted in R utilizing analysis of variance procedure (ANOVA). Main effects and interactions were tested for at  $\alpha$ =0.05, when insignificant data were pooled over insignificant factors.

**RESULTS AND DISCUSSION:** Overall yield and first pass quality was average to slightly above average for both locations in all years of testing. Black bean yields were statistically different for multiple interactions between factors. When means were separated for the interaction of most interest (Variety \* Year) little differences existed between varieties in yield potential with the exception of Zorro, such that Zorro trended to produce yields lower than the other five entries tested. When combined over locations, seed weight ranged from 1744-2388 seeds per pound. Most measurements of canned bean quality were statistically significant for the interaction of Variety\* Location\* Year (three-way interaction). This includes hydration coefficients (HC) or the ratio of blanched seed weight (90 second blanch) to the original dry seed weight. Numerically, HC were greatest from Black Beard at 1.36 in the East growing location in 2021. However, all HC ratios ranged between 1.05-1.36 with the varieties of Black Beard, Spectre, and Nimbus grouping in the top statistical group in at least one Location by Year interaction. Black bean color was evaluated by visual ratings by trained evaluators and empirically using a colorimeter. Both methods of evaluation produced a three-way interaction as previously described. This indicates that there is are genetic by environmental effects in the color retention of black beans. When values from the colorimeter were analyzed L\* (darkness) varieties sorted into two general groups. Dark: Zenith and Black Beard (lowest L\* values: 12.9-17.1); Medium: Zorro, Spectre, Nimbus and Adams (16.7-20.6). In a similar response to visual color ratings, appearance ratings were also impacted by the same three-way interaction. Overall, all varieties scored as average (3.0-4.0) in at least one location by year combination. However, except for Zenith, below average scores were also noted in specific locations by year combinations for all varieties. A more detailed report and all data tables can be found in the 2023 Michigan Dry Bean Research report available on-line at Michiganbean.com (https://michiganbean.com/2023-michigan-dry-bean-research-report/)

**CONCLUSIONS:** From 2020-2022 results, it appears that new varieties in the black bean market class have maintained commercially acceptable canning quality while surpassing the older market standard for yield (Zorro). However, when analyzing comprehensive measurements of canning quality, it becomes apparent that high levels of variability exist as a result of the production environment. This is a large challenge for the canning industry when consistent quality/canned bean products are the goal. Future efforts in variety development will need to focus on the *stability of canning traits* across complex environments not only in Michigan, but across North America if we are to make progress on the overall improvement and consistency of canning quality. We would like to thank Bush Brothers Company (Knoxville, Tennessee) and the Michigan Bean Commission for supporting this research.

## WHITE BEANS ARE A PROMISING MARKET CLASS FOR DELIVERING MORE BIOAVAILABLE IRON TO CONSUMERS IN GHANA

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**INTRODUCTION**: The common bean (*Phaseolus vulgaris* L.) is considered a suitable food vehicle for delivering absorbable iron into diets. Although farmed and consumed extensively in many parts of Africa, common beans are not grown in Ghana. Instead, they are sold as imported canned beans and marketed primarily in urban areas. The CSIR-Crops Research Institute has an active bean breeding program in Ghana, which aims to promote the cultivation and consumption of drought-tolerant and iron-rich common beans that deliver more bioavailable iron; particularly in rural communities where the prevalence of iron deficiency remains persistently higher nationwide. There is little information on the iron nutrition of dry beans produced in Ghana, and their ability to provide additional dietary iron for consumers in West African nations where dry beans may not be a traditional staple. Therefore, the goal of this research was to evaluate the possibility of growing common bean genotypes with high seed iron concentration and high iron bioavailability in Ghana.

**MATERIALS AND METHODS**: The common bean test panel included 36 advanced breeding lines adapted to Ghana and 4 officially released varieties from diverse bean market classes, all belonging to the CSIR-CRI germplasm collection (Table 1). Since common bean is not locally grown, two widely consumed commercial cowpea varieties (Tona and Zamzam) were included as closest local checks for each measured trait (Table 1). All the bean / cowpea genotypes were grown in Ghana at the CSIR-CRI research station during the minor rainy season (Sept – Dec) of the 2022 cropping season. The seeds were stored in a cold room  $(18 – 20 \,^{\circ}\text{C})$  for 3 months after harvest and transported (via DHL<sup>®</sup>) to the R.W. Holley Center (Ithaca, New York) for analysis. To measure cooking time, raw bean / cowpea seeds were soaked in distilled water for 12 h prior to determining the number of minutes to reach 80% cooking time with an automated Mattson pin-drop device. Cooked bean / cowpea samples were freeze-dried and milled into powder with an analytical hammer mill for ICP-AES mineral analysis and iron bioavailability, which was assessed by an *in vitro* digestion/Caco2 cell culture model of the human intestinal epithelial barrier, according to the methods described in Glahn, 2022 (Glahn, 2022 JoVE, 182: e63859).

**RESULTS AND DISCUSSION**: Cooking in 10 and 14 minutes, both cowpea varieties cooked 3-6 times faster than all the bean breeding lines or released varieties in the test panel (Table 2). Nonetheless, this research shows that it is possible to grow beans with comparatively higher seed iron content (e.g. cranberry beans) and higher iron bioavailability (e.g. white beans) than the popularly consumed cowpea varieties in Ghana (Table 2). There was no correlation (r = -0.169, P = 0.285) between iron concentration and iron bioavailability of cooked entries. However, the Caco-2 cell culture model revealed that white beans have high iron bioavailability when produced in

Ghana, despite having low iron concentrations (Table 2). Furthermore, fast cooking entries had significantly higher (r = -0.544, P = 0.001) iron bioavailability compared to slower cooking entries. This research demonstrates that to increase iron nutrition from beans, bean breeders should consider targeting specific quality traits and market classes that combine to improve the iron bioavailability of beans after cooking, as shown with the white beans in our panel. Thus, the intended nutritional benefits to bean consumers can be ensured.

Market Class	Entry name
Cowpea	Zamzam (white) and Tona (red)
White	Adoye (released variety), Ennepa (released variety) and 4c-1c-1c-80
Red Mottled	GH-CRI-20
Cranberry	GH-CR4-20, GH-MR23-20, GH-MR26-20, GH-MR43-20, GH-MR60-20, GH-
	MR65-20, GH-RN5-20, GH-RN7-20 and GH-RN10-20
Black	SCN 24
Purple	Jesca
Beige	Semanhyia (released variety), GH-MR18-20, GH-MR34-20, GH-MR48-20, GH-
	MR62-20, GH-MR79-20, GH-RN1-20 and SEF 29
Red	Nsroma (released variety), GH-MR9-20, GH-MR2-20, GH-MR7-20, GH-MR74-
	20, GH-MR11-20, GH-MR13-20, GH-MR14-20, GH-MR17-20, BFS 39, BFS 55, SEF
	44, SEF 17, SEF 47, SEF 55, SEF 60 and SEF 64

**Table 1.** Market classes and descriptions of cowpea and common bean genotypes produced in Ghana<sup>1</sup>

<sup>1</sup>Common bean genotype entries are grouped into seven distinct bean market classes based on seed coat colour. Descriptions are based on names of cowpea / common bean genotypes at the CSIR-CRI.

	Number of	Cooking	Uncooked bean	Cooked bean	Iron bioavailability <sup>2</sup>
	Entries	time	iron <sup>1</sup>	iron <sup>1</sup>	
Market Class		(min)	(µg)	(µg)	(% of white bean control)
Cowpea (white)	1	10	47.06	46.78	89.90
Cowpea (red)	1	14	58.64	59.22	59.18
White	3	38	56.85	58.66	123.93
Red Mottled	1	42	56.22	53.78	20.52
Cranberry	9	59	68.25	66.51	21.16
Black	1	61	58.19	60.88	8.28
Purple	1	61	47.57	52.96	50.19
Beige	8	66	60.00	62.75	29.69
Red	17	67	60.81	61.70	33.34

**Table 2.** Mean cooking time, iron concentration and iron bioavailability of cowpea and common beans produced in Ghana

<sup>1</sup>Mean concentration of iron is expressed as micrograms per gram of a lyophilized/milled powder representing a homogenous mixture of either 50 cooked or 25 uncooked seed for all genotypes in each market class. <sup>2</sup>Mean iron bioavailability in cooked lyophilized/milled whole seed expressed as percentage of in vitro Caco-2 cell ferritin formation (ng ferritin / mg total cell protein) relative to a commercial white kidney bean (cv. Snowdon) from the United States, which is used as reference standard with each bioassay. Caco-2 cell ferritin values for Snowdon averaged 10.06 ng ferritin / mg total cell protein. Iron bioavailability values that are  $\geq$  75% of Snowdon are considered to have potential for high iron bioavailability.

#### ENHANCING THE IRON NUTRITION OF BEANS: UTILIZING THE SLOW AND NON-DARKENING TRAITS TO REDUCE PROANTHOCYANIDINS IN MULTIPLE MARKET CLASSES OF DRY BEANS

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**INTRODUCTION**: Reducing proanthocyanidin concentrations (inhibitors of iron absorption) in bean seed coats via alleles in the non-darkening (j gene) or slow darkening (P<sup>sd</sup>) gene will enhance iron (Fe) bioavailability across multiple market (color) classes of dry beans.

**MATERIALS AND METHODS:** Dry beans (*Phaseolus vulgaris* L.) with slow-darkening (SD), non-darkening (ND), and regular darkening (RD) traits were grown at research sites in North America (U.S. and Canada) and Africa (Zambia). The Caco-2 cell culture bioassay was used to determine the iron bioavailability of cooked beans. Mineral analysis was conducted by ICP-ES and phytate measured with a Megazyme<sup>TM</sup> kit. Flavonoids (>25 compounds) were measured using a newly developed Acetone-Methanol (acidified) extraction methodology before analysis with UPLC/MS.

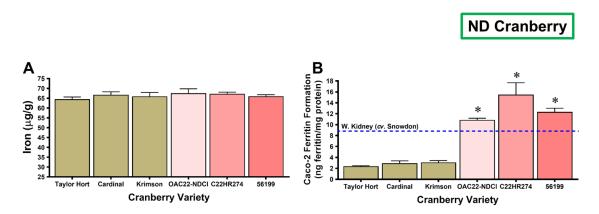
#### **RESULTS AND DISCUSSION: SD**

pinto beans from North Dakota provide 2-7x more bioavailable iron than RD pinto varieties. Iron and phytate concentrations were similar between SD and RD pinto beans, however, flavonoid analysis revealed 4x more proanthocyanidins were detected in RD pintos after cooking. Similar findings were demonstrated in ND yellow, cranberry and purple beans produced in North America and Africa. The absence or low levels of proanthocyanidins in the seed coats of ND beans correlated with 5-7x more bioavailable iron than RD beans. The greatest enhancement was observed in the yellow bean market class with a ND variety exhibiting 200% of a white

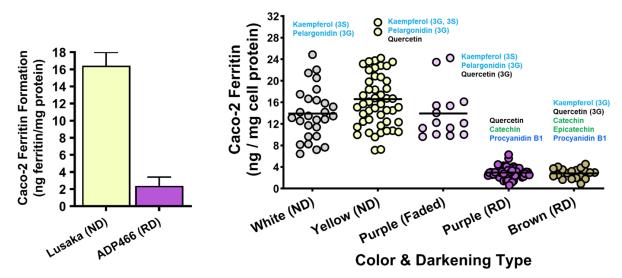


**Figure 1.** Iron bioavailability (A) and flavonoid compositions (B) of 50 dry bean varieties in the Historic Bean Collection produced in Hatton, North Dakota. Each market class is represented by 3 - 6 different varieties, each measured in triplicate. Hyphenated lines indicate the average iron bioavailability of a white kidney bean reference control run with each Caco2 bioassay.

kidney bean reference control. There was no relationship between iron bioavailability, phytate or iron concentrations in cooked beans. However, there was a strong (P < 0.001) association between iron bioavailability and proanthocyanidins across all market classes.



**Figure 2.** Iron concentrations (A) and iron bioavailability (B) of regular darkening (RD) and slow darkening (ND) cranberry beans produced in North America. Values are the means (SD) of three replicate samples each measured in triplicate (n = 6).



**Figure 3.** Bar graphs of parental lines Lusaka (yellow bean) and ADP466 (Kabulangeti, purple bean) comparing iron bioavailability of non-darkening (ND) and regular darkening (RD) RILs produced from a cross between Lusaka (yellow) and ADP466 (kabulangeti). Iron bioavailability is measured as Caco-2 cell ferritin formation (ng ferritin / mg total cell protein) after exposure to an in vitro digestion of cooked, drained, lyophilized and milled beans. Flavonoid concentrations were measured in cooked, drained, lyophilized and milled beans (dry weight).

**CONCLUSIONS**: In addition to a brighter seed coat appearance after storage, this study reveals that downregulating the synthesis of proanthocyanidins with the slow darkening  $(P^{sd})$  or non-darkening (j) gene could be a novel and a sustainable strategy to improve the iron bioavailability of dry beans, especially those susceptible to darkening, including yellow, pinto, purple and cranberry beans.

**ACKNOWLEDGEMENTS:** Funding from the U.S. Department of Agriculture, Agricultural Research Service.

## WATER UPTAKE PATTERNS AND HARD-TO-COOK PHENOMENA OF BEAN VARIETIES AND ADVANCED LINES BRED IN EASTERN AFRICA

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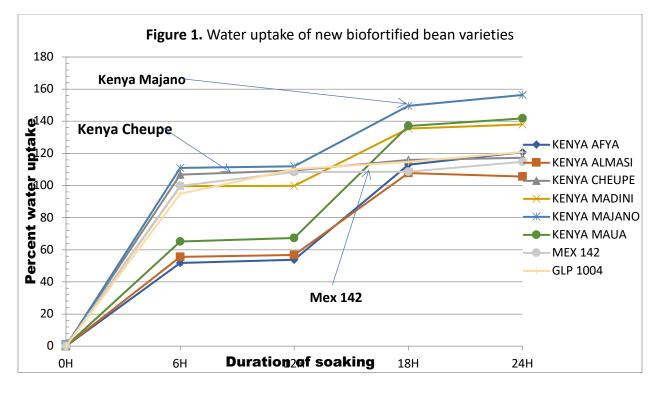
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**INTRODUCTION:** Rapid water uptake is a critical aspect for fast cooking pulses and a requirement for beans destined for the processing industry. Slow water uptake is associated with hard-to-cook and hard-shell defects. Beans with less than 90% water uptake after 16 hours of soaking are considered unsuitable for processing. Hydration is important to optimize the cooking parameters and to obtain a product that presents desired moisture content, texture, and taste (Abu-Ghannam, 1998; Zhang and McCarthy, 2013). Several studies indicated that bean cultivars with high water absorption capacity are fast cooking (Elia et al., 1997; Boros and Wawer, 2003; Castellanos and Guzntán-Maldonado, 1994). Water absorption patterns of commercial cultivars grown in eastern Africa, and its relationship with cooking time is unknown. The objective of this study was to determine prevalence of hard-to-cook phenomena and water uptake patterns of commercial cultivars of diverse market classes, recently released biofortified varieties types and advanced canning bean lines developed in Kenya.

MATERIALS AND METHODS: Study materials were 10 commercial varieties, seven recently developed biofortified cultivars, and 17 advanced lines representing the Andean and Mesoamerican gene pools and the major market classes grown in East, Central and Southern Africa. They included seven red mottled, six red kidney, four speckled sugar, seven navy, five small red, and five pinto, carioca and purple genotypes. Water absorption was determined by weighing and soaking five seeds in 25ml of distilled water for 6, 12, 18, and 24 hours, at a room temperature (25  $^{0}C$  +/- 2). Water absorption was expressed as the percent weight gained by beans on dry weight basis, according to equation: A= [(soaked weight-dry weight)/dry weight] \*100. The experiment was replicated three times. To determine hard-shell defect, seed samples stored for five months to simulate seed hardening defect. Seeds were cleaned manually for any damage and foreign material. Duplicate 100-seed samples were counted and soaked for 16 hours in tap water and the seeds were visually verified for water absorption. Seeds that did not absorb water were counted. The hard-shell seed percentage was expressed as a ratio of grains that did not absorb water after soaking in relation to the total number (Corrêa et al., 2010). Cooking was determined using a Mattson cooker following procedures described by Jackson and Marston (1981). Data was subjected to analysis of variance using Genstat software (version 15). Fisher's protected least significant difference was used for mean separation.

**RESULTS AND DISCUSSION:** Analysis of variance showed that there were significant differences in water uptake among the soaking periods. Mean water uptake for all genotypes increased from 0 to 94.6 % after 6 hours of soaking. Water uptake further increased to 110.2% after 12 h, 123.6 % after 18h and 129.7% after 24h. This implied that the rate of water uptake was highest during the first six hours of soaking, resulting to an increase of nearly 100% in volume of beans. However, rate of water uptake decreased for the next 6 h showing an increase of 2.6% per hour, compared with 15.8% per hour during the first 6 hours. Rate of water uptake was 2.2% per

hour between 12 and 18 hours of soaking and further decreased to 1% per hour between 18 and 24h of soaking. This implied that most genotypes had reached their maximum water absorption capacities after 18h. These results suggest that the first six hours were the most critical for water uptake. Results showed that there were highly significant (P<0.001) time and genotypic differences among the study genotypes. Differences were most conspicuous after 6 and 12 hours of soaking (Fig.1). Among the biofortified bean varieties, water uptake after 12h varied from 53.7% (Kenya Afya) to 109.4% (Kenya Cheupe). However, after 18h, all the biofortifed varieties had more than 100% water uptake. In contrast, except for BCB 1-245, all the new advanced lines had exceeded 100% after only 12 hours of soaking. For these lines, water uptake varied from 104.7% (KCB13-02) to 154.9% (KCB13-10). Correlation between soakability and cooking time varied from r=0.03 for speckled sugar, to r=-0.82\*\* for red kidney genotypes. The highest percentages of hard-shell seeds were recorded in small red and mixed-colour market classes, while the lowest was found in red mottled and speckled sugar lines. Except for red mottled and speckled sugar genotypes, highly significant correlation (r= $0.661^{**}$  to  $0.924^{**}$ ) was found between water absorption and hard-shell. About 30 lines from different market classes with fast-cooking (<35 minutes), high water-holding capacity (>90%) and zero percent hard-shell seeds were identified. These results indicated that most of the new lines had improved water uptake, cooking time and met criteria for processing compared with existing commercial varieties and industry reference variety, Mex 142.



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## **IMBIBITION AND GERMINATION IN P. COCCINEUS L. SEEDS**

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**INTRODUCTION:** Seed germination begins with imbibition and is divided in three phases: first, cells are hydrated and physiological activities can begin before tissues are fully hydrated; second, water content is constant, metabolic activity increases with transcription of new genes and the radicle emerges through the surrounding structures; third, water uptake occurs as the seedling establishes itself using increased stored reserves (Bewley et al., 2006). The hypothesis proposed is the existence of differences in the time and percentage of imbibition in germinated seeds of domesticated and wild ayocotes.

MATERIALS AND METHODS: Three Mexican native varieties from: Zitlaltepetl, Tlax., Nombre de Dios, Dgo. and Tlayacapan, Mor.; the Blanco Tlaxcala improved variety, and two wild forms collected in Ocotitlan, Mor., and Tetzcutzinco, Méx. The 12 seeds from each material were sterilized with 5% hypochlorite, placed between a layer of Alstrom No. 541 filter paper and a layer of absorbent paper in 9 cm diameter Petri dishes. They were hydrated with 10 mL of distilled water and kept at a constant temperature of 25°C in an ESCO Isotherm incubator until the radicle emerged through the testa. The sowing date was July 19 and the imbibition seed weight was recorded every four hours from sowing to visible germination on a Sciencetech analytical balance model SA 120. A total of 72 seeds were used in six Petri dishes. The percentage of imbibition was calculated with the formula:

(Fresh weight 2-fresh weight 1)/(Fresh weight 2)\*100, where: Fresh weight 1 =first weight of imbibed seed and Fresh weight 2= second weight of imbibed seed. The time to determine how long germination takes to complete was determined when, after the seed had been imbibing, the radicle of the embryonic axis breaks the testa and is visible to the naked eye. The experiment design was completely randomized. The experimental unit was each germinated seed. Independence, normality and homogeneity of variance were verified; when these assumptions were not met, the variables were transformed using a log transformation, subjected to ANDEVAS and Tukey's mean comparison tests ( $P \le 0.05$ ) with the SAS statistical package (SAS, 2012), and graphed with Sigma Plot 14.0 software (Systat, 2017).

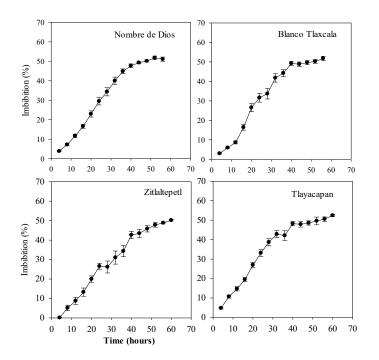
nbibition (%) of four	domesticated	and two w	'ild Phaseolus co	<u>occineus L</u>
Ayocote	Time (h)	SD	% Imbibition	SD
Nombre de Dios	50.90 b	10.9	51.95 a	2.7
Blanco Tlaxcala	53.90 b	14.4	52.15 a	2.3
Zitlaltepetl	66.66 b	20.9	51.18 a	1.8
Tlayacapan	74.91 b	14.7	53.57 a	3.2
Ocotitlan	240.0 a	110.3	28.26 b	13.7
Teztcutzingo	340.0 a	150.8	28.14 b	13.6
	Ayocote Nombre de Dios Blanco Tlaxcala Zitlaltepetl Tlayacapan Ocotitlan	AyocoteTime (h)Nombre de Dios50.90 bBlanco Tlaxcala53.90 bZitlaltepetl66.66 bTlayacapan74.91 bOcotitlan240.0 a	AyocoteTime (h)SDNombre de Dios50.90 b10.9Blanco Tlaxcala53.90 b14.4Zitlaltepetl66.66 b20.9Tlayacapan74.91 b14.7Ocotitlan240.0 a110.3	Nombre de Dios50.90 b10.951.95 aBlanco Tlaxcala53.90 b14.452.15 aZitlaltepetl66.66 b20.951.18 aTlayacapan74.91 b14.753.57 aOcotitlan240.0 a110.328.26 b

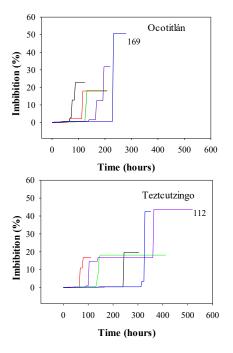
Table 1. Seed imbibition	%) of four domesticated and two wild Phaseolus	s coccineus L.
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Different letters indicate difference between avocotes (Tukey 0.05). has=hours after sowing, SD=standard deviation.

**RESULTS AND DISCUSSION:** Domesticated seeds started imbibition 12 hours after sowing, and the wild ones still had no imbibition. At 52 hours after sowing, the imbibition in the domesticated seeds was about 50 % and it was just starting in the wild seeds.

<u>Domesticated ayocotes</u> were homogeneous in the number of hours and imbibition percentage required to complete germination (Fig. 1). But the <u>wild</u> ones were heterogeneous, and only five of the 12 wild seeds of each material completed germination: three seeds had similar number of hours and percentage of imbibition, and two required more time and had higher percentage of imbibition to germinate. Variation in time and percentage of imbibition in the wild collections was evident in seed 112 (Teztcutzingo) with 520h to germination and 44 percent imbibition; and seed 169 (Ocotitlan) with 232 h to germination and 51 percent imbibition (Figs 1 and 2).





**Figure 1**. Percentage of seed imbibition from sowing to visible germination of native *Phaseolus coccineus* L. varieties.

**Figure 2**. Percentage of seed imbibition from sowing to visible germination of two wild collections of *Phaseolus coccineus* L.

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## LOCI MAPPED TO Pv04 AND Pv01 ARE REQUIRED FOR ROUND POD SHAPE IN SNAP BEAN (*PHASEOLUS VULGARIS*)

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**ABSTRACT:** Wild and domesticated dry bean pods have an oval cross-section and strong pod wall fiber deposition, while snap beans typically have round cross section and lack wall fiber. The two traits co-segregate and may be controlled pleiotropically. Intriguingly, snap beans frequently revert to the ancestral condition for both traits simultaneously, creating a commercial issue with an unresolved genetic basis. Here, we used a dry bean x snap bean RIL population to identify two loci, on chromosomes Pv04 and Pv01, both required for a round pod shape. The most significant SNP was in the promoter of the HD-ZIP IV gene model *Phvul.004G143500*, with homologs involved in floral patterning and fiber deposition. Candidate genes on Pv01 involved in similar pathways have also been identified. Our results provide insight into the inheritance of a commercial trait and will help determine the source of its instability.

**INTRODUCTION:** Oval pod cross-section shape and extensive wall fiber are the ancestral conditions in *Phaseolus*. Pod wall fiber is critical for pod shattering and seed dispersal in the wild and is required for the efficient threshing of dry beans. In snap beans (green beans), however, this woody fiber deposition and the accompanying oval shape are extremely undesirable. Along with pod strings, these form the major phenotypic distinctions between snap beans and dry beans. Pod shape and wall fiber frequently revert, at a rate of approximately 0.5% to 2.25% (1), and these reversions typically happen together, implying pleiotropic control. Between one and three classically-described genes have been implicated in pod wall fiber formation [reviewed in (2)]. Hagerty et al. (1) identified a single Pv04 locus implicated in regulating both pod shape and wall fiber. Subsequently, mapping of pod morphology in the SnAP population identified a major QTN on Pv04, with numerous other SNPs of limited effect across all 11 chromosomes (3). In a population segregating for flat and oval pods (without round types), pod cross-sectional shape was mapped to Pv01 and Pv06 (4). To date, the loci and gene models governing pod shape remain elusive.

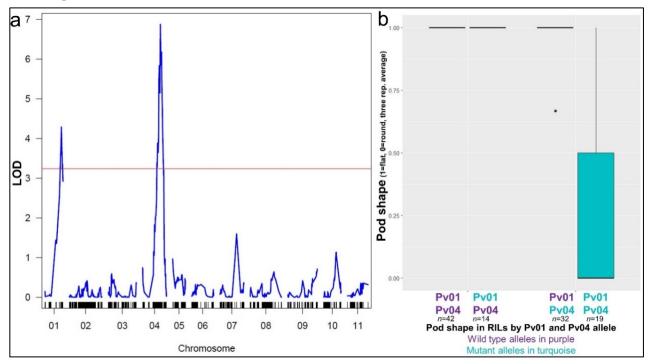
**MATERIALS AND METHODS:** A 110-member  $F_{6:7}$  recombinant inbred population was developed by hybridizing 'A195' (flat-podded dry bean, with wall fiber) and 'OSU 6137' (round podded snap bean, lacking wall fiber). The RIL population was field-grown in Corvallis, Oregon, USA in three replicate plots. For each plot, pods were scored as either round or flat. Genotyping was conducted according to Arkwazee (5). DNA was extracted from leaves of each  $F_6$  RIL. Library preparation for genotyping-by-sequencing used the *Ape*KI enzyme. Tassel5 was used for SNP data generation and Beagle4.1 was used for imputation. The ASMap R package was used for linkage mapping and QTL mapping was conducted with 5,678 markers in rQTL using the Haley-Knott method and scanone() and scantwo() functions.

**RESULTS AND DISCUSSION:** Two major QTLs were identified for the control of pod shape (Fig. 1). On Pv01, the QTL interval ranged from 50,184,110 (scantwo() LOD=18.1,  $R^2$ =0.349;

coordinates based on G19833 v2.1) to 50,479,138 bp, with several collocated SNPs between these. On Pv04, the most significant SNP was at 44,419,468 bp (scantwo() LOD=23.8  $R^2$ =0.529) with flanking markers at 44,157,994 and 44,563,598 bp. The Pv01 x Pv04 interaction was highly significant (P=4\*10<sup>-15</sup>,  $R^2$ =0.246), indicating epistasis between the loci. The snap bean allele is required at both loci for round pods (Fig. 1b).

The genetic mapping of two epistatically-acting genes for round pod shape is unique to this study. Our most significant Pv04 SNP is found in the promoter of Phvul.004G143500, a HD-ZIP IV gene previously considered a candidate for pod shape regulation (1, 3). HD-ZIP IV genes in Arabidopsis affect floral development and suppress fiber formation (6). Candidate genes on Pv01 include *Phvul.001G253101*, an *EPIDERMAL PATTERNING 1-LIKE* ortholog, and *Phvul.001G254000*, an HD-ZIP I gene. *Phvul.004G143500* and *Phvul.001G253101* are involved in specifying dermal cell fate, and may achieve their effects by over-specifying superficial and dermal tissue identity into deep structures such as the fiber layer (6). Future analyses could test these hypotheses through gene expression studies and sequencing of diverse and revertant lines.

**Figure 1.** Two genes govern pod shape differences between snap and dry bean. A) A single QTL scan of pod shape in the A195/OSU 6137 population shows significant QTLs on chromosomes Pv01 and Pv04. Candidate genes have been identified at each locus, including transcription factors governing internal vs. external floral tissue identity. B) Boxplot showing snap bean alleles are mandatory at both genes to achieve round shape.



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### VALIDATION OF IMPROVED BEAN LINES BASED ON GRAIN QUALITY UNDER IRRIGATION CONDITIONS

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**INTRODUCTION:** The common bean (*Phaseolus vulgaris* L.) is one of the cultivated species of high importance in Northern Mexico. In the state of Durango, in the period between 2018 and 2022, an average of 208,000 hectares were planted with beans annually and 112,000 tons of grain were produced annually (SIAP, 2023), with only 1,875 hectares cultivated under irrigated conditions and the rest was planted in rainfed conditions. During the same period, the average yield obtained under rainfed conditions was 376 kg ha<sup>-1</sup>, while under irrigation it was 1,588 kg ha<sup>-</sup> <sup>1</sup>. The bean crop faces different problems during its cultivation, such as water stress, temperature variations and limited soil fertility, as well as diseases caused by different pathogenic organisms and damage caused by various pest insects. A strategy to solve these productive problems includes genetic improvement and adjustment of the agronomic management of the crop. Durango is a leader in the generation of bean varieties with a high impact from a productive and commercial point of view. Outstanding results have been shown in terms of increasing the productivity and quality of bean grain obtained in the state. Furthermore, the generation of varieties is considered a profitable activity, since varieties have been developed with high value for economic variables, such as the benefit/cost balance, net present value (NPV) and IRR (internal rate of return). The objective was to validate a group of four improved bean lines with possibilities for registration as high commercial quality varieties in Durango.

**MATERIALS AND METHODS**: A group of four improved bean lines with different commercial types of grain were planted on July 10th, 2023: pinto (PT, 2) and opaque black (NGO, 2) in the State of Durango, which were compared with commercial controls (PID 1 and NOD 1) and two improved varieties (Pinto Saltillo and Negro San Luis).

The trial was planted to determine the adaptation to the normal planting period in Durango (spring-summer cycle). A completely random design was used (paired strips), with an experimental plot of four, 50 m long rows and a useful plot of two 5 m long rows, 0.81 m apart (8.1 m2) and five replications. It was fertilized at the time of the first weeding with the dose 35-50-00 for nitrogen (N), phosphorus (P<sub>2</sub>O<sub>5</sub>) and potassium (K<sub>2</sub>O) (INIFAP, 2017). Six applications of insecticide were made (one of spinetoram Palgus® and two of dimethoate: Danapyr®) to control the mexican bean beetle (*Epilachna varivestis*) and pod weevil (*Apion spp.*). Three relief irrigations were applied to avoid water stress in the bean plants. The yield, weight of 100 seeds, crude protein content and ash were evaluated, the last two according to the methodology reported by AOAC (1990). The data obtained was analyzed in a completely randomized design with five repetitions for the field variables and two repetitions for the laboratory variables. The comparison of means was carried out with the Tukey test ( $p \le 0.05$ ).

**RESULTS AND DISCUSSION**: No statistical differences were observed for grain yield, the late variety Negro San Luis (3,370 kg/ha) and the lines PT14053 (3,270 kg/ha), NGO14013 (3,247 kg/ha) and PT14055 (3,010 kg/ha) (Table 1). Highly significant differences were observed for the weight of 100 seeds. The highest average weight was observed for PT14053 (40.0 g/100 seeds), which was statistically equal to the Negro San Luis control (39.9 g/100 seeds) and the lines PT14055 (39.7 g/100 seeds) and the PID 1 control (38.1 g/100 seeds). The lines with opaque black grain showed low values for grain weight, although this was high based on what is required for its commercial class (25 g/100 seeds). In the content of crude protein and ash, no statistical differences were observed; with a trial average for crude protein of 24.48% and ash of 5.08%. These values agree with those reported by Rosales-Serna et al. (2019) for lines and varieties of the same type of grain.

Line/	Yield	<sup>1</sup> P100S (g)	Crude protein	Ash (%)
Variety	kg/ha		(%)	
<sup>2</sup> NGO14014	2,847	34.3 <sup>b</sup>	24.44	5.17
PT14053	3,270	$40.0^{\mathrm{a}}$	24.32	5.17
NGO14013	3,247	33.1 <sup>b</sup>	23.44	5.20
PT14055	3,010	39.7 <sup>a</sup>	23.49	5.22
NOD 1	2,755	29.4°	24.34	5.04
PID 1	2,616	38.1ª	25.60	5.25
Pinto Saltillo	2,723	35.3 <sup>b</sup>	25.00	4.61
Negro San Luis	3,370	39.9 <sup>a</sup>	25.24	5.00
General average	2,980	36.2	24.48	5.08

**Table 1.** Yield and grain quality traits in bean lines and varieties grown under irrigation in Durango, Mexico.

<sup>1</sup>P100S= weight of 100 seeds. <sup>2</sup>NGO = small opaque black, PT = pinto, NOD = opaque black developed in Durango and PID = pinto developed in Durango. <sup>a-b</sup>Different letters in the column denote significant differences based on Tukey ( $p \le 0.05$ ).

**CONCLUSIONS**: It was possible to select outstanding improved lines, similar to the commercial controls Pinto Saltillo and Negro San Luis. The seeds were larger in size compared to the Pinto Saltillo control, which can improve the commercial value and facilitate the marketing of the beans produced in Durango. The evaluated lines did not present differences with respect to the control varieties in terms of crude protein and ash content, in consequence it is possible that these lines could be selected for the generation of improved bean varieties with high productivity and commercial value in Mexico.

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# NUTRITIONAL QUALITY OF BEANS FROM DIFFERENT COLLECTIONS IN DURANGO, MEXICO

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**INTRODUCTION:** The common bean (*Phaseolus vulgaris* L.) is one of the most important cultivated species in the state of Durango, which is considered one of the main centers of genetic diversity and possible domestication of the species (INAH, 2023). The increase in the area planted with improved varieties of beans, such as Pinto Villa and Pinto Saltillo, has caused a loss of diversity of the species in Durango. It is less and less common to see traditional native varieties, such as the national pinto, negro bola (Negro San Luis), bayo rata, bayo blanco, canario (chickpea), flor de mayo and ojo de cabra, which were cultivated for years by the producers of the state. Different observation and collection points of wild bean germplasm have been recorded in this state (Debouck, 1979; Cárdenas et al., 1996; Wallander et al., 2022). Therefore, the objective was to compare the protein and ash content among ten collections of wild bean, eight weedy bean collections, four criollo beans varieties and four improved bean varieties in order to obtain options of bean crop varieties for producers and to match the quality of those commonly planted.

**MATERIALS AND METHODS:** The experimental site was located in the municipality of Durango, at 23° 59' 15" N, 104° 37' 17" W and an altitude of 1,879 m. Seeds were collected of individual plants obtained at random from wild populations of beans growing naturally in the area near the archaeological site of La Ferrería, Dgo., and on the edge of the Tunal River. It was possible to differentiate between collections of wild germplasm (10) and intermediate forms (8) of beans; In addition, four criollo varieties, four improved varieties and one variety of patol (*Phaseolus coccineus* L.) were included as references. Planting was carried out on July 10th, 2023 and the agronomic management recommended in Durango for bean cultivation was applied (INIFAP, 2013). The dependent variables were protein and ash content, which were determined using the methodology proposed by AOAC, 1990. A completely randomized design with factorial arrangement was carried out. An ANOVA and a means comparison by Tukey test ( $\alpha$ = 0.05) was performed in the SAS statistical package.

**RESULTS AND DISCUSSION:** Highly significant differences (p < 0.01) were found between the different groups for crude protein content and statistical equality for ash content. In the improved varieties, crude protein was higher (26.96 %), followed by wild (25.89 %) and intermediate (25.61 %), while native varieties had the lowest value (24.13 %). The average of the criollo varieties was similar to that reported by Solano-Cervantes et al. (2009) for criollo varieties of black beans (23.57%) and white bean varieties (24.68%) from the state of Guerrero. Ash content ranged from 4.78 to 5.19 % (Table 1). Ash content ranged from 4.78 to 5.19 %. These results agree with those reported by Rosales-Serna et al. (2019) for improved bean lines.

**Table 1**. Average values of the ANOVA for protein and ash content in grain of wild bean collections grown in Durango according to their status.

Status	Crude Protein (%)	Ashes (%)
<sup>1</sup> Landrace	24.13 <sup>b</sup>	5.19
Improved	26.96 ª	5.05
Weedy	25.61 <sup>ab</sup>	4.86
Wild	25.89 <sup>ab</sup>	4.78

<sup>1</sup>Landrace = Criollo; Improved = enhanced; Weedy = Intermediate; Wild = Silvestres

**CONCLUSIONS:** The protein content of the varieties evaluated in this work was influenced by their status, the wild and intermediate varieties can be matched to improved varieties, while ash content showed the same behavior regardless of variety status.

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## STARCH GRANULES IN COTYLEDON OF DOMESTICATED AND WILD GERMINATING SEEDS OF *PHASEOLUS VULGARIS* L.

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**INTRODUCTION:** In developing seeds of domesticated *P. vulgaris,* starch granules are formed in an elongation and a thickening stage, and in mature seeds, are elliptical or spherical in shape and measure between 33 and 10  $\mu$ m (Nazaki *et al.*, 2002). Wani, cited by Punia et al., (2020), using scanning electron microscopy, indicated that starch granules in a domesticated bean have a 6-32 width range and a 8-42 $\mu$ m length range. We counted and measured the starch grains of germinated seeds in domesticated and wild *P. vulgaris* L. to learn if modifications at the anatomical level occurred as a result of the domestication process.

**MATERIALS AND METHODS**: Three improved varieties: OTI, race Mesoamerica; Cacahuate-72, race Nueva Granada; and Canario-G15, race Jalisco, and three wild forms were collected in: Tepoztlán, Mor. Cholula, Pue. and Arcelia, Gro., Mexico. Four seeds of each variety were disinfected, scarified with a scalpel on the opposite side of the hilum, placed in 9 cm diameter Petri dishes with filter paper discs, moistened with distilled water and kept at a constant temperature of 25 °C. Once the radicle was visible in each seed, four cross-sectional cuts were made in the center of the cotyledon. The sections were fixed in FAA, gradually dehydrated in alcohol, embedded and infiltrated in paraffin in an automatic tissue exchanger (TISSUE-TEK II). The sections were made with a microtome (ERMA INC) at a thickness of 10 microns. They were then dyed with peryodic acid and Schiff's reagent (Johansen, 1940). The number of cells and starch granules was determined in 40x fields under an optical microscope (Zeiss, model Axioscope 2), and the number per mm<sup>2</sup> was determined. Images were obtained with a digital camera (Amscope) and the length, diameter and area of each starch granule were estimated with Image J software. The attributes of the starch grains were examined with an ANOVA, and a comparison test of Tukey means (0.05) (SAS, 2012).

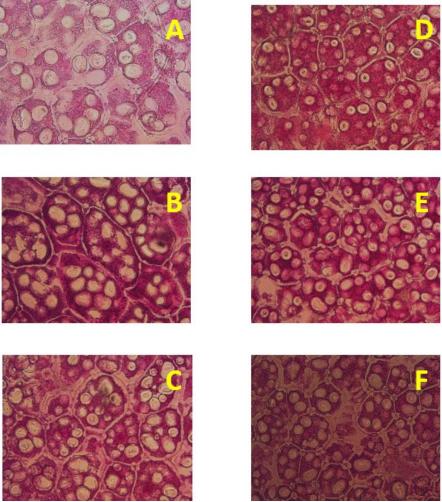
**RESULTS AND DISCUSSION:** Cotyledons from wild bean seeds had 1.7 times more cells and 1.4 times more starch granules per mm<sup>2</sup> than cotyledons from seeds of domesticated varieties. Starch granules from seed cotyledons of domesticated varieties were, however, 1.18 times longer and 1.15 times wider than cotyledon granules from wild bean seeds. In the germination process, anatomical changes occurred in the cotyledon cells and starch granules of *P. vulgaris*. The size of starch granules from domesticated materials match the range reported by Wani (Punia *et al.*, 2020) for kidney beans.

Ш	improved and wind forms of <i>P. vulgaris</i> L.								
	Number mm <sup>-1</sup>			m <sup>-1</sup>	Starch granules				
	Form	Name	Cells	Starch	Length µ	Diameter µ			
_				granules					
	Domesticated	OTI	1246 c	3476 c	9.4 a	7.8 a			

**Table 1**. Cell number and number and size of starch granules in germinated seed cotyledons of improved and wild forms of *P. vulgaris* L.

	Cacahuate-72	1224 c	4090 bc	8.2 b	6.2 bc
	Canario G-15	1924 b	6231 a	7.6 b	6.2 bc
Wild	Tepoztlán	2536 a	6056 ab	7.4 bc	6.0 bc
	Cholula	2121 ab	6035 ab	6.3 c	5.2 c
	Arcelia	2915 ab	7674 a	7.7 b	6.3 b
Domesticated	Mean	1465 b	4599 b	8.4 a	6.7 a
Wild		2524 a	6589 a	7.1 b	5.8 b

Data are the mean of 12 domesticated and 12 wild materials. Different letters in columns indicate significant difference (Tukey  $\leq 0.05$ ).



**Figure 1**. Starch granules in cotyledon cells of visibly germinating seeds (1-2 mm visible radicle) of domesticated beans: A, B and C) varieties OTI, Cacahuate 72 and Canario G-15; and wild beans: D, E and F) Cholula, Arcelia and Tepoztlán.

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### NOTICE OF NAMING AND RELEASE OF PID 2, A PINTO CULTIVAR WITH HIGH YIELD AND ENHANCED GRAIN QUALITY

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**INTRODUCTION:** The Valle del Guadiana Experiment Station of the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP; National Research Institute for Forestry, Agriculture and Livestock) announces the naming and release of PID 2, a new pinto common bean cultivar. This new common bean cultivar has an indeterminate semi-erect growth habit (type III) and is considered a well-adapted and high yielding cultivar for irrigated and rainfed conditions in the highlands of México, where it has proven to be resistant to diseases, especially anthracnose [*Colletotrichum lindemuthianum* (Sacc. & Magnus) Lambs. Scrib.] and rust [*Uromyces appendiculatus* (Pers.) Unger. var. *appendiculatus*].

**MATERIALS AND METHODS**: PID 2, which was tested as the improved line PT14053, originates from a multiple cross (PTBayacora/Maverick///PTClaro/PTSaltillo// PTSaltillo/PTVilla-2-6) made in 2010. The cross was developed to increase the seed size of the cultivar Pinto Saltillo, which is used as a recurrent parent in crossing blocks.

Pinto Saltillo is a mid-season pinto bean cultivar with indeterminate growth habit (type III) adapted to the highlands of México. Despite its popularity for commercial cultivation in Durango, Chihuahua and Zacatecas, the seed size of Pinto Saltillo (30-34 g/100 seeds; Sánchez et al., 2009) has been classified as medium (CIAT, 1987), which limits its acceptance in national and international markets, where larger seeds (> 35 g/100 seeds) are preferred. Pinto Bayacora is an erect, indeterminate (type III) cultivar that was also developed at INIFAP's Valle del Guadiana Experiment Station. It was considered as a gene source for higher seed size (37-46 g/100 seeds) and also shows resistance to anthracnose and rust. Pinto Villa was included as a parent showing wide adaptability, phenological plasticity and drought resistance. The pinto cultivars Maverik and Claro were included to increase seed size, maintain an erect canopy, and strengthen the slow darkening traits of the coat in the resulting improved lines.

In 2011, the  $F_1$  plants were bulk advanced under field conditions in the State of Durango, located in the semi-arid highlands of México. In 2012, the  $F_2$  populations were sown directly in the field in Durango and individual  $F_{2:3}$  plants were selected based on plant vigor, pod load, disease resistance and grain quality (seed size and color). During 2013, the  $F_{3:4}$  families were bulk advanced at the Valle del Guadiana Experiment Station and individual plants were selected in the F4 generation based on disease reaction, earliness, plant vigor and commercial grain quality (seed size, color, and shape).

**RESULTS AND DISCUSSION**: In 2014, a uniform population was coded as an improved line (PT14053) that was extensively tested under irrigation in the State of Durango (trials were conducted at locations above an elevation of 1,800 m). PID 2 was selected for its yield performance and agronomic traits at 17 locations from 2014 to 2023. In the experimental plots under irrigated conditions, PID 2 recorded high yields averaging 2,783 kg/ha, with variations between 1,161 kg/ha

to 5,365 kg/ha. PID 2 grown in semi-commercial plots achieved an average yield of 2,783 kg/ha and outperformed the local control Pinto Saltillo (2,509 kg/ha) by 10%. The seed size of PID 2 averages 36 g/100 seeds, and the highest value was registered in 2017 with 43 g/100 seeds, compared to Pinto Saltillo (33 g/100 seeds).

PID 2 has an intermediate growth cycle (matures 108 days after sowing), is photoperiod sensitive and its seed colors include brown stripes on a cream background and a slow darkening seed coat, which extends the shelf life of the grain. Growers said that varieties such as PID 2 are needed to increase productivity, improve grain quality, and promote the commercialization process of pinto beans produced in northern México.

The breeder and foundation seed classes are produced and maintained by the INIFAP's Valle del Guadiana Experiment Station. For further information and seed samples for experimental purposes, please contact: Dr. Rigoberto Rosales-Serna. Carretera Durango-El Mezquital km 4.5. Durango, Dgo., México. C. P. 34170. Tel. (52) 5538718700, 01 (800) 088-22-22. Ext. 82714.

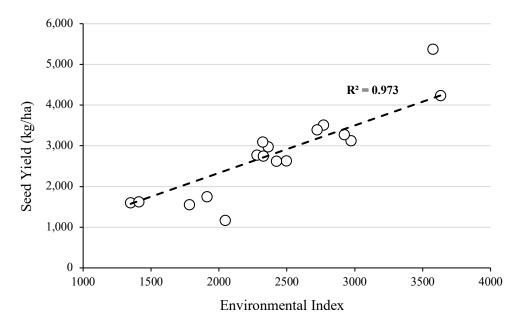


Figure 1. Seed yield for PID 2 compared to the average of 32 common bean improved lines and cultivars sown in 17 environments (2014-2023) in northern México.

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## EVALUATION OF THE AGRONOMIC PERFORMANCE OF FOUR VARIETIES OF COMMON BEAN IN CENTRAL COTE D'IVOIRE

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## ABSTRACT

The aim of this study was to evaluate the agronomic performance of four dry bean varieties. To do this, the trial was conducted using a Fisher block design and data were collected on germination rate, number of nodes and pods, and seed yield, and pod to seed ratios. Among the varieties evaluated, HARI04/BKE18 performed best.

## **INTRODUCTION**

The common bean occupies an important place in tropical Africa after groundnuts and cowpeas. In 2008, Côte d'Ivoire produced 4,761 tons of green beans (PNIA, 2018). Unlike green beans, dry beans have remained marginalized in production areas. Moreover, dry beans are mainly used for family consumption and their cultivation has therefore been relegated to second place. The varieties used by growers are traditional, with low yields (Meminader, 2009). To revive its cultivation, it is therefore necessary to propose high-performance varieties that are adapted to the production areas. The aim of this study was to assess the agronomic performance of four common bean varieties in central Côte d'Ivoire.

## **MATERIALS AND METHODS**

The plant material consisted of four common bean varieties (Figure 1). The trial was set up using a Fisher block design with 3 replicates. Each replicate consisted of four elementary plots spaced 1 m apart. Each elementary plot consisted of 4 lines, 3 m long. The seed was sown at a density of 50 cm  $\times$  20 cm. One seed was sown per plot to a depth of about 3 cm. This sowing density corresponds to 300,000 plants/ha. Two manual weeding operations were carried out, the first 21 days after sowing and the second 40 days after sowing. Agronomic evaluation of the varieties included percent germination, number of nodes, pod and seed yield, and the ratio of seed weight/pod weight. The data collected were subjected to a one-factor analysis of variance (ANOVA) using Statistica version 7.1 software.

## **RESULTS AND DISCUSSION**

The mean values for germination rate, number of nodes, pod and seed yields, and ratio of seed weight/pod weight for the four common bean varieties evaluated are given in Table 1. The results showed that there was a significant difference between the varieties for all the parameters measured. The variety HARI04/BKE18 had the highest germination rate (42.21), while the lowest rate was observed in the variety HARI19/GHA19 (7.33). The number of nodes varied from 12 to 23.50. The highest number of nodes was recorded in the variety HARI06/BON18 and the lowest in the variety HARI19/GHA19. In terms of pod yield, HARI04/BKE18 had the highest yield (2.24 t/ha), followed by HARI03/FER18 with a yield of 2.11 t/ha. The variety HARI06/BON18

produced the lowest yield (1.41 t/ha). Grain yields ranged from 0.63 to 1.90 t/ha. The variety HARI04/BKE18 was the most productive with 1.90 t/ha and the variety HARI6/BON18 was the least productive with 0.63 t/ha. Finally, the ratio of seed weight/pod weight varied from 0.78 to 0.83, with the highest values observed in HARI19/GHA19 and HARI03/FER18. Of the varieties studied, HARI04/BKE18 performed best and could be considered a promising variety.



HARI06/BON18 HARI04/BKE18 Figure 1. Images of pods and seed of four dry bean varieties tested

Table 1. Mean values for germination rate, number of nodes, pod yield, grain yield and the ratio
of seed weight to pod weight for four common bean varieties.

Varieties	Germination rate (%)	Number of nodes	Pod yield (t/ha)	Seed yield (t/ha)	Ratio seed weight /pod weight
HARI03/FER18	$12.66 \pm 4.66^{b}$	$19.00\pm4.24^{\mathrm{a}}$	$2.11\pm0.45^{\rm a}$	$1.21\pm0.45^{\rm a}$	$0.83\pm0.01^{\rm a}$
HARI19/GHA19	$7.33 \pm 3.33^{\circ}$	$12.00\pm5.63^{\mathrm{b}}$	$1.92\pm0.73^{\rm a}$	$1.28\pm0.73^{\rm a}$	$0.83\pm0.05^{\rm a}$
HARI06/BON18	$14.22\pm2.35^{b}$	$23.50\pm5.20^{\mathrm{a}}$	$1.41\pm0.48^{\text{b}}$	$0.63\pm0.41^{\text{b}}$	$0.79\pm0.13^{\rm a}$
HARI04/BKE18	42.21±5.97 <sup>a</sup>	$12.50\pm4.94^{\text{b}}$	$2.24\pm0.41^{\text{a}}$	$1.47\pm0.49^{\rm a}$	$0.78\pm0.03^{\rm a}$
Means	19.11	16.75	1.92	1.15	0.81
Significances	0.02	0.04	0.047	0.027	0.325
CV (%)	21.34	29.87	26.95	45.22	6.80

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## GRAIN YIELD AND ITS RELATIONSHIP WITH ITS COMPONENTS IN CLIMBING *PHASEOLUS* SPECIES

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## **INTRODUCTION**

The common bean (*Phaseolus vulgaris* L.) is a basic crop for Mexico and Latin American due to its nutritional and medicinal properties. In spring-summer sowing of beans under rainy conditions, the national average grain yield (GY) is 0.593 t ha<sup>-1</sup> (SIAP, 2017), which is insufficient to satisfy national demand. Different growth habits are known in beans (Escalante and Kohashi, 2015). Planting the indeterminate climbing growth habit type IV (HIT) is less common, because it requires trellises. In Mexico, planting is with corn, as a trellis, under rainy conditions and in small areas. One problem is the reduction of up to 40% of the GY of beans by corn. There are several species of *Phaseolus*, such as *P. vulgaris* (common bean), and *P. lunatus*, known as comba in warm regions for forage and grain purposes. The *P.coccineus*, known as ayocote, is found in temperate zones and used for food purposes and has a GY of 184 g m<sup>-2</sup> in association with corn (Rojas et al.2015.) Given the importance of *Phaseolus*, studies that lead to knowing the growth of this species and its management would help in the search for an increase in GY. The objective of the study was to research GY and its components in *P. vulgaris*, *P.lunatus*, and *P.coccineus*, planted with corn as a trellis, in a temperate climate.

#### **MATERIALS AND METHODS**

The study was carried out under rainy conditions in Montecillo, Mpio de Texcoco, Edo., Mexico, Mexico, (19°29'N and 98°53'W and 2250 meters above sea level) with a temperate climate. The sowing of the HIT *Phaseolus vulgaris* L., Amarillo and Negro, comba (*P. lunatus*), and Ayocote (*P. coccineus*), was on May 18 with an Azul corn trellis, with 5 plants m<sup>-2</sup> (0.30 m x 0.80 m) and fertilization with 100-100-00 (NPK). The experimental design was randomized blocks with four replications. The days to emergence (E), flowering (F) and physiological maturity (PM) were recorded. GY (g m<sup>-2</sup>) at 8% humidity), number of grains m<sup>-2</sup> (GN), grain size (GY/GN, g), number of pods m<sup>-2</sup> (PN), and number of grains per pod (GP) were recorded. An analysis of variance (ANDEVA) and Tukey's test were applied using the SAS 9.0 package. During the development of the crop, the average maximum and minimum temperature (° C) and the sum of rainfall (mm) were recorded.

#### **RESULTS AND DISCUSSION**

The emergence of the cultivars was 8 days after sowing (das), the flowering (F) for Amarillo was 50 das and for Negro was 70 das and the PM for Amarillo was at 120 das and for Negro at 130 das, and the PM of ayocote and comba was 130 and 140 das, respectively. The average maximum and minimum temperature during the development of the crop was 28°C and 10 °C, respectively. The precipitation was 450 mm during the crop cycle. The GY of the *Phaseolus* and its components such as GN, GS, GP and PN, showed significant differences between the species (Table 1). The Amarillo cultivar presented the highest GY and its components, which was 354 g m<sup>-2</sup>; followed

by Negro with 187 g m<sup>-2</sup>, comba with 164 g m<sup>-2</sup>, and Ayocote with 160 g m<sup>-2</sup>. This indicates that the environmental conditions were more favorable for the Amarillo cv to present a greater expression of GY and its components.

Cultivars	GY	GN (m <sup>-2</sup> )	GS (g)	GP	PN
	(g m <sup>-2</sup> )				
Amarillo	354a	1140a	0.35b	3.5 a	325a
Comba	164b	410b	0.49a	2.7 a	154b
Negro	187b	218b	0.31b	2,4b	60 c
Ayocote	160b	80b	0.47a	33b	54 c
Mean	216	540	4.51	2.9	448
Tukey (0.05)	102	280	0.094	0.61	64

**Table 1.** Grain yield and its components of *Phaseolus* species in Montecillo Mpio of TexcocoMexico. Summer. 2020

GY=grain yield; GN=grain number; GS=grain size; GP= grains per pod; PN=pod number

## CONCLUSIONS

The *Phaseolus* species under study presented differences in grain yield and its components. The Amarillo *P. vulgaris* stands out, while Comba and Ayocote presented the lowest values.

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## DYNAMICS OF POD PRODUCTION, YIELD AND BEAN COMPONENTS IN WARM CLIMATE

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## **INTRODUCTION**

The bean (*Phaseolus vulgaris* L.) is an important crop for the diet due to its nutritional and medicinal properties. In Mexico, 87% of the planted area is rainfed, which makes it a crop highly dependent on climatic conditions. The average yield of the Iguala Gro region is 0.91 g m<sup>-2</sup> (SIAP,2021), which is not enough to cover internal consumption. A strategy to increase yield is the search for greater pod set since during the reproductive cycle more pods are produced than reach physiological maturity, so knowing the number of pods during the cycle and reducing their abscission could result in higher grain yield. The objective of the research was to learn about the dynamics of pod appearance, grain yield and yield per pod to evaluate the loss of yield due to pod abscission, in bean cultivars (*Phaseolus vulgaris* L.) of growth habit type I in a hot climate.

## **MATERIALS AND METHODS**

The sowing of the cultivars (CV) Canario 107 (Canario) and Cacahuate 72 (Cacahuate) with a type I growth habit was on July 15, 2017, at a population density of 4.2 plants m<sup>-2</sup> under rainfall in Iguala, a warm climate (Aw0, García, 2005). The soil is a clay loam, with a pH=8.4, MO of 3.5% and assimilable N of 45 kg ha<sup>-1</sup>. Using a randomized block design with four repetitions, fertilization was 100-100-00 (NPK). For pod production dynamics m<sup>-2</sup>, three plants per experimental unit were taken at 35, 48, 62 and 80 days after sowing (das). The days to emergence, flowering (F) and physiological maturity (PM) were recorded. At harvest (PM), the maximum grain yield (GY), grain size (GS, g grain<sup>-1</sup>) and number of pods (PN) were recorded per m<sup>-2</sup>, and at harvest, the expected yield based on the maximum PN, yield per pod (PY. g p<sup>-1</sup>) and the reduction in GY obtained with the difference between the expected GY and that of the harvest. An analysis of variance (ANDEVA) and the comparison of means test (Tukey  $\alpha = 0.05$ ) were applied with the SAS statistical package.

#### **RESULTS AND DISCUSSION**

The F and MF were 37 and 80 days for both CV, respectively, and the average minimum and maximum temperature during the cycle was 23 °C; and 38 °C, respectively. The total rainfall was 750 mm. In both CVs, the pod production trend was adjusted to a 2nd degree polynomial, where the maximum PN was at 48 das. A similar response was reported by Escalante *et.al* (1999), The GY, GS, PN, PY, maximum number of pods and expected GY, the % reduction in GY was higher in Canario in comparison with Cacahuate. The maximum PN was 216 and 163 pods m<sup>-2</sup> and at harvest it was reduced to 120 and 91 m<sup>-2</sup> for Cacahuate and Canario, respectively. This caused a reduction in the GY of 60 and 57%, respectively (Table 1). Therefore, strategies must be sought to reduce pod drop and thus achieve a higher GY.

Cultivars	GY g m <sup>-2</sup>	GS (mg)	PN m <sup>-2</sup>	PY(g V <sup>-</sup> <sup>1</sup> )	Máx.PN (48dds)	GY (gm <sup>-2</sup> ) máximo	Reductión (g m <sup>-2)</sup>
Canario 107	125 a	225 a	120 a	1.04	216a	208a	83 b (60%)
Cacahuate 72	102 b	249 b	91 b	1.1	163b	179b	77 a(57%)
Mean	113	237	105	4.5	189	193	80 (58%)
Tukey (0.05)	19	20	20	1	20	20	5

**Table 1**. Grain yield (GY) and its components in bean cultivars with growth habit type I. Iguala Gro. Summer 2017.

Note: In columns, values with similar letters are statistically equal according to Tukey (0.05)

## CONCLUSIONS

The days to flower and physiological maturity did not show changes due to cultivars. The cultivars under study presented differences in yield, grain size and number of pods at physiological maturity (harvest). In the maximum number of pods, maximum expected yield and in the reduction in yield and pods at harvest Canario 107 surpassed Cacahuate 72. The dynamics of pod production showed a trend that adjusted to a polynomial of 2nd degree.

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#### INCREASE IN BEAN PRODUCTIVITY BY ESTABLISHING DOUBLE CROPPING IN THE VALLE DEL MEZQUITAL, HGO., MEXICO

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#### **INTRODUCTION**

In the irrigated area of Valle del Mezquital, in the State of Hidalgo, common beans have traditionally been sown only in the PV season, under rainfed conditions. However, there is a great opportunity to increase bean production by obtaining two harvests of dry beans in the same year, optimizing the use of the production window, free of frost, mainly between March and October. To achieve two cropping cycles it is necessary to identify the appropriate genotypes to use in the first planting date and which in the second. The objective of this work was to test various bean genotypes under irrigated conditions, evaluating several planting dates, in the Mezquital Valley in the State of Hidalgo.

#### MATERIALS AND METHODS

During seven years, 2009, 2010, 2017, 2018, 2019, 2020 and 2021, two growing seasons were evaluated in the same year. For the first growing season, the planting date was from February to April, and for the second planting the June-July period. The varieties Flor de Durazno, Jamapa, Bayomex, Canario-107, Albicampo, Huitel-143, Xicuco-10, Primavera 28, Negro 8025, Mayomex, Peanut-72 and Azufrado Regional 87 were planted in rows spaced at 0.80 m. The genotypes were planted in semi-commercial plots on producer's land. In each plot the number of genotypes varied, according to the availability of land and the preference of the producers. Samples were taken from two five-meter-long rows to estimate the yield per hectare. With the data obtained, an analysis of variance was carried out each year under a randomized complete block design with four replications. The comparison of means was made through the DMS test ( $\alpha = 0.05$ ). Field days (demonstrations) were held to present to the producers of this region the adaptation of the evaluated genotypes.

#### **RESULTS AND DISCUSSION**

The obtained yields in several years indicated that it is possible to have two growing seasons under irrigation conditions in the Valle del Mezquital. The first growing season planting date would be recommended in the first half of March; although it is possible to make the first planting date at the end of February. For the second growing season, the planting date would be between the second half of June and the first half of July, using intermediate and early season varieties, with resistance to diseases, such as Primavera-28, Negro-8025, Albicampo, Huitel-143, Xicuco-10, Mayomex, Flor de Durazno. The possibility to achieve a second growing season depends on the harvest opportunity of the first growing season, very good results have been obtained with the varieties Flor de Durazno, Bayomex, Jamapa, Primavera-28, Canario 107, Cacahuate 72, Azufrado Regional-87, Mayomex, Huitel 143 and Xicuco 10, with Jamapa, Primavera-28 and Azufrado Regional-87 showing superior performance, sown in the first half of March, with yields exceeding 4.5 tons per hectare (Table 1).

growing seas		nung u		i i cordar j						
Planting dates	Flor de Duraz no	Bayo mex	Jamapa	Primaver a	Canari o 107	Cacah uate 72	Azufrado Reg-87	Mayo mex	Huitel 143	Xicuco 10
28 Feb 2019	1,482		1,988		855	842				
8 March 2018	2,849	1,798	6,335							
11 March 2021	3,716		2,473	3,537			4,580	2,961		
14 March 2020	0		4,548	6,422						
21 March 2017	0	2,443	3,288		3,081					
29 March 2020	3,186								3,880	2,922
20 April 2018	1,955			2,003						
25 April 2009	2,531	2,207	2,205							
25 April 2010	1,568	3,352	3,853		3,800	3,605				

**Table 1**. Bean yields (t ha-<sup>1</sup>) for different planting dates, in Valle del Mezquital, Hgo. 1<sup>st</sup> growing season. Planting dates from February to April.

For the second cycle, the varieties Flor de Durazno, Primavera-28, Cacahuate 72, Xicuco 10, Negro 8025 and Albicampo were superior. This highlights sowing during the month of June and the first half of July (Table 2).

<b>Table 2</b> . Bean yields (t ha-1) for different planting dates, in Valle del Mezquital, Hgo. 2 <sup>nd</sup> growing								
season. Planting dates from June to July.								
Planting Dates	Flor de Durazno	Negro 8025	Primavera	Albicampo	Xicuco 10	Cacahuate 72		
1 June 2020		2,537	2,765	2,473				
12 June 2021	2,102				2,344			
22 June 2020	2,370							
14 July 2020			1,608					
18 July 2021	1,753				2,478	2,270		
19 July 2010	1,415	1,113						
21 July 2017	2,743							

For the conditions of the High Valleys of the Central Plateau (Table 2), it is not recommended to use for the second growing season, (planting date, June-July), genotypes introduced from other regions, such as Jamapa and Azufrado Regional 87, due to their susceptibility to diseases in this region of the country. The new varieties, such as Bayo Azteca, Altiplanomex, Huitel-143, Mayomex, and Azufradoro, will continue to be evaluated for the plantings in the second growing season of the year, to select only those with the highest performance taking as a reference 1.8 t ha-<sup>1</sup>. This yield is the lowest average obtained by these varieties under rainfed conditions, in locations in the High Valleys of the Central Plateau.

#### CLOSE-SPACED ARRANGEMENT TO INCREASE SEED YIELD IN COMMON BEAN IMPROVED LINES

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**INTRODUCTION:** The adjustment of planting density is necessary to optimize seed yield in improved cultivars of common bean (*Phaseolus vulgaris* L.), which have a compact plant canopy compared to traditional landraces. In the semi-arid highlands of northern México, different plantings were studied to analyze their effects on the seed yield of common bean. The arrangement with six rows of plants (0.30 m apart) in a 1.5 m wide planting bed was selected (Osuna *et al.*, 2013) to achieve higher yields in compact plant canopies of pinto beans. Assuming that more space is available between rows and plants, 1.5 m wide planting beds with 0.30 m spacing and four rows of plants with 0.30 m spacing could be suitable. The objective was to evaluate the effect of close spacing on seed yield of improved common bean cultivars developed in the semi-arid highlands of northern México.

**MATERIALS AND METHODS:** In 2023, eight common bean cultivars and improved lines were sown using two planting methods. The germplasm included different seed colors [pinto, opaque black, flor de mayo (pink) and azufrado (sulfur)] and growth habits (I, II and III) (CIAT, 1986 and 1987). The planting consisted of traditional (0.75 m between each row of plants) and 1.5 m in wide planting beds and four centered rows of plants (0.30 m apart). The experimental plots consisted of four rows (traditional) and one planting bed with a length of 50 m and four replications per cultivar. Data were recorded for days to flowering, disease reaction, days to physiological maturity and seed yield. Seed yield data were used for analysis of variance in a completely randomized design with factorial arrangement (cultivar and planting method). The Tukey test was used to compare the mean values ( $p \le 0.05$ ). Both methods were performed using the SAS ver. 9.4 computer program.

**RESULTS AND DISCUSSION:** Intermediate values were recorded for the number of days to flowering (Table 1), with values ranging from 41 to 45 days after planting (DAP). The results were related to the genetic breeding of common bean to achieve an intermediate onset of the reproductive phase, with an adequate balance between biomass accumulation and mobilization to the pods and grains. Symptom-free (1) to medium (5) levels of disease incidence were observed in all the improved cultivars. Some cultivars that showed disease resistance in Durango, where most cultivars and lines were developed, showed symptoms of anthracnose and rust due to the variability and virulence of the pathogens observed in México (Araya *et al.*, 2004; Rodríguez *et al.*, 2006). An intermediate life cycle length with maturity values from 96 to 102 DAP was also observed in most cultivars, which is related to the adaptability of the common bean to the agroclimatic conditions of the Mexican semi-arid highlands.

Significant differences were observed in seed yield between cultivars and planting methods  $(p \le 0.05)$ . High planting density increased seed yield by 39% (2,328 kg/ha) compared to single-row planting (1,412 kg/ha) (Table 1). Effects on growth habit and seed color were observed for all

the cultivars. The results showed that higher planting densities are recommended to increase seed yields in common bean cultivars with small plants, semi-erect bush and short to medium vine length. The higher yielding cultivars in both planting densities were PT14053 (PID 2) with 2,743 kg/ha in the four-row bed and 1,613 kg/ha in the single-row traditional method and NGO14013 (2,567 kg/ha and 1,558 kg/ha, respectively). Similar results were obtained in previous trials where high densities were associated with an increase in seed yield (Osuna et al., 2013). A lower response was observed in upright determinate type I bush plants (AZ16010) and semi-upright type II plants with short vine growth (NOD 1). Different responses to the planting method were observed in common bean cultivars depending on the origin of the germplasm, mainly due to a lower adaptation to the semi-arid highlands of northern México.

Line/Cultivar	DF*	А	R	В	DPM	Yield 4	Yield 1
						(kg/ha)	(kg/ha)
NOD 1	44	2	2	4	101	1988 <sup>d</sup>	1292 <sup>g</sup>
NGO14013	42	1	2	4	100	$2567^{ab}$	1558 <sup>e</sup>
NGO14014	42	1	2	4	102	2269°	1337 <sup>g</sup>
PT14053	41	1	1	4	96	2743 <sup>a</sup>	1613 <sup>e</sup>
PID 1	42	2	2	5	98	2214°	1343 <sup>g</sup>
PT14055	42	1	1	4	98	2308 <sup>b</sup>	1353 <sup>g</sup>
FM14002	43	2	1	4	100	2393 <sup>b</sup>	1467 <sup>f</sup>
AZ16010	45	2	2	4	101	2145°	1333 <sup>g</sup>
Average						2328 <sup>A</sup>	1412 <sup>B</sup>

**Table 1**. Mean values of the evaluated traits of improved common bean cultivars sown in Pabellón<br/>de Arteaga, Ags., México in 2023.

\*DF= days to flowering; reaction to anthracnose (A), rust (R) and B = common bacterial blight; DPM = days to physiological maturity; Yield 4 = planting bed and four plant rows, yield 1= single row. Different letters within the same column indicate statistically significant differences (Tukey  $p \le 0.05$ ) among cultivars (<sup>a-d</sup>) and planting method (<sup>A-B</sup>).

**CONCLUSIONS:** It is recommended to plant modern common bean cultivars at high densities to increase seed yield and economic benefits. Higher water productivity could also be achieved at high planting densities by reducing evaporation due to soil shading.

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## YIELD RESPONSE OF DRY BEANS TO ORGANIC AND INORGANIC FERTILIZERS AND BIOFERTILIZER

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**INTRODUCTION:** Common bean is widely adapted to a wide range of environments, grown in latitudes between 52°N to 32°S; in humid tropics, in the semi-arid tropics and even in the cold climatic regions (Beebe, 2012). By 2050, an increase in cereal food supply is required to feed the predicted world population of 9.8 billion people. Common bean like other crops had its yield affected by many external and internal factors (soil fertility degradation, less fertilizer use, soil properties, irrigation, weed and pest control, genetic improvements etc.), that decrease yield potential (Rurangwa et Bernard, 2020). The objective of this study undertaken by the Vegetable and Protein Research Program (VPRP) of the National Center for Agronomic Research (CNRA) and PABRA was to assess the response of dry bean to different levels of organic, inorganic fertilizers and biofertilizer.

**MATERIALS AND METHODS:** Seven treatments were used on three bean varieties [SMR53 (Hari25/GHA19), Roba1 (Hari35/GHA19) and Zabra (Hari36/GUI20)] as follow: Dose 0 (Control), Dose 1 (100 kg of NPK/ha before sowing), Dose 2 (50 kg/ha Urea 2 weeks after sowing), Dose 3 (reference dose,100 kg/ha of NPK before sowing + 50 kg Urea 2 weeks after sowing), Dose 4 (5 t of organic manure/ha before sowing), Dose 5 (10 t organic manure/ha before sowing), Dose 6 (Green Humico 500 ml for 16 l of water ; this corresponds to 12 l of Green Humico/ha).







SMR53 (HARI25/GHA19) Roba1 (HARI35/GHA19) Zabra (HARI36/GHA19)

Figure 1. Seeds of three dry bean varieties proposed for the fertilizer tests.

**RESULTS AND DISCUSSION:** For the variety SMR53, yields varied from 0.44 to 1.13 t/ha. The best performances were found with Dose 4 (5 t organic manure/ha before sowing) and Dose 6 (Green Humico 500 ml for 16 l of water; 12 l/ha) which produced respectively 1.07 t/ha and 1.13 t/ha (Table 1). Concerning the variety Roba, yields ranged from 0.46 to 1.22 t/ha. The highest yields were obtained with only Dose 2 (50 kg/ha of Urea for 2 weeks after sowing) which generated 1.22 t/ha (Table 2). As for variety Zabra, the yields have evolved from 0.5 to 1.25 t/ha. The optimal doses were Dose 3 (100 kg/ha of NPK before sowing + 50 kg Urea 2 weeks after sowing) and Dose 4 (5 t organic manure/ha before sowing) with yield of respectively 1,25 t/ha and 1,11 t/ha (Table 3).

Variety	Treatment	Flowering (JAS)	time Nodules number/pla	ant weigh	seed Yield nt (g) (t/ha)	
SMR53	DO	36±0,00	4,66±2,60	24,33	±2,88 0,61±0,3	3
SMR53	D1	36±0,00	9,66±7,75	25,33	±1,15 0,44±0,0	7
SMR53	D2	36±0,00	10,33±7,35	5 28±3,	.46 0,72±0,7	5
SMR53	D3	36±0,00	07,00±1,73	3 25,66	±0,57 0,56±0,3	9
SMR53	D4	36±0,00	09,66±4,63	3 24,66	±3,21 1,07±0,4	2
SMR53	D5	36±0,00	09,00±2,08	3 26,33	±1,52 0,72±0,3	2
SMR53	D6	36±0,00	10,66±0,33	3 25,66	±1,52 1,13±0,1	5
	Means Probabilit CV (%)	36±0,00 	7,56±1,25 0,987365 16,52	27,11 0,000 24,42	,	
able 2. Fl	owering time, num		· ·			ety Ro
Variety	Treatment	Flowering ti (JAS)	ime Nodules number/plant		ed weight Yield (t/ha)	
ROBA 1	DO	32±0,00	3,66±3,66	25,00±	9,64 0,79±0	,31
ROBA 1	D1	32±0,00	$1,00\pm0,93$	18,66±	2,08 0,69±0	,24
ROBA 1	D2	32±0,00	1,66±1,66	20,00±	2,00 1,22±0	,15
ROBA 1	D3	32±0,00	14,33±13,83	21,66±	3,21 0,96±0	,20
ROBA 1	D4	32±0,00	9,33±6,98	21,00±	2,64 0,95±0	,58
ROBA 1	D5	32±0,00	14,66±14,16	20,66±	0,57 0,96±0	,33
ROBA 1	D6	32±0,00	3,66±3,17	20,00±	2,64 0,46±0	,17
	Means	32±0,00	7,56±1,25 0,987365	27,11±		
	Probabilitie CV (%)	es -	-	0,0000	-	10
	~ /	-	16,53	24,42	7,14	_
	owering time, num				8	ety Za
/ariety	Treatment	Flowering time (JAS)	Nodules number/plant	100 seed weight (g)	Yield (t/ha)	
ZABRA	DO	32±00	10±10,00	34,33±4,04	0,763±0,22	
ZABRA	D1	32±00	4,33±2,03	33,33±5,53	0,92±0,13	
ZABRA	D2	32±00	5,00±,100	34,66±4,16	0,87±0,72	
ZABRA	D3	32±00	2,00±0,95	37,33±2,52	1,25±1,23	
ZABRA	D4	32±00	9,00±4,04	34,66±2,52	1,11±0,66	
ZABRA	D5	32±00	11,33±7,33	37,66±3,06	$0,75\pm0,46$	
ZABRA	D6	32±00	8,00±6,00	32,00±00,0	$0,50\pm0,46$	
	Means	32±00	7,56±1,25	27,11±6,62	0,83±0,51	
	Probabilities	-	0,987365	0,000000	0,900816	
	CV (%)	-	16,53	24,42	61,45	

Table 1. Flowering time, number of nodules per plant, 100-seed weight and yield for the variety SMR53

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## COMPARATIVE STUDY OF THE NUTRITIONAL COMPOSITION OF *PHASEOLUS VULGARIS* (COMMON BEAN), *VIGNA UNGUCULATA* (COWPEA) AND *VIGNA RADIATA* (MUNG BEAN)

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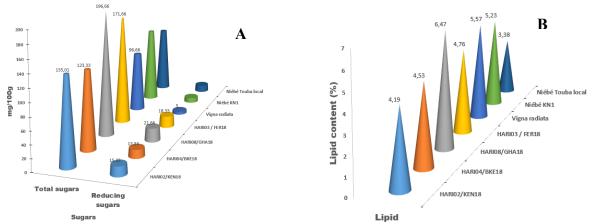
**INTRODUCTION:** Malnutrition is a condition that occurs when one's diet is unbalanced with under-nutrition, over-nutrition, or both, all of which could coexist and result in adverse health effects (WHO, 2021). In 2020, the World Health Organization (WHO) reported that 149.2 million children under five years of age were stunted, 45.4 millions were wasted, and 38.9 millions children were overweight (McClements & Grossmann, 2021). To combat malnutrition and ensure food sovereignty, the Ivorian government has encouraged crop diversification adoption. Thus, the Vegetable and Protein Crops Program of the University of Abomey Calavi and CIAT have undertaken the selection of high-yielding bean varieties with good agromorphology and nutrition attributes. Results of this study will be useful in promoting bean consumption as part of a healthy diet and support new breeding initiatives towards nutritional and health benefits.

**MATERIALS AND METHODS:** Laboratory based nutritional analysis on 4 dry common bean varieties HARI02/KEN18 (Kenya), HARI04/BKE18 (Central Ivory Coast), HARI03/FER18 (Northern Ivory Coast and HARI08/GHA18 (Ghana); 2 cowpea varieties KN1 and Touba local (Ivory Coast); anda of *Vigna radiata* variety (Ivory Coast). Nutritional parameters studied were total and reducing sugars, lipids, proteins, calcium, potassium, magnesium, iron, and zinc.

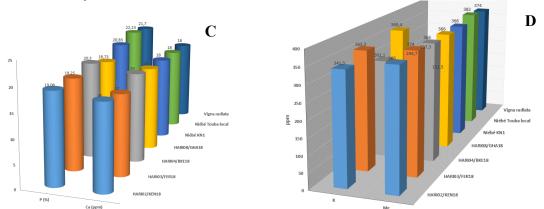


Figure 1. Seeds of 4 dry bean, 2 cowpea and 1 Vigna varieties used for nutrition analysis in this study.

**RESULTS AND DISCUSSION:** The nutrient analysis showed that the protein content was the same both bean and cowpea varieties. Same results were observed for iron and zinc content in dry beans, but slightly higher for zinc values. For lipid content, the highest value was found in one common bean variety (HARI08/GHA18) and the lowest in Vigna.



**Figure 2.** Sugar (A) and lipid (B) content in 4 dry bean varieties, 2 cowpea varieties and 1 Vigna radata variety



**Figure 3.** Protein and Calcium (C) and Potassium and Magnesium (D) content in 4 dry bean varieties, 2 cowpea varieties and 1 Vigna radata variety

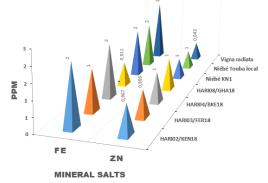


Figure 4. Iron and Zinc content in 4 dry bean varieties, 2 cowpea varieties and 1 Vigna radata variety

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#### **BREEDING COMMON BEANS FOR RESISTANCE TO BRUCHIDS**

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#### 1. Overview

The common bean weevil (*Acanthoscelides obtectus* Say) and the Mexican bean weevil [*Zabrotes subfasciatus* (Boheman)] are important storage pests of common beans (*Phaseolus vulgaris* L.) in tropical climates (Myers et al, 2021; Tigist et al., 2021). Both species belong to the chrysomelidae family, Bruchinae subfamily; the source of the generic term 'bruchid'. This subfamily includes approximately 1,650 species and is found worldwide (Yus-Ramos, 2014). The Mexican bean weevil is a serious bean pest in the Americas and East/Central Africa whereas the common bean weevil can infest beans in most tropical and subtropical regions. Back and Duckett (1918) noted that commercial bean production in the U.S. was concentrated in the northern States, in large part, to the avoid the damage caused by infestation of the common bean weevil in warmer regions of the country.

Cardona (1989) described important differences between the common bean weevil and the Mexican bean weevil. The Mexican bean weevil is better adapted to warmer temperatures and is more common in lower altitudes of the tropics. The common bean weevil is more frequently found at higher altitudes and latitudes, although it is also a pest in some warmer climates such as Puerto Rico. Araújo Soares et al. (2015) reported optimal temperature for pre-adult development of the common bean weevil was 30° C and optimal temperature for reproduction was 24° C. Another important difference noted by Cardona (1989) is oviposition behavior. Infestation and damage by the Mexican bean weevil only occur in storage. The Mexican bean weevil attaches eggs to the surface of the seed and does not oviposit in the field, whereas the common bean weevil scatters unattached eggs among stored seed or infests seed in the field by laying eggs on growing pods. Both weevils are short-lived (35-45 days) and mate and oviposit soon after emergence.

Seed infestation by the Mexican bean weevil can be managed in storage with appropriate sanitation techniques such as fumigation and/or storage in bruchid-proof containers (Myers et al., 2021). The chemicals used for seed fumigation are highly toxic and too expensive for use by smallholder farmers. The common bean weevil represents a greater challenge to manage than the Mexican bean weevil because infestation can begin in the field. Harvesting as soon as the bean crop reaches maturity can help to reduce infestation of the common bean weevil in the field (Schmale et al., 2002).

The development of bruchid resistant common bean cultivars will help to reduce postharvest losses and contribute to a more stable supply of bean seed in developing countries. Mishra

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et al. (2018) noted that the use of resistant cultivars should form the backbone of integrated bruchid pest management practices. The following is a description and review of research techniques that have been used to breed beans for resistance to both the Mexican bean weevil and the common bean weevil.

## 2. Screening methods for resistance to bruchids in common beans

a. <u>Mexican bean weevil</u> - Z. subfasciatus (Boheman)



Z. subfasciatus (Boheman)

Source: Willow Warren, Department of Agriculture Western Australia, CC BY 3.0 AU, https://creativecommons.org /licenses/by/3.0/au/deed.en>, via Wikimedia Commons.



Eggs of the Mexican bean weevil on the surface of bean seed.

Source: Juan Carlos Rosas

Schoonhoven and Cardona (1982) conducted trials to evaluate expressions of resistance under different infestation levels of the Mexican bean weevil and found that infesting 50 seeds with 7 pairs of newly emerged adults provided a sufficient level of infestation to detect differences in resistance between bean lines. Single replicates of each common bean germplasm accession were infested. Each group of 100 accessions included five replicates of the susceptible check cultivar 'Diacol-Calima'. Once an estimated 50% of the adults had emerged from the susceptible check cultivar, the samples were frozen, and the number of emerged adults were counted. The most resistant lines were retested using three replicates of 50 seeds infested with seven pairs of adults. Oviposition per female, adult emergence (counted every other day), percent emergence, duration of developmental stages, and dry weight of progeny were recorded. The data were analyzed using the log (x + 0.5) transformation.

Kornegay et al. (1993) conducted a study of the inheritance of resistance to the Mexican bean weevil by infesting small containers of seed with three adult pairs of weevils for 48 h. Number of eggs laid per seed was limited to four with excess eggs removed. About six days after infestation, individual seeds were examined to count the number of larvae that had hatched and penetrated the seed coat. The number of hatched eggs, number of emerged adults, and days to adult emergence from oviposition were recorded for each seed. The data were used to determine

percentage adult emergence and to calculate a reproductive index (RI) based on number of emerged adults, number of hatched eggs and days to adult emergence.

Blair et al. (2010) screened common bean lines for Mexican bean weevil resistance by infesting 15 seeds of each line with nine pairs (female and male) of adults from an insect colony maintained at CIAT. The seed was evaluated in mesh-covered, clear plastic vials (9-cm high  $\times$  1.7 cm in diameter). The walls of the vials were covered with sandpaper (No. 150) facing inward to prevent adults from laying eggs on the plastic surface of the container rather than the bean seed coat. Three replications of each line were evaluated in a rearing chamber at 27°C and 70% RH. Adults were removed 5 days after infestation when the counting of the number of eggs laid was initiated. The number of emerged adults was then evaluated over a 70-day period. The percentage of adult emergence was calculated based on the total number of adults emerged relative to the number of eggs laid.

Tigist et al. (2021) screened bean lines in Ethiopia for resistance to the Mexican bean weevil. Adult weevils were collected from infested seed to initiate the infestation and an unidentified susceptible bean cultivar was used to develop and maintain the colony. The mass rearing of the bruchid was conducted under an average room temperature of 27°C and a relative humidity of 70%. The bean seed to be evaluated was frozen at -20°C for four weeks to eliminate any prior infestation of weevils. Fifteen seeds of each bean line were placed in 6 cm x 7 cm transparent plastic jars. The lids of the plastic jars were perforated to provide ventilation and covered with mesh to prevent the escape of weevils. Each jar was infested with three pairs (female and male) of newly emerged adult weevils. A randomized complete block design (RCBD), with three replications was used. Ten days after infestation, the adult weevils were removed and the number of eggs on the surface of the seed was counted. The jars were monitored daily for the emergence of the next generation of adult weevils. After the first appearance of bruchids, the jars were monitored every 2 days, for recording purposes, and for the removal of newly emerged bruchids. The number of eggs per adult female (NE), the number of adult bruchids emerged (NAE) and seed damaged (% of seed with holes) were recorded. The percentage adult weevil emergence (PAE) was calculated, based on the total number of adults emerged compared with the number of eggs laid. The level of resistance of the bean lines was based on the percentage of adult emergence as described by Blair et al. (2010). The genotypes with adult emergence from 0-15% were classified as highly resistant (HR), those from 15-30% as resistant (R), those from 30-50% as intermediate resistance (IR) and those from 50-100% as susceptible (S). To ensure the homogeneity of variance, the data, based on count and percentage values, were transformed using natural log and arcsine transformation, respectively.



Damage to bean seed caused by the common bean weevil.

Source: James Beaver

Acanthoscelides obtectus Say Source: Udo Schmidt, CC BY-SA 2.0 <https://creativecommons.org/licenses/bysa/2.0>, via Wikimedia Commons

Kornegay and Cardona (1991) conducted an inheritance study by screening individual seeds for reaction to the common bean weevil. Individual seeds were infested in small glass vials with three eggs. Glass balls (0.5 mm diameter) were mixed with the seed to improve larval penetration and prevent escapes. The number of adults that emerged from each seed was recorded and were collected as they emerged, oven-dried (24 hours at 50°C) and weighed. The CIAT breeding program evaluated at least 200 individual  $F_2$  seed to be able to identify a few highly resistant segregants and suggested the development of a serological test for routine screening after more was learned about the biochemical basis of this resistance. It should be noted that the availability of effective molecular markers associated with common bean weevil resistance would also allow early generation selection for resistance.

Kusolwa and Myers (2011) multiplied and maintained a colony of the common bean weevil in Oregon using seed of the susceptible cultivar 'Rojo' to produce adult weevils for infestation. In screening for resistance, 15 adults were placed into glass containers containing 20-30 seeds. The caps of the containers were not completely closed to allow aeration but prevented the escape of adult weevils. The containers containing seeds and adult insects were placed in incubation trays and remained undisturbed for 12 days at  $25 \pm 3^{\circ}$ C and ambient relative humidity, until the first adult emerged. After 12 days, the number of eggs laid was estimated using a magnifying lens, and adults were removed. In cases where no eggs were visible, samples were re-inoculated with new adults. The presence of a powdery/floury appearance on the surface of the beans was noted to confirm larvae penetration into the seeds and to identify escapes from infestation. The emergence of adults in each glass container was inspected daily and counted until 72 days after infestation. Data were collected included the total number of adult weevils emerged after infestation, number of days for first adult emergence (DAE), number of days for 50% of total adults emerged, number of perforated seeds, severity of damage expressed by the number of seeds with 5 or more holes and percent seed weight loss. Frequency of adult emergence was determined by the total number adults emerged per day for the period of 72 days.

In Puerto Rico, Beaver et al. (2023) maintained a population of the common bean weevil by infesting a mixture of seed of the susceptible common bean cultivars 'Verano' and 'Badillo'. One quart (946 cc) Mason jars were partially filled with seed. The metal seals of the caps of the Mason jars were replaced with metal screens to allow aeration. At least 100 adults were placed in each Mason jar to maintain the colony and avoid inbreeding in the population. A layer of petroleum jelly was applied at the base of the plastic box containing the mason jars to prevent predation of the weevils by ants and other insects. The days to the first emergence of adults varied depending on the ambient temperature. Screening began when large numbers of adults emerged, usually about 45 days after infestation. Grain dockage sieves with holes larger than the weevils but smaller than the bean seed were used to easily separate the adult weevils from the bean seed. Tapping the metal base of the sieve made the adults weevils temporarily quiescent and facilitated the handling of the insects. Placing the adults in a refrigerator for a short period of time also temporarily reduced their mobility. At the time of emergence of adults, new Mason jars of clean seed were infested with weevils.



Mason jars and small plastic containers are used in Puerto Rico to screen bean lines for resistance to the common bean weevil. Source: James Beaver

The technique used by Kusolwa et al. (2016) to screen beans for bruchid resistance involved infesting beans with at least one adult weevil per seed. Adult weevils were placed in 118-cm<sup>3</sup> plastic containers containing 10-25 seeds of each line. It is highly unlikely that females would be absent when infesting a container with at least 10 adults. The caps of the containers had 2 cm diameter holes covered by screens to allow aeration. The white bean cultivars 'Verano' and 'Morales' were included as susceptible checks. Two replications per bean line were used for the screening. Thirty days after infestation, the dead adults from the initial infestation were removed from each container. Seed of susceptible lines can show translucent panels of perforations at 30 days after infestation. In this case, the number of seeds with and without perforations were counted in each container. Additional evaluations of damaged seed were conducted between 45 and 60 days after infestation. In some studies, initial seed weight and seed weight at 60 DAI were measured to calculate percentage seed weight loss.

Schmale et al. (2002) sampled bean seed from small-scale farms over a 3-year period in the Cauca Valley of Colombia. Infestation of the seed with the common bean weevil was frequent ( $\sim 90\%$ ) although the average initial level of infestation was only 16 weevils per 1000 seed.

Emergence of adult weevils in the samples occurred over a three-week period with a normal distribution which suggested that initial infestation occurred during the last few weeks before harvest.

When screening beans for resistance, infesting seed at a rate of one adult common bean weevil per seed is a much higher level than expected under natural conditions. For a 100 seed weight of 20 g, this represents an initial density of 5,000 adults per kg of seed. At these high rates of infestation, genotypes that show no damage at 30 days after infestation are considered in Puerto Rico to have useful levels of resistance.

Screening seed in the laboratory for resistance does not allow the identification of mechanisms of resistance to field infestation of the common bean weevil. It also does not provide a measure of the effectiveness of resistance to what would be expected to be much lower levels of infestation shortly after the harvest of beans. Thus, a study was conducted in Puerto Rico over three seasons to measure the level of seed damage due to natural infestation after 90 days of storage. Samples of up to 100 g of seed were taken from each experimental unit shortly after harvest of field trials and placed in 946 cc plastic food storage containers from which the weevils could not enter or escape. After 90 days of storage, the percentage of damaged seed was measured. Resistant lines had a significantly lower frequency and level of seed damage than susceptible cultivars (Beaver et al., 2024).

Baldin et al. (2017) maintained a population of the common bean weevils in a chamber (25  $\pm$  2° C, RH = 70  $\pm$  10%, and photoperiod of 12:12 h L:D) to supply adults for bean screening trials in Brazil. Transparent glass flasks (750 mL) were used for rearing the weevil colony. The flasks were closed at the top with a screw-on lid with a circular opening covered with a fine-mesh nylon screen for aeration. Each flask contained approximately 0.3 kg of the susceptible bean cultivar 'Bolinha' and approximately 300 unsexed adults. Emerging adults from the seed in the flasks were periodically sifted and used to re-infest seed to maintain the colony.

In trials to screen bean lines for resistance, Baldin et al. (2017) used transparent plastic containers (5.0 cm height x 3.0 cm diameter) with fitted lids. 10 g of seed of each bean line was placed in a container. Each plastic container was infested with six adult weevils that were no more than 48 h old. The containers were capped and placed in a chamber. At 7 days after initial infestation, both dead and alive adult weevils were removed from the plastic containers. Beginning 15 days after initial infestation, the seed in the plastic containers were evaluated daily to observe the developing weevils. The seed in each container was sifted with a sieve, and the number of emerged insects was recorded. The emerged adults were placed in small glass vials (5.0 cm height x 2.2 cm diameter) and were immediately frozen to avoid the loss of weight. At the end of the emergence period, the weevils in the small glass vials were oven-dried at 50° C for 2 days and weighed. The experimental design was completely randomized with eight replications per bean line. Each plastic container was an experimental unit. The Carioca cultivar 'Pitoco' was used as a susceptible check and a line having Arcelin 2 was used as a resistant check (Baldin and Lara, 2004). The total number of eggs was counted with a stereoscopic microscope at 20 days after initial infestation to determine ovipositional preference. 25 days after the initial infestation, the numbers of emerged adults and the periods of development (egg-adult) were also determined. The dry weights of the weevils and the consumption of seed were measured when the emergence of the adults was complete. The seed in the vials at the end of emergence were oven-dried at 50° C for 2 days and weighed. The initial and final weights of the seed were adjusted according to the weights of the controls, and the difference in dry weight (consumption) was calculated.

Baldin et al. (2017) also conducted a greenhouse trial to evaluate natural infestation of the common bean weevil. Eight replicates were used per genotype in a completely randomized design. The experimental unit was an individual plant in a pot. After the plants in the greenhouse had reached harvest maturity, a general cleaning was performed on each pot by removing dried leaves, petioles, and branches. The number of pods/plants was counted, and the plants were individually caged within tubular metallic structures (35 cm diameter x 60 cm height). Two Adult (48 h old) common bean weevils per pod were released in each cage. The tubular structures were covered with organdy fabric to prevent the escape of weevils. The infestation was maintained for 25 days, after which the pods were removed from the plants, placed in paper bags, and placed in the chamber (under conditions described for the previous tests). Sixty days after the initial infestation, the bags were opened, and the pods and seed of the different bean lines were evaluated. The variables analyzed were the number of pods per plant, number of damaged pods per plant, number of seeds per pod.

Li et al. (2021) used an indoor weevil infestation method to screen common bean lines for resistance to the common bean weevil. The common bean weevils used in the study were collected from several locations in the Yunnan Province of China where severe bruchid damage had been observed. Two petri dishes of each line containing 20 seeds were screened for resistance. The seed of each bean line, and susceptible and resistant checks, were placed into 9-cm-diameter petri dishes and randomly placed on shelves. All the shelves were covered with a fine net. Thousands of the collected common bean weevil adults (male and female) were placed inside the net to mate freely to ensure that all the seeds were infested under the same conditions. The room temperature was maintained at  $18 \sim 25$  °C. The humidity was maintained at approximately 70%. After 100 days of exposure to the common bean weevil, the level of damage of each bean line was determined. The percentage of damaged seeds (PDS) and the total number of perforations per experimental unit were calculated. It should be noted that 100 days of exposure is sufficient for two generations of the weevil to be completed.

#### 3. Sources of resistance to bruchids

#### a. Common beans (Phaseolus vulgaris)

Schoonhoven and Cardona (1982) screened at CIAT more than 4,000 accessions of common beans for resistance to the Mexican bean weevil. Although significant differences among genotypes were observed using different resistance criteria, the levels of resistance were considered to be too low to have economic value.

The complex APA locus in some small-seeded wild common bean germplasm accessions includes genes for the seed proteins Arcelin (Arc), the lectin Phytohemagglutinin (PHA) and  $\alpha$ -Amylase Inhibitor ( $\alpha$ -AI) that provide insecticidal properties (antibiosis) to bean seed by reducing larval development and insect fertility and growth (Blair et al., 2010). Cardona (1989) reported that these wild common bean germplasm accessions possess different variants of the arcelin gene. Sources of the eight different variants include G 12882, G 24390 (*Arc-1*); G 12866 (*Arc-2*); G 12891, G 12895 & G 12942 (*Arc-3*); G 24371, G 24370, G 24369, G 24368, G 23676, G 23675, G 24391, G 12949, G 12952 & G 12953 (*Arc-4*), G 02771 (*Arc-5*), G 11051 (*Arc-6*), G 24582, G 24584 (*Arc-7*). A wild bean collected in Mexico identified as QUES was reported to the have *Arc-8* and resistance to both the Mexican and common bean weevil (Zaugg et al., 2012). Unfortunately, this report has limited value to bean breeders because no additional information was provided on how to obtain this potentially valuable genotype. Zaugg et al., (2012) did note that QUES had APA components like G12949 (*Arc-7*).

The different variants of arcelin have different effects on the Mexican bean weevil and confer distinct levels of resistance (Kornegay et al., 1993; Acosta et al., 1998; Tigist et al., 2021). Hartweck et al. (1997b) noted that the ranking of resistance of arcelin types to the Mexican bean weevil varied between studies. It was not determined if these differences were due to variability in virulence between biotypes of the Mexican bean weevil or to differences in screening techniques. The presence of the arcelin protein is inherited as a single dominant trait, where the homozygous state provides a higher level of resistance to bruchids (Blair et al., 2010).

Kornegay and Cardona (1991) reported that the wild bean accession G12492 (*Arc 4*) had high levels of resistance to the common bean weevil. Antibiosis resulting in delayed and reduced adult emergence, high mortality of late first instar larvae and reduced female fecundity were identified as the mechanisms of resistance. Results from an inheritance study suggested that resistance was conferred by two recessive complementary genes. Baldin et al. (2017) identified *Arc-1S* to have a strong antibiosis to the common bean weevil that resulted in a delay in the development of immature stages and a reduction in the number of emerged adults.

Bean breeders at CIAT developed numerous RAZ (Resistant to *Zabrotes subfasciatus*) bean breeding lines by backcrossing *Arc-1* into different market classes of beans (Cardona et al, 1990). Marker-assisted selection was used to develop MAZ lines. Tigist et al. (2018) reported that RAZ-11, RAZ-36, RAZ-2, RAZ-44, RAZ-120, RAZ-40 and MAZ-203 had high levels of resistance to the Mexican bean weevil in Ethiopia. Tigist et al. (2021) developed the Mexican bean weevil resistant common beans using the Mesoamerican line RAZ 168 as the source of resistance. Broad sense heritability of traits related to Mexican bean weevil resistance ranged from 68.5%–93.9% and several breeding lines having high levels of resistance were identified.

Osborn et al. (2003) utilized the recurrent parents 'Sanilac' and 'Porillo 70' and backcrossing to develop white (SARC) and black (PARC) bean lines having different arcelin variants (*Arc-1, Arc-2, Arc-3, Arc-4*). The lines with *Arc-1* from the wild bean germplasm G12882, SARC1 and PARC1, expressed high levels of resistance to the Mexican bean weevil but low levels of resistance to the common bean weevil (Harmsen, 1989). In an attempt to increase arcelin content in the seed, SARC1 was crossed with the common bean breeding line MB11-29 that lacked phaseolin in the seed due to the introgression of a recessive allele from *P. coccineus* (Hartweck et al., 1997b). The breeding line SMARC-PN1 (PI 628627) from this cross expressed moderate levels of resistance to the common bean weevil and high levels of resistance to the Mexican bean weevil. The seed storage protein (phaseolin) deficiency found in SMARC-PN1 was reported by Taylor et al. (2008) to have resulted in improved sulfur amino acid content. The bruchid resistant germplasm line AO1012-29-3-3A which combines arcelin resistance from the wild tepary bean accession G40199 and the seed storage protein deficiency of SMARC-PN1 also showed higher levels of sulfur amino acids in the seed (Kusolwa et al., 2016).

Baldin et al. (2017) reported that in the field, female common bean weevils lay eggs on mature pods producing larvae that bore into seeds. They suggested that physical barriers, such as trichomes, surface waxes, and hardened or thicker tissues in pod walls may provide partial resistance.

In the highlands of Central America, another pest, the bean pod weevil (*Trichapion godmani* Wagner), lays eggs in the mesocarp of pods that produce larvae that burrow into immature bean seed (Cardona, 1989). It has not been determined if resistance mechanisms found in bean genotypes selected for resistance to the bean pod weevil (Blair et al. 2016; Garza et al., 2001) might provide some protection against field infestation of the common bean weevil and vice versa.

#### b. Tepary beans (Phaseolus acutifolius)

Mbogo et al. (2009) reported that the wild tepary bean line G40199 was resistant to both the common and Mexican bean weevil. This source of resistance was successfully used to develop common bean breeding lines with enhanced levels of resistance to this pest (Kusolwa et al., 2016).

Porch and Beaver (2022) screened thirty-four cultivated and 122 wild tepary bean genotypes from the tepary diversity panel (TDP) for resistance to the common bean weevil. All the cultivated common bean and tepary beans were susceptible at 60 days after infestation (DAI) whereas seven wild genotypes in the TDP had  $\leq 10\%$  damaged or soft seed at 90 DAI. Loss in initial seed weight ranged from 0.0-3.0% among the resistant wild tepary genotypes. The authors noted that valuable genetic diversity for bruchid resistance may have been lost during domestication of tepary bean. Introgression of this high level of resistance into common bean may be possible using bridging-parents that facilitate interspecific crosses (Barrera et al., 2020). The wild tepary bean germplasm lines G40199, G40087 and G40253A were reported by Bornowski et al. (2023) to exhibit no damage by the common bean weevil at 60 days after infestation. Results from a GWAS conducted by Bornowski et al. (2023) found bruchid resistance to be associated with two cupin-1 domains on Pa07. Introgression of this novel source of resistance from tepary beans into common bean may be useful to complement the bruchid resistance conferred by the APA locus.

#### 4. Conventional plant breeding techniques

Breeding for bruchid resistance in common bean using conventional techniques requires a long-term commitment. Simple backcrossing of arcelin variants in the APA locus has not proven effective in introgressing high levels of resistance to bruchids into common beans. Results from breeding efforts and QTL analyses suggest that additional genetic factors may contribute to high levels of resistance to bruchids (Mateo, 2016; Kamfwa et al., 2018). Although wild common bean lines containing Arc-4 were reported to be highly resistant to the common bean weevil (Cardona et al. 1989), backcrossed common bean lines having Arc-4 had low levels of resistance to this weevil (Harmsen, 1989). Kamfwa et al. (2018) reported that only 7% of the RILs derived from crosses with AO-1012-29-3-3A had high levels of resistance to the common bean weevil. This result is consistent with a low recovery rate of resistance to the common bean weevil in a cross between resistant wild common bean and cultivated common bean (Kornegay and Cardona, 1991). Similar low recovery rates of resistance have been obtained when crossing AO-1012-29-3-3A with ~13 African Andean bean landraces, with only 5% of the progenies being resistant (Mazala, 2023). Therefore, successful breeding for resistance to the common bean weevil may require the screening of larger populations of common beans and evaluations for resistance in later generations.

After selecting beans for highly heritable traits such as seed type, relative maturity and growth habit, breeding lines could be initially screened for bruchid resistance in the  $F_4$  or later generations without replications but using multiple susceptible checks to confirm that the weevils uniformly infested the seed samples. The smaller number of lines expressing resistance can be confirmed in the following generations in replicated trials. Several cycles of selection may be necessary to overcome linkage drag in breeding for bruchid resistance that can result in progeny having lower yield potential or poor agronomic characteristics (Blair et al., 2010).

Identity	Species	Source(s) of resistance	Bruchid	Reference
G12882, G24390	P. vulgaris	Arc-1	Z. subfasciatus	Cardona (1989)
RAZ-168	22	Arc-1	55	Tigist et al. (2018)
	22	Arc-2	22	Cardona (1989)
G12891, G12895, G12942	33	Arc-3	"	33
G24371, G24370, G24369, G24368, G23676, G23675, G24391, G12949, G12952, G12953	3	Arc-4	3	3
	55	Arc-5	33	25
	77	Arc-6	"	>>
G24582, G24584	"	Arc-7	>>	>>
SMARC-PNI (PI 628627)	33	<i>Arc-1</i> , lack of phaseolin from an interspecific cross with <i>P. coccineus</i>	Z. subfasciatus, A. obtectus	Osborn et al. (2003)
G 40199	P. acutifolius	APA complex locus on Pv04	Z. subfasciatus, A. obtectus	Mbogo et al. (2009)
AO1012-29-3-3A (red kidney)	Interspecific (Pv x Pa)	G 40199, SMARC-PN1	Z. subfasciatus, A. obtectus	Kusolwa et al. (2016)
PR1303-129 (black), PR1743-44 (small red)	P. vulgaris	AO1012-29-3-3A	Z. subfasciatus, A. obtectus	Beaver et al. (2024)
SUA-Red (dark red kidney), SUA-Karanga (light red kidney), SUA-Rosa (red speckled kidney)	P. vulgaris	AO1012-29-3-3A	Z. subfasciatus, A. obtectus	Myers et al. (2021)
Yellow and red mottled Andean bean breeding lines	P. vulgaris	AO1012-29-3-3A	Z. subfasciatus, A. obtectus	Mazala (2023)
G40199, G40087, G40253A	Wild P.acutifolius	Two cupin-1 domains on Pa07	A. obtectus	Bornowski et al. (2023)

Table 1. Summary of sources of resistance to bruchids.

Phenotypic screening of beans for resistance requires the maintenance of a colony of weevils. To avoid the buildup of pests and waste products in the colony, recently emerged adults should be transferred into containers with clean and uninfested seed. Sufficient numbers of adults should be transferred to avoid negative effects of genetic drift. Ideally, adults collected from the field can be occasionally added to the weevil colony to avoid inbreeding depression in the population. There is scarce information available regarding the population structure and genetic diversity of these bruchid species (e.g., biotypes). Ideally, promising breeding lines should be tested across different locations to ensure the identification of broad levels of resistance.

#### 5. Mechanisms of resistance and molecular markers

Ishimoto et al. (1995) noted that the APA locus in cultivated bean genotypes have genes coding only for phytohaemagglutinin (PHA) and alpha-amylase inhibitor ( $\alpha$ -AI) and are not resistant to the common and Mexican bean weevils. Blair et al. (2010) observed that the lack of success in breeding for bruchid resistance utilizing arcelin from the APA locus in wild beans may be due to a lack of understanding about the mechanisms of resistance and the organization of the APA locus. The complex APA locus in bean genotypes possessing arcelin can have gene duplications and deletions (Lioi et al. 2003) and copy number could be a factor in genetic resistance.

Blair et al. (2010) used phenotypic data from an Andean population segregating for *Arc-1* that showed a highly significant association with markers in the region of the APA locus where bruchid resistance was measured by the percentage of Mexican bean weevil adult emergence (PAE). The presence of the arcelin protein is inherited as a single dominant trait whereas the homozygous genotypes possess higher levels of resistance to bruchids (Kornegay et al. 1993). Blair et al. (2010) identified co-dominant and other microsatellite markers from the APA region that are potentially useful for marker-assisted selection for *Arc-1*. He noted that these markers may not be effective for other arcelin genes.

Using a gene-based approach, Mazaheri (2018) developed an  $\alpha$ -AI-1 INS45 indel marker based on the annotated sequence of the  $\alpha$ -amylase inhibitor gene linked to the complex APA locus, which amplified a DNA fragment that showed a 45 base pair insertion in the middle of a lectin Leg b domain. Mazala et al. (2023) reported that the  $\alpha$ -AI indel marker was 100% accurate in identifying Andean bean lines resistant to the common bean weevil using the AO-1012- 29-3-3A source of resistance.

Kamfwa et al. (2018) identified three QTL for resistance to the common bean weevil in the interspecific line AO-1012- 29-3-3A on chromosomes Pv04 and on Pv06. One of the QTLs on Pv04 was previously reported as the arcelin, phytohemagglutinin and a-amylase, (APA) locus. Li et al. (2022) also reported a major QTL for resistance to the common bean weevil on Pv06 in a black bean germplasm accession from China encoding a bifunctional  $\alpha$ -amylase/protease-inhibited protein. Kamfwa et al. (2018) noted that these results support previous studies (Kusolwa and Myers, 2011) that other resistance factors, in addition to arcelins encoded by the APA locus, from wild common and tepary bean, may confer resistance to the common bean weevil. Pandurangan et al. (2016) reported that a single nucleotide polymorphism was likely introduced from *P. coccineus* into SMARC1-PN1 associated with the genotypic differences in  $\beta$ -phaseolin accumulation. Viscarra-Torrico et a. (2021) identified SNP markers on chromosomes Pv04 and Pv07 associated with the introgression of phaseolin and lectin deficiency into common beans.

Tigist et al. (2021) noted that laboratory screening for bruchid resistance is tedious and time-consuming, thus marker-assisted selection would be more efficient and cost effective and may allow selection for resistance in earlier generations than conventional breeding methods. Researchers at CIAT recently developed MAZ lines by crossing RAZ lines with different market

classes of beans. The KASP marker BRU\_00261 (Intertek ID: snpPV0007), located in the arcelin locus on the end of Pv04, was successful in identifying about 95% of the lines with resistance to the Mexican bean weevil in an F<sub>4</sub> Mesoamerican bean population derived from the cross 'SR 15 and MAZ 200' (Tigist et al., 2021).

#### 6. Challenges

Bruchid resistant bean cultivars have not been widely deployed, therefore questions remain concerning the effectiveness of resistance when exposed to different biotypes of common bean and Mexican bean weevils. The bruchid resistant Andean bean germplasm AO1012-29-3-3A selected in Puerto Rico for resistance to the common bean weevil has proven to be an effective source of resistance to common bean weevil populations in Tanzania (Myers et al., 2021) and Zambia (Mazala, 2023). Beaver et al. (2024) reported that the bruchid resistant black bean breeding line PR1303-129 expressed resistance to the common bean weevil in Puerto Rico and Guatemala and the Mexican bean weevil in Honduras and the Dominican Republic.

It has also not been determined if or how quickly the weevils may develop tolerance and reproduce in the seed of resistant cultivars. Mayunga et al. (2023) found little genetic intraspecific variability among samples of the common bean and Mexican bean weevils collected in Tanzania. The authors noted that the lack of variability may be due in part to commercial trade of beans and the movement of infested seed within the country.

Molecular markers need to be effective in selection for resistance in different gene pools and market classes of beans. A better understanding of the mechanisms and genetics of sources of resistance is needed to develop more effective selection strategies. Agronomic practices such as timely harvesting and threshing of the crop and morphological traits such as cultivars having determinate (type I), or erect indeterminate (type IIA) growth habits may help to avoid exposure of the bean crop to the common bean weevil in the field and may complement genetic resistance by reducing initial pest population pressure during storage.

The potential effects of the introgression of bruchid resistance on the expression of other traits merits further study. Kusolwa et al. (2016) reported that the seed of the bruchid resistant Andean line AO-1012-29-3-3A had greater levels of threonine, proline, alanine, valine, lysine, methionine, and crude protein compared with the check cultivar 'Badillo'. Mazala et al. (2023) reported that bruchid resistance did not affect cooking time of Andean bean lines. However, additional sensory studies (taste, texture, etc.) may be needed to ensure that these resistant varieties will not have consumer acceptability issues.

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## SUBJECT MATTER INDEX – Volume 67

Abiotic Stress, Drought, Heat	1,21,23,25,39,69,71,73,75,77,127,155
Acutifolius	
Angular leaf spot	
Anthracnose	
BNF, Fertility, Nutrients, Tillage	
Bruchids, Seed Storage	
Coccineus	
Common Bacterial Blight, Brown Spot	
Cooking, Nutrition, Quality	27,29,65,67,131,133,135,137,139,141,143,145,147,149,163
Cropping Systems	
Databases, Collections, Information Systems, Sequencing	
Diversity, Phaseolus species, wilds	
Genetics, Genomics, Breeding	5,7,9,11,13,17,19,21,25,37,45,47,51,57,63,81,115,123,141
Insects	
Interspecifics	
Lunatus	
Macrophomina	
Markers and Mapping	
Nematodes	
Organic, Green manure	
Phenotyping, Phenomics, Machine learning	9,19,59,61,69,115,125,127,129,141
Root Rots	
Rust	
Snap Beans	
Varieties, Testing and Releases	
Viruses	
Web blight	
White Mold, Sclerotinia	
Yield	

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## 2023 FINANCIAL STATEMENT BEAN IMPROVEMENT COOPERATIVE

BALANCE AS OF January 1, 2023	\$ 36,725.19
INCOME	
2023 Membership Dues	\$465.70
BIC meeting sponsorships	\$11,420.25
Bank Interest	\$0
TOTAL INCOME	\$11,885.95
EXPENSES	
Labor charges	\$0.00
Postage, Copy Charges and Office Supplies	\$20.00
Pdf & Book editing and publishing fees	\$515.00
BIC Student travel awards (2023 meeting)	\$19,650.00
BIC Student awards (2023 meeting)	\$600.00
PayPal Fees	\$119.15
Bank Fees	\$136.00
TOTAL EXPENSE	\$21,040.15

BALANCE AS OF December 31, 2023 \$ 27,570.99