BEAN IMPROVEMENT COOPERATIVE

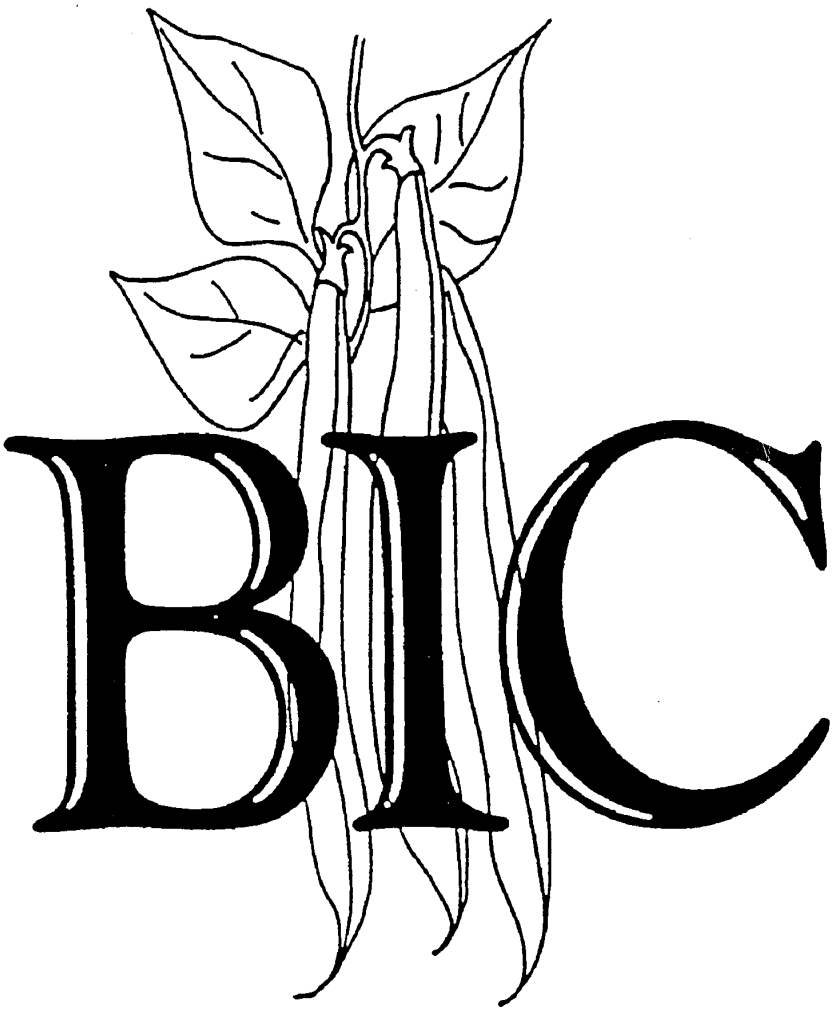
BIC

2025

VOL 68

ANNUAL REPORT OF THE

BEAN IMPROVEMENT COOPERATIVE



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Report of The

BEAN IMPROVEMENT COOPERATIVE

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Coordinating Committee

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

TABLE OF CONTENTS

RESEARCH PAPERS FOR 2025

THE 68th ANNUAL REPORT OF THE BEAN IMPROVEMENT COOPERATIVE

It is both an honor and a pleasure to coordinate the 68th Annual Report of the BIC as the new president of this important organization. I can say without doubt that across my career, the BIC has been the most important and productive scientific network I belong to. I'll strive to continue maintaining the excellence of this group of individuals focused on the improvement of the bean crop worldwide, which is an important task. I also want to thank Dr. Tim Porch on behalf of all the BIC community for his time, effort, leadership, and unconditional service as past president during the last five years. Dr. Porch will continue to be active both as my mentor in this new role and also as an active member of our executive committee.

The Bean Improvement Cooperative (BIC) will celebrate its 33rd Biennial Meeting from the 3-6 of November 2025. This will be a concurrent meeting with our colleagues in the North American Pulse Improvement Association (NAPIA), with BIC as the principal organizer of this meeting. The local meeting organizers are: Dr. Carlos Urrea, dry bean breeder at the Univ. of Nebraska-Lincoln, but stationed at the Scottsbluff Research and Extension Center, and Connie Hansen (UNL Event Planner). A workshop focused on Root Rots is planned for November 6. In addition, the Phaseolus Crop Germplasm Committee (PCGC), BIC Genetics Committee (BGC) and the Regional W-4150 Committee are scheduled to meet on November 6. All these meetings are open to everyone interested. A tour of UNL campus facilities is also planned for November 6. Additional details regarding registration, abstract submission information, and other updates from the organizers are available on the Meeting website: https://agronomy.unl.edu/bic-napia/.

Please review the call for nominations for the BIC Meritorious Service Award, BIC Achievement Award, and new BIC Technical Merit Award, and forward your nominations to the Awards Committee Chairperson, Carlos Urrea (currea2@unl.edu) by July 15, 2025. The Frazier-Zaumeyer Distinguished Lectureship will also be awarded and will honor our founding members. Nominations for this Lectureship should be sent to Carlos Urrea. A current membership list of BIC Committees and those who have received awards throughout the history of the BIC is provided in the current issue and on the BIC website to assist you in nominating colleagues for these awards.

Please share information about the BIC with interested colleagues who might like to attend the 2025 meeting or who would like to join the BIC as members. Also, feel free to contact us with any new ideas, contributions, or updates for the BIC website or this Annual Report. We are especially interested in receiving new or updated methods that can be shared with the general community for the Research Techniques page of the BIC website.

The BIC continues to conduct business by email, postings on the webpage, and through the online publication of this Annual Report. We are always open to new ideas to make the BIC a more effective organization and any suggestions can be shared with members of the Coordinating Committee.

We wish you a fulfilling and successful year. Warm regards,
Juan M. Osorno, 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, **Coyne**, Dean, Jorgensen, Polzak, Zaumeyer
1972 Burke, **Coyne**, Dean, Jorgensen, Kiely, Polzak, Zaumeyer
1974 Ballantyne, Bravo, Burke, **Coyne**, Dickson, Emery, Evans, Kiely, Saettler, Zaumeyer
1977 Ballantyne, Bliss, Coyne, **Dickson**, Emery, Evans, Graham, Meiners, Morris, Saettler, Zaumeyer
1978 Atkin, Ballantyne, Bliss, Coyne, **Dickson**, Graham, Meiners, Morris, Saettler, Sprague
1979 Atkin, Bliss, **Dickson**, Graham, Hagedorn, Meiners, Morris, Sprague, Wallace
1980 Atkin, Bliss, **Dickson**, Hagedorn, Morris, Sprague, Steadman, Temple, Wallace
1982 Atkin, Coyne, **Dickson**, Hagedorn, Sprague, Steadman, Temple, Wallace, Wyatt
1983 Coyne, **Dickson**, Hagedorn, Saettler, Silbernagel, Steadman, Temple, Wallace, Wyatt
1985 Coyne, **Dickson**, Mok, Saettler, Silbernagel, Steadman, Temple, Wallace, Wyatt
1986 Coyne, **Dickson**, Mok, Saettler, Schoonhoven, Schwartz, Silbernagel, Steadman, Wallace
1988 Brick, Dickson, Emery, Magnuson, Roos, **Schwartz**, Singh, Steadman, Uebersax
1992 Dickson, Emery, Grafton, Magnuson, **Schwartz**, Singh, Stavely, Steadman, Uebersax
1994 Antonius, Dickson, Grafton, Magnuson, Park, **Schwartz**, Singh, Stavely, Uebersax
1996 Antonius, Grafton, Park, **Schwartz**, Singh, Stavely, Myers, Kotch, Miklas, Riley
1998 Antonius, Park, Schwartz(ex officio), Singh, Myers, Kotch, Miklas, Riley, Beaver,Vandenberg,**Kelly**
2000 Antonius, Beaver, **Kelly**, Kotch, Miklas, Myers, Park, Riley, Schwartz, 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, Kmiecik, 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
2022 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
2024 Brown, Dohle, Ferreira, Gepts, Gomez, Goncalves-Vidigal, Kelly, McClean, Miklas, Osorno, **Parker** (Chair), Porch, Urrea

RECIPIENTS of BIC AWARDS for MERITORIOUS SERVICE, ACHIEVEMENT, TECHNICAL MERIT & FRAZIER-ZAUMEYER DISTINGUISHED LECTURESHIP

Year	Recipients
1970	Melvin E. Anderson- Rogers Bros. Seed Co., Plant Pathologist
	William A. Frazier- Oregon State Univ., Horticulturist (**BIC Founder** & **Coordinator**, 1957-67)
	Walter H. Pierce- Asgrow Seed Co., Plant Pathologist
	William J. Zaumeyer- USDA, Plant Pathologist
1971	Walter H. Burkholder- Cornell Univ., Plant Pathologist
	James R. Douglass- USDA, Entomologist
	Howard S. Gentry- USDA, Plant Explorer
	Charles W. Hungerford- Univ. of Idaho, Plant Pathologist
	Herbert A. K. Lamprecht- Pl. Breeding Inst. of Sweden, Geneticist
	John J. Natti- Cornell Univ., Plant Pathologist
	Melbourne C. Parker- Gallatin Valley Seed Co., Plant Breeder
	Francis L. Smith- Univ. of California, Agronomist
	Robert E. Wester- USDA, Plant Breeder
1973	Leslie L. Dean- Univ. of Idaho, Plant Pathologist
	Nicolaas Hubbeling- Inst. of Phyto. Res.- Netherlands, Plant Pathologist
1975	M. Wayne Adams- Michigan State Univ., Plant Breeder
	Dermot P. Coyne- Univ. of Nebraska, Plant Breeder (BIC **Coordinator**, 1968-76)
	Shigemi Honma- Michigan State Univ., Plant Breeder
	Max. L. Schuster- Univ. of Nebraska, Plant Pathologist
1977	Douglas W. Burke- USDA, Plant Pathologist
	Roelof Prakken- Utrecht Univ. of the Netherlands, Geneticist
	Clibas Vieira- Univ. Federal de Vicosa of Brazil, Agronomist
1979	Barbara J. Ballantyne- New South Wales, Plant Pathologist
	Donald J. Hagedorn- Univ. of Wisconsin, Plant Pathologist
	Marshall LeBaron- Univ. of Idaho, Agronomist
1982	Eelco Drijfhout- Agr. Inst. of the Netherlands, Plant Breeder
	Donald H. Wallace- Cornell Univ., Plant Breeder
	Donald R. Wood- Colorado State Univ., Plant Breeder
1983	Leland W. Hudson- USDA, Horticulturist
	Roger F. Sandsted- Cornell Univ., Horticulturist
1987	Michael H. Dickson- Cornell Univ., Plant Breeder (BIC **Coordinator**, 1976-87)
	Aart van Schoonhoven- CIAT, Entomologist
	Frederick A. Bliss- Univ. of Wisconsin, Plant Breeder
	Matt J. Silbernagel- USDA, Plant Pathologist
1989	Axel L. Andersen- Michigan State Univ., Plant Breeder/Pathology
	John D. Aktin- Asgrow Seed Co., Plant Breeder
	Colin L.A. Leakey- England, Geneticist
	Alfred W. Saettler- USDA/ARS, Plant Pathologist
	Arthur P. Sprague- Del Monte, Plant Breeder
	James R. Steadman- Univ. of Nebraska, Plant Pathologist
	J. C. "Mike" Tu- Agriculture Canada, Plant Pathologist

1991	Iver L. Jorgensen- Northrup King & Co., Plant Breeder
	John L. Morris- Rogers/NK Seed Co., Plant Breeder
	Rosario Provvidenti- Cornell University, Plant Pathologist
1991, continued	Shree P. Singh- CIAT, Plant Breeder
	J. Rennie Stavely- ARS/USDA-Beltsville, Plant Pathologist
	Daniel Debouck- IBPGR, Rome, Plant Geneticist [Achievement Award]
	Paul L. Gepts- Univ. of Calif.-Davis, Plant Geneticist [Achievement Award]
	Pat Barnes-McConnell- Bean/Cowpea CRSP, Director [Achievement Award]
1993	Hubert L. Bannerot- INRA, Versailles, Plant Breeder
	Cesar Cardona- CIAT, Entomologist
	Robert B. Colville- Del Monte Foods, Variety Development
	George L. Hosfield- ARS/USDA, East Lansing, Genetics/Nutrition
	Oswaldo V. Voysest- CIAT, Agronomy/Germplasm Evaluation
	James S. Beaver- Univ. of Puerto Rico, Plant Breeder [Achievement Award]
1995	Howard F. Schwartz- Colorado State University, Plant Pathologist (BIC **President**, 1988-97)
	Kenneth F. Grafton- North Dakota State University, Plant Breeder [Achievement Award]
1997	George Emery- Ferry Morse, Plant Breeder
	James D. Kelly- Michigan State University, Plant Breeder (BIC **President**, 1998-2009)
	Steve Magnuson- Harris Moran, Plant Breeder
	David Nuland- University of Nebraska, Bean Extensionist
	Phillip Miklas-USDA-ARS, Prosser, WA, Plant Geneticist [Achievement Award]
1999	James R. Baggett - Oregon State University, Plant Breeder
	James S. Beaver - University of Puerto Rico, Plant Breeder
	Phillip McClean - North Dakota State University, Geneticist [Achievement Award]
	James Myers - Oregon State University, Plant Breeder [Achievement Award]
2001	Dermot P. Coyne - University of Nebraska, Plant Breeder [Frazier-Zaumeyer Distinguished Lectureship]
	Mark J. Bassett – University of Florida, Plant Geneticist
	Soon J. Park – Agriculture and Agri-Food Canada, Plant Breeder
	Mark A. Brick – Colorado State University, Plant Breeder [Achievement Award]
	Ron Riley – Syngenta, Plant Breeder [Achievement Award]
	Juan Carlos Rosas – Escuela Agricola Panamericana, Honduras, Plant Breeder
2003	Fredrick A. Bliss – Seminis Seeds, Plant Breeder [Frazier - Zaumeyer Distinguished Lectureship]
	Steve Beebe – CIAT, Colombia, Plant Geneticist
	Paul Gepts – University of California, Plant Geneticist
	Marcial A. ‘Talo’ Pastor-Corrales – USDA-ARS, Beltsville, Plant Pathologist
2005	Perry B. Cregan – USDA-ARS, Beltsville, Geneticist, Soybean Genomics [Frazier - Zaumeyer Distinguished Lectureship]
	Jorge A. Acosta Gallegos, INIFAP, Mexico, Plant Breeder
	Phillip N. Miklas, USDA-ARS, Prosser, Plant Geneticist (BIC **President**, 2010-2019)
	David M. Webster, Seminis Seeds, Plant Breeder
	A. ‘Bert’ Vandenberg, University of Saskatchewan, Plant Breeder [Achievement Award]
2007	Molly Jahn – University of Wisconsin, Plant Geneticist and Dean CALS [Frazier - Zaumeyer Distinguished Lectureship]
	Robert L. Gilbertson, University of California-Davis, Plant Pathologist

	Walter Edwin (Ed) Kee Jr. University of Delaware, Vegetable Specialist Hans Henning Muendel, Agriculture and Agri-Food Canada, Lethbridge, Plant Breeder Matthew W. Blair, CIAT, Colombia, Plant Breeder [Achievement Award]
2009	Maurice Bennink, Michigan State University, Nutritionist [Frazier - Zaumeyer Distinguished Lectureship] Henry Thompson, Colorado State University, Nutritionist [Frazier - Zaumeyer Distinguished Lectureship]
2009 continued	Mark Brick, Colorado State University, Plant Breeder
2011	Phillip McClean, North Dakota State University, Geneticist [Frazier - Zaumeyer Distinguished Lectureship] Kenneth F. Grafton, North Dakota State University, Plant Breeder and Dean, Director, & Vice President of Agriculture Juan Jose Ferreira Fernández , SERIDA Spain, Plant Breeder [Achievement Award] Timothy G. Porch, USDA-ARS, Mayaguez, Plant Geneticist [Achievement Award] Carlos A. Urrea Florez, University of Nebraska, Plant Breeder [Achievement Award]
2013	James D. Kelly, Michigan State University, Plant Breeder [Frazier - Zaumeyer Distinguished Lectureship] James Nienhuis, University of Wisconsin, Plant Breeder K. Peter Pauls, University of Guelph, Plant Geneticist Kirstin E. Bett, University of Saskatchewan, Plant Geneticist [Achievement Award] Thomas Smith, University of Guelph, Research Technician [Technical Merit]
2015	Paul Gepts, University of California-Davis, Plant Geneticist [Frazier - Zaumeyer Distinguished Lectureship] Karen A. Cichy, USDA-ARS, East Lansing, Plant Geneticist [Achievement Award] Juan M. Osorno, North Dakota State University, Plant Breeder [Achievement Award]
2017	David M. Kramer, Michigan State University, Photosynthesis and Bioenergetics [Frazier - Zaumeyer Distinguished Lectureship] Maria Celeste Gonçalves-Vidigal, Plant Geneticist [Meritorious Service Award] Gregory V. Varner, Research Director [Meritorious Service Award] Irvin E. Widders, Director of the Legume Innovation Lab [Meritorious Service Award] Deidré Fourie, ARC Grain Crops Institute, Plant Pathologist [Achievement Award] Clare Mukankusi Mugisha, CIAT Uganda, Plant Breeder [Achievement Award] Rian Lee, Research Technician [Technical Merit Award] Evan M. Wright, Research Technician [Technical Merit Award]
2019	James Beaver, University of Puerto Rico, Plant Breeder [Frazier - Zaumeyer Distinguished Lectureship] Juan Carlos Rosas, Zamorano University, Plant Breeder [Frazier - Zaumeyer Distinguished Lectureship] James R. Myers, Plant Breeder and Geneticist [Meritorious Service Award] Sara F. Rose, Vice President at Bush Brothers and Company [Meritorious Service Award] Frédéric Marsolais, Research Scientist [Achievement Award] Albert Jody Vander Wal, Research Technician [Technical Merit Award]
2021	Biennial meeting held online due to COVID. No awards.
2023	Karen Cichy, USDA-ARS, MI, Plant Breeder/Geneticist [Frazier - Zaumeyer Distinguished Lectureship]

Raymond Glahn, USDA-ARS, NY, Research Physiologist [Frazier - Zaumeyer Distinguished Lectureship]
Daniel G. Debouck, International Center for Tropical Agriculture (CIAT), Bean germplasm curator [Meritorious Service Award]
Antonio de Ron, Spanish National Research Council (CSIC), Pontevedra, Spain, Bean breeder [Meritorious Service Award]
Emmalea Ernest, University of Delaware Newark, Delaware, lima bean breeder [Achievement Award]
Consuelo Estevez the Jensen, University of Puerto Rico-Mayaguez (UPRM), Bean pathologist [Achievement Award]
Aldemaro Clara, CENTA San Salvador, El Salvador, Bean breeder/agronomist [Achievement Award]
Antonia Palkovic University of California, Davis Davis, California, Research Technician [Technical Merit Award]

Please consider nominating your colleagues for the 2025 BIC Awards. Details on nominating colleagues are provided below

2025 BIC AWARDS – NOMINATION REQUEST

The Bean Improvement Cooperative has proudly acknowledged outstanding contributions made by many of its members to bean research and education. The **Meritorious Service Award** has been presented to over 75 of our colleagues during the 66-year history of the BIC. These recipients have devoted many years of their illustrious careers to bean research and education, and have consistently provided outstanding service to our organization.

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

The BIC Coordinating Committee and Awards Committee proudly also present the **Frazier-Zaumeyer Distinguished Lectureship**. The purpose of this lectureship is to honor an individual who has contributed significantly to bean research over the past 5-10 years or longer. The recipient will provide the keynote address and a short publication (maximum of 6 pages) for the BIC report. The recipient is not excluded from receiving the BIC Achievement Award or the Meritorious Service Award. Further details can be acquired from the BIC Awards Committee Chair.

The Technical Merit Award recognizes outstanding and long-standing contributions made to bean research, extension and education by bean program support personnel. Criteria used in selection by the BIC Awards Committee, with approval by the BIC Coordinating Committee, are as follows:

- minimum of 10 years of service as a Bean Program Technician, Associate, Assistant
- membership for 5 years in the BIC is desirable
- 1-page typewritten summary giving place of birth, date and name of institution granting each degree, career history
- summary of accomplishments, innovations, and impacts made by the nominee to the Bean Program and stakeholders locally, nationally and/or internationally
- evidence of participation in development and release of at least one improved germplasm line and/or cultivar; or contribution to the development and application of a technique or process to further knowledge related to pulse crops, pathogens or pests, nutritional uses
- evidence of participation in publication of at least one refereed research and/or extension article

Nomination for these awards should be sent to the BIC Awards Committee Chair (see below). The awards will be presented at the next BIC Biennial Meeting to deserving candidates nominated by their peers and selected by the BIC Awards Committee. Award recipients will be acknowledged at the thirty-third Anniversary of the BIC/NAPIA Biennial Meeting at Lincoln, Nebraska, on the 5th of November 2025.

BIC AWARD NOMINATION
Return by July 15, 2025 to:

Carlos Urrea
Panhandle Research & Extension Center
University of Nebraska- Lincoln
4502 Avenue I
Scottsbluff, NE 69361
currea2@unl.edu

The other Awards Committee member is Dr. James Myers

Nominee: Name: _____

Address: _____

_____

_____

_____

Discipline: _____

Nominated for: _____ Meritorious Service Award

_____ Achievement Award

_____ Frazier-Zaumeyer Distinguished Lectureship

_____ Technical Merit Award Nomination

Submitted by: _____

Date of Submission:_____

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

FIRST ANNOUNCEMENT FOR THE BIENNIAL BIC/NAPIA 2025 MEETING

The 33rd BIC Meeting will be hosted by Clemson University
Nov 3-6, 2025

Our BIC/NAPIA meeting website is up and running: https://agronomy.unl.edu/bic-napia/. This should be your central hub for all meeting information. Special thanks to Carlos Urrea and his local team who put this together nicely. Now with the website up, I have few items to mention:

1- Meeting dates/location: November 4-6, 2025, Embassy Suites Hotel, 1040 P Street, Lincoln, Nebraska. A draft/general agenda/schedule is available online (https://agronomy.unl.edu/extension-outreach/conferences/bic-napia-biennial-meeting/bic-napia-biennial-meeting-agenda/). Additional meetings include a root rot workshop, the W-5150 annual meeting, the BIC Genetics Committee, and the Phaseolus Crop Germplasm Committee.

2- Accommodations: A block of rooms has been secured for $139/night + taxes. Please make your reservation as soon as possible and no later than Oct. 13, 2025. In order to guarantee a room at this price, please use the following link to make a reservation: https://www.hilton.com/en/attend-my-event/beanimprovementcooperativenorthamericanpulseimprov/

3- Registration and abstracts for poster or oral presentations forms are also open in the website.

   a. Conference registration: https://cvent.me/qDNZmZ
   b. Poster/Oral abstract submission deadline for abstracts is August 31st 2025: https://agronomy.unl.edu/extension-outreach/conferences/bic-napia-biennial-meeting/bic-napia-biennial-meeting-2025-abstract/

4- Graduate students can apply to travel awards using the link available in the website. Submission deadline is August 10th 2025: https://forms.gle/sT1WVEzA6hgiKRs89

5- If you need a letter of invitation for your institution or for visa purposes, please send an email to Carlos Urrea (currea2@unl.edu) and he will provide you one.

Business meetings
Business meetings, including the W-4150, the Phaseolus Crop Germplasm Committee, and the BIC Genetics Committee Meetings, will be held on Thursday Nov 6. In addition, a workshop focused on Root Rots will also be held on the same day (please check our website for details). All these meetings are open to anyone interested.

Awards
Please consider your colleagues for the Frazier-Zaumeyer Distinguished Lectureship and for the Distinguished Achievement, Meritorious Service, or Technical Merit Awards.

Contacts
Our local BIC host are organizing the meeting:
Dr. Carlos Urrea, dry bean breeder at UNL (currea2@unl.edu)
BIC business contacts: Dr. Juan M. Osorno (BIC President and Treasurer; juan.osorno@ndsu.edu)

Minutes
BIC Genetics Committee Meeting
University of Delaware, Carvel Research & Education Center, 16483 County Seat Hwy, Georgetown, DE 19947
Tuesday, 08/20/2024, 4:40pm to 6:00pm

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

Participating in person: Sandra Branham, Karen Cichy, Travis Parker, Juan Osorno, Christine Diepenbrock, Ruifeng (Ray) He, David Gang, Emmalea Ernest, Tim Porch
On-line: Phil Miklas, Phil McClean, Celeste Goncalves-Vidigal, Paul Gepts, Jenna Hershberger, Valerio Hoyos, Carlos Urrea

A. Old Business

The Genetics Committee minutes from the 2023 BIC meeting were approved (1st and 2nd) and were published online at:

B. New Business:

1. Review gene list amendments (3:32-3:50)
    a. Seed color updates from Travis:
        i. *T*: Self-colored seed coat and colored flowers (Emerson 1909a; Lamprecht 1934b; Shaw and Norton 1918). *T* is located on Pv09 (McClean et al. 2002). *Phvul.009G044700, a* WD40-repeat gene model homologous to *TRANSPARENT TESTA GLABRA1* in Arabidopsis, has been proposed for control of *T* (Parker et al. 2024, McClean et al. 2024). At least seven characterized independent putative mutations have been identified at the locus.
        ii. t^{cf}: superscript cf, *colored flower*: a seed coat gene (from PI 597984) for partly colored patterns without pleiotropic expression for white flowers; necessary for expression of the two-points pattern (Bassett et al. 1999a). Whole-genome sequencing has identified a putative 22 Mb inversion in the 3' UTR of the *T* candidate gene model in types with the t^{cf} mutation (Parker et al. 2024).
        iii. *Bip: bipunctata* (Latin): *Bip* and *bip* combine with *Arc* and *arc* to form seed coat patterns based on the hilum; extends seed coat color in partly colored seeds (Lamprecht 1932d, 1940b). Genotype *t z bip* expresses the bipunctata pattern of partly colored seed coats; whereas *t z Bip* expresses virgarcus pattern (Bassett 1996c; Schreiber 1940). *Bip* is linked to *J* and is located on Pv10 (McClean et al. 2002). *Phvul.010G098500*, a bHLH-encoding gene model with sequence homology to *P* in common bean, was proposed for its control (Parker et al. 2024).
        iv. bip^{ana}: Anasazi pattern of partly colored seed coats is expressed by genotype *t Z* bip^{ana}; whereas *t z* bip^{ana} expresses the Anabip pattern

(Bassett et al. 2000). A non-synonymous SNP in a conserved residue of *Phvul.010G098500* has been proposed for control of the allele (Parker et al. 2024).

v. p^{hbw}: stippled seed coat (different from p^{stp}) and violet flowers with the lower (superscript hbw) *half* of the *banner* petal *white* (Bassett 1996a, 2003a). A 612 bp intron deletion in the *P* gene model is found in types with this allele and was proposed to control the pattern (Parker et al. 2024). The intron deletion eliminates conserved sequence motifs and is associated with a 20-fold reduction in *P* expression (Parker et al. 2024).

Co genes

1. Celeste has contacted him about Indian paper--Chrom. 10 (Indian group)—New *Co-18* gene symbol requested (used race 3) from KRC-5 (Lateef et al., 2024)
2. *Co* genes and locations—*Co-5* no location others (Pv07 Susa et al.).
3. Contact author after checking on *Co-11, Co-8, Co-12*?—allelism tests.
4. Celeste tried to make *Co-11* and *Co-12* crosses, but no results
5. No isolate information included; Reference information not provided
6. Clusters of genes differ from genotype to genotype, so would need to sequence them.
7. To do: Disclaimer that not confirmed, but do add to gene list.
8. (Travis contacted the authors to discuss in more detail

CoPv01^CDRK/PhgPv01^CDRK: on Pv01; Nomenclature not according to standards

1. From CDRK (Gonzalvez-Vidigal et al., 2020; Lovatto et al., 2023)
2. Separate gene or same cluster?—why not identify it as a separate as Co gene. Need to adhere to consistent nomenclature
3. Transcriptome or QTL mapping results?
4. Review outside of the meeting: Decide by email (Travis will send out the papers and suggestions). Continue with nomenclature, add in other information as details or retain the same symbols as in the publication; Miklas--Indicate that in the Co-1 complex—a bunch of candidate genes that part of the cluster; Several 211kb away from phg-1, so not very highly linked; Organize resources to map out genes/QTL so can serve as a community resource.

2. Discussion: Integrating genomics-based discoveries with classical nomenclature (3:50-4:00)
    a. Genes increasingly being named after homologs or gene models with no other name… how to integrate?
    b. How to handle large-effect QTLs? Should these be included at all?
    c. List in table format? E.g., (*St* | *PvINDEHISCENT* | *Phvul.002G271000* | [description] | Pv02 | flanking markers | population, environment, test on panels across gene pools—MDP/ADP, selectable markers--separate….)
        i. This could possibly help in assessing problematic/useful linkages

1. List in a table format the genes and their locations in a table format to have a more useful resource and reference (from the List of Genes only).

a. Like the Tm shift table. Keep list for description.
b. No QTLs—only validated genes from list.
c. Having two studies confirm results—suggested from other crops.
d. In Excel to sort and work with and make it useful—Travis will start table and then others can contribute to.
e. Soltani dormancy genes in grey zone
f. Shattering could be included…

3. Preferred genotyping strategies (4:00-4:05)?
    a. Best methods for SNP sets in particular; future status of 12K BeadChip
    1. Bead chip great resource, but we are losing these resources and not so available any longer.
    2. Genome sequencing—cheaper, Kmer analysis
    3. SNP chips—Thermo Fisher chip is about $10/sample for 50-100 markers
        a. Agroseq panel; per marker price; Chickpea 5,000 (85% amplifying regularly). Ion torrent based…
        b. Thermo Fisher--50k for 6,000 samples and then $10/sample cost. 4,000 SNPs for NDSU for trait-based selection
        c. GS—narrow down to 500 markers certain programs (trait-based efforts); working on 25 traits in barely (quality)—reduce field testing by half.
        d. Illumina chip—more for mapping, broad diversity
        e. Hudson Alpha—been slow for UC Davis; not clean necessarily
        f. Whole genome sequencing—good, but cost higher
        g. Pan-Genome—Valerio using 12k chip work
        h. PACE and KASP markers useful as well
    4. GBS platform another option

4. Discussion (4:05-4:25): Is it worth pursuing a larger grant to take on some of the bigger genetics questions in a coordinated and systematic way?
    a. For example, SCRI SREP? https://www.nifa.usda.gov/sites/default/files/2023-10/FY24-SCRI-Pre-App-RFA-508-P_0.pdf
    b. Topics: divide-and-conquer on the major disease resistances and seed type genes?
    c. Pre-breeding to move a set of major resistances into each major market class?
    d. Improve genomic resources?
        i. Whole-genome sequencing → Expanded MDP, ADP, DDP, SnAP, etc.?
        ii. Improved G19833 reference?
    e. Other priority areas suggestions?
    1. SCRI Popping beans grant has come through
    2. SCRI for Lima bean
    3. AFRI small bean project also funded--shelling
    4. Other opportunities with opportunities for dry beans
        a. Unlikely to get another SCRI after popping bean project
    5. NSF plant genome grant opportunity
    6. Whole-genome sequencing—extracting out MDP, ADP, DDP, SnAP coordinates

a. Expand work with whole-genome sequencing
b. Trait of interest—select markers to move phenotype based on background
c. Include genes that identified—seed coat color McClean.

7. Improved G19833 reference—telomere to telomere sequence (Phil McClean already working on)

C. Membership updates and other business (4:25-4:30)

-Sandra, Ray, Christine new members

-motioned Juan and 2nd by Tim

FLOWERING TIME OF *Phaseolus coccineus* OVERWINTER ROOT RESPROUTS PLANTS IN MEXICO

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INTRODUCTION. This work was based on field data obtained in 2013-2014. It is one of a series of results obtained by the study of plant morphology, phenology, and their interaction with the original collection sites climate of 500 *P. coccineus* Mexican native varieties. This is a 'snapshot' of plants developed from early flowering root resprouts.

In Mexico, the photoperiod required of *P. coccineus* landraces plants to bloom depends on the planting date. However, there is no information about the photoperiod required for overwinter plants arising from root resprouts (Vargas-Vázquez *et al*, 2024). We compared: 1) the photoperiod and temperature required to initiate flowering time of seed plants and root resprouts plants, 2) registered the early flowering landraces developed from root sprouts, and 3) identified its original collection sites to know where do they come from.

MATERIALS AND METHOD. In Texcoco, Mex. on April 23, 2023, 70 *P. coccineus* landraces were planted in a completely randomized design with seven replications. The seeds were harvested between September and October, leaving the thick and fleshy tap-root inside the soil (Vargas-Vazquez 2023), and on March 10, 2014, identified those whose roots developed shoots from root-sprouting plants that already had flower buds and even open flowers. We compared the photoperiod, maximum and minimum ambient temperature on the day of flowering (García and CONABIO 2024) in plants growing from seeds in summer 2013, and those developed from root resprouts in late winter/early spring, March 10, 2014. An ANOVA and a Tukey mean comparison analysis were done with the SAS program.

RESULTS.

Plant type	**Flowering time**	**Natural photoperiod h: min**	**Photoperiod Decimal mean value**	**Maximum temperature Mean value °C**	**Minimum temperature Mean value °C**
Seeds plants in 2013	June 3 to June 30	13:15 to 13:18	13.26 **a**	28.42 **a**	8.05 **a**
Root resprouts plants in 2014	March 12 to April 28	11:58 to 12:49	12.18 **b**	25.70 **b**	7.33 **b**

Planting date: April 22, 2013

In seedling and root regrowth plants, the first sign of reproductive development was the appearance of flower buds. The *P. coccineus* seedling plants required longer photoperiod and higher temperature to initiate flowering time than the root regrowth ones.

(a) (b) (c)

P. coccineus native varieties plants: (a) seed plants, (b) root regrowth plant without flowers, and (c) an early flowering root regrowth plant.

These results suggest root regrowth influences the plant's response to photoperiod signals to start its reproductive stage earlier than seed plants, and what benefits does this provide to the plant?

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García E, and Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (COBNABIO). 2024. Temperatura Máxima Promedio. Catálogo de Metadatos Geográficos. Escala 1:11,000,000. http://www.conabio.gob.mx/informacion/gis/?vns=gis root/clima/temper//tmaxplmgw

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FLOWERING RESPONSE TO PHOTOPERIOD OF *Phaseolus coccineus* L. MEXICAN NATIVE VARIETIES

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INTRODUCTION. Species with food potential must have experienced a long natural selection to reach, in harmony with photoperiod and temperature, the flowering phase and develop fruits that ripened at the appropriate time in a given location (Aitken 1974). The aim of this study was to register the flowering time of *P. coccineus* native varieties, to explore under different planting dates, the plant response to photoperiod in Texcoco Mexico. Central and southern *P. coccineus* native varieties, included in this work, could contribute to expand the species cultivation in the world for human feeding, as dry seeds or green vegetable pods. Moreover, in certain rural Mexican markets, flowers are sold and consumed as vegetables in soups.

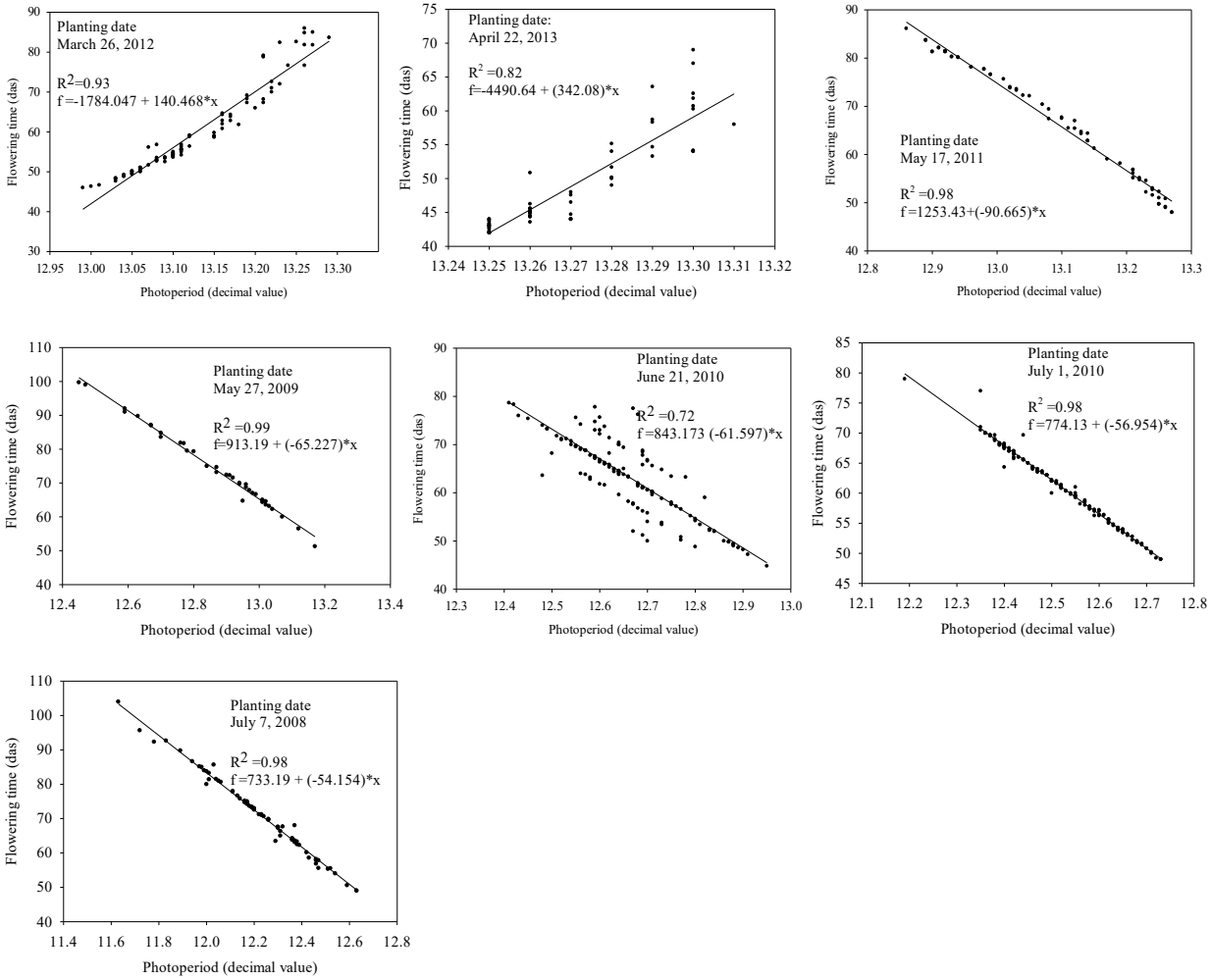
MATERIALS AND METHODS. In Texcoco, Méx., period 2008-2013, 403 seed landraces were planted. In 7 yearly sets there were sown different native varieties from Mexican highlands. Each year, different ones were planted, and planting dates varied by year. The experimental plot by landrace was a five m length with a 0.80m row separation, plots were distributed randomly. Seven plants per landrace were registered. **Variables**: flowering initiation time (the first visible flower bud has its whitish or reddish color) and photoperiod registered that day (https://doi.org/103389//fpls.2020.599426).We made graphs and regression analyzes by planting date with the Sigma Plot statistical program (SigmaPlot (RRID:SSR_003210).

RESULTS. In one year, photoperiod in Texcoco is from 10:59 h in Jan1st, increasing gradually until 13:19 h in June 19-22, and decreases again until 10:59 h in Dec. 31. The climate is temperate and semi-arid with a median temperature of 15.9 °C. Few frosts. Rains come between Jun and Oct (https://en.wikipedia.org/wiki/Texcoco_de_Mora#:~:text=The%20remains%20of%20Lake%20Texcoco,months%20of%20June%20and%20October)

Planting dates description. In Texcoco (19°29' NL, 98°53° WL) according to its photoperiod, the planting dates were classified as early, intermediate and late planting dates.

In **March 26 and April 22** planting dates, flower initiation was in May 7-20, when photoperiod was increasing through time, these were considered **early planting dates**; In **May 17 and May 27** planting dates, flowering initiation was on June-July, when photoperiod has just begun to decrease after reaching its peak on June 21, and these were considered **intermediate planting dates**; The **June 21, July 1 and July 7** planting dates promoted flower initiation until August when photoperiod continues to decrease down the course of descent, these were considered **late planting dates**.

There was a highly significant interconnection between photoperiod and flowering initiation time. Regression analysis (flowering time *vs* photoperiod) was all highly significant. In March and April sowings, plants reached their flowering time in May and June, as the photoperiod increased, reached their peak and began their decline. In sowings made in May-July, the plants flowered from early June until mid-September, when the photoperiod has already reached its maximum and is in decline.



Photoperiod and flowering time in plants of Mexican native varieties of *P. coccineus* grown in seven planting dates. Different genotypes each planting date.

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PERFORMANCE OF DETERMINATE LIMA BEAN LINES IN PUERTO RICO AND HONDURAS

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Green-shelled bean (*Phaseolus vulgaris* L.) production is common in Puerto Rico. Landrace varieties of lima bean (*Phaseolus lunatus* L.) in Puerto Rico are indeterminate and generally photoperiod sensitive (Montero-Rojas et al., 2013. *Genet Resour. Crop Evol.* 60: 2241–2252). The availability of an adapted photoperiod insensitive, determinate, white-seeded lima bean cultivar would provide farmers with an alternative crop for the local green-shelled bean market and gardeners with a lima bean that would be easier to manage. Nine breeding lines derived from a cross between 'Sieva', an indeterminate, white-seeded cultivar, and 'Beseba' (G27525), a determinate landrace from Haiti, and the check cultivar 'Henderson's Bush' were tested in Puerto Rico and Honduras. Beseba was chosen as a parent because the cultivar had been reported to express resistance to *Bean golden yellow mosaic virus* (BGYMV) in Honduras. The nine breeding lines were insensitive to the range of photoperiods in Puerto Rico (11-13 hours) which would allow green-shelled lima beans to be produced throughout the year. In fact, the highest seed yields were obtained from the trial planted at the Isabela Substation in June 2021 (Table 1). The lima bean lines set pods over a longer period than common beans. Greater seed yields were obtained when two harvests were conducted (Table 1). This may be a useful trait for home gardeners by allowing the harvest of fresh pods over several weeks. In the trials where one harvest was conducted mean seed yields ranged from 833 to 875 kg/ha. PR2126-81 and PR2126-40 had mean seed yields > 1,000 kg/ha in the three trials with single harvests. In the trials where two harvests were conducted, mean seed yields ranged from 1,458 to 2,374 kg/ha. PR2126-10 and PR2126-40 had average seed yields > 2,000 kg/ha in the three trials with double harvests. Hundred seed weights of the nine lines ranged from 28.9 to 33.3 g and days to maturity ranged from 82 to 85 days after planting in a drought trial at the Juana Diaz UPR Substation in February 2023. Asian bean flower thrip (*Megalurothrips usitatus*) damage to flowers and pods was observed in the trials harvested at the Isabela Substations in 2023, 2024 and 2025. In general, the lima bean breeding lines showed less thrip damage than the common beans planted in the same fields. In 2024, application of insecticide was suspended about 6-7 weeks after planting which allowed an increase in leafhopper (*Empoasca* spp.) pressure. Significant differences were observed in reaction to the leafhoppers. 'Henderson's Bush', had the most severe leafhopper symptoms whereas several of the lima bean breeding lines had moderate resistant scores ranging from 4.0 to 4.2. The lima bean lines were exposed to BGYMV pressure in a trial planted at Zamorano University in 2024. Significant differences were noted between lines in BGYMV symptoms during the vegetative stage of development. A sample of 10 seeds of each lima bean line was infested with the common bean weevil (*Acanthoscelides obtectus*). At 30 days after infestation, the lima bean lines had less weevil damage than the susceptible common bean lines 'Badillo', 'Verano' and 'Bella' (Table 1). It has not yet been determined if the differences in weevil damage between lima bean lines were related to HCN content in the seed.

Table 1. Performance of determinate lima bean lines in trials planted in Puerto Rico and Honduras.										
Line	2021 Isabela two harvests	2022 Isabela one harvest	2023 Isabela one harvest	2023 Fortuna one harvest[1]	2024 Isabela two harvests[2]	2025 Isabela two harvests[1]	Mean seed yield	2023 bruchid at 30 days after infestation[3]	2024 Isabela leafhopper score[4] (1-9)	2024 Honduras BGYMV score[4] (1-9)
	Dry seed yield (kg/ha)									
PR2126-8	3086	567	823	624	1473	1430	1334	3.0/10	4.6	5.8
PR2126-10	2974	774	1064	651	2098	1592	1526	3.0/10	4.2	5.6
PR2126-61	2527	939	805	776	1920	1488	1409	3.5/10	4.0	4.5
PR2126-81	2415	779	1075	1355	1724	1316	1444	0.5/10	4.6	6.0
PR2126-46	2389	962	842	861	1616	1522	1365	2.5/10	4.0	5.8
PR2126-40	2296	1258	941	904	2127	1679	1534	5.0/10	5.2	7.0
PR2126-22	2270	941	807	776	1494	1428	1286	0.0/10	5.4	2.4
PR2126-41	2217	876	752	674	1898	1324	1290	0.0/10	4.0	4.0
PR2126-59	2211	662	787	870	2065	1469	1344	0.5/10	4.2	7.6
Henderson's bush	1354	992	936	879	1258	1335	1126	1.0/10	8.2	7.6
Mean	2374	875	833	837	1767	1458	1366		4.8	5.6
LSD (0.05)				NS	594	NS	NS		0.7	2.3
CV (%)				23.3	26.2	15.9	18.9		12.1	31.4
							Badillo	9.5/10		
							Verano	9.0/10		
							Bella	6.0/10		

[1] Drought trial

[2] High infestation of the Asian bean flower thrip.

[3] Number of seeds with weevil damage/Total number of seed.

[4] Rated using the CIAT (1-9) scale where 1 = no damage and 9 = very severe damage (van Schoonhoven, A., and M.A. Pastor-Corrales, editors. 1987. Standard system for the evaluation of bean germplasm. CIAT, Cali, Colombia).

GENETIC DIVERSITY OF BLACK BEAN BREEDING LINES AND CULTIVARS EXPRESSED BY MORPHOAGRONOMIC TRAITS

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INTRODUCTION: The common bean (*Phaseolus vulgaris* L.) is the main legume consumed in Brazil, standing out as a source of proteins and nutrients, especially iron and zinc. Black beans present protein content ranging from 17.9 to 31.1 %. They are notable for their abundance of essential amino acids, isoflavones, and anthocyanins. In addition to vitamin E, and carotenoids, increasing nutritional and functional properties (Guerrero-Becerra et al., 2025). The evaluation of morphoagronomic traits allows the identification of genetic variability within the genotypes and the selection of the most promising ones for the development of new cultivars. Thus, the aim of this study was to evaluate the contribution of the morphoagronomic descriptors to the genetic diversity of black common bean cultivars and breeding lines developed by the breeding program of Rural Development Institute of Paraná State– IAPAR-EMATER (IDR-Paraná).

MATERIAL AND METHODS: Twenty genotypes were evaluated, of which 15 were breeding lines developed by the IDR‑Paraná breeding program and five cultivars - IPR Tuiuiú, IPR Uirapuru, IPR Urutau, BRS Esteio e IAC Veloz. The experiment was established in the 2023/24 rainy season, at the IDR‑Paraná experimental station in Londrina, Paraná State, Brazil located at 23°55'46" S and 50°52'23" W, with an altitude of 508 meters. The design used was a randomized block with three replications, plots of four rows, four meters long, with 0.5 m between lines. Also a population of 12 plants per linear meter, with the two central rows being considered as a useful plot. The morphoagronomic characterization was performed using the 56 minimum descriptors proposed by the Ministry of Agriculture, Livestock and Supply ‑ MAPA through the National Cultivar Protection System – SNPC, and 9 descriptors related to yield components. To assess genetic diversity between accessions, from the data obtained, principal component analysis (PCA) and hierarchical grouping using the Gower dissimilarity matrix and the Ward´s grouping method were performed. The analyzes were conducted using the R software (R CORE TEAM, 2020).

RESULTS AND DISCUSSION: The first two components of the PCA together explained 51,9% of the variability present between the breeding lines and cultivars evaluated in this study (Figure 1). The variables that contributed most to the formation of the first component were days to florewing (Q1), plant length (Q2), number of locules per pod (Q10), number of nodes (Q9), and number of grains per pod (Q11). In the formation of the second component, the characteristics that contributed most were number of grains per plant (Q13), insertion height of the first pod (Q8), and the number of pods per plant (Q12).

The genetic divergence analysis grouped the genotypes into four main groups (Figure 2). The first group was formed by IAC Veloz and LP 6, genotypes with the shortest cycles to flowering (37 days). The shortest plants (61 cm), and the higher number of pods per plant (23). Six breeding lines comprised the second group, presenting a greater 100-grain mass (26 g) and a higher insertion

height of the first pod (25 cm). The third group consisted of four genotypes that showed the largest number of grains per plant (64). Finally, the fourth group was composed of six breeding lines and two cultivars, which showed the highest yield, with an average of 2.325 kg ha^{-1}. In particular, the L9 genotype presented the highest yield, 2.729,89 kg ha^{-1}.

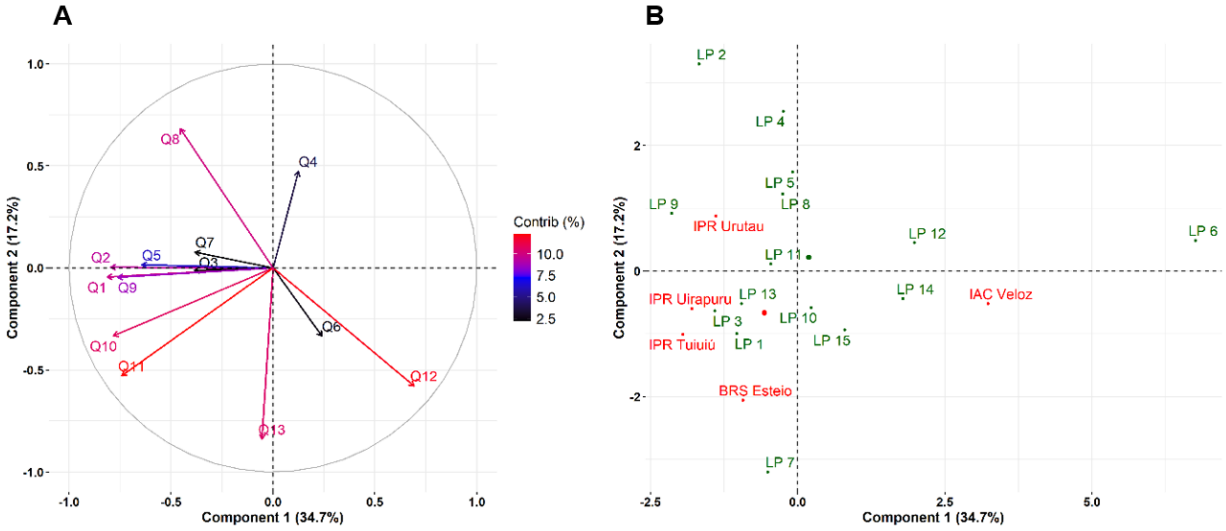

Figure 1. Principal component analysis (PCA) of black common bean cultivars and breeding lines Contribution of variables (A) and distribution of accesses in relation to the first two components (B). Q1: days to flowering, Q2: plant length (cm), Q3: pod length (cm), Q4: mass of 100 grains (g), Q5: total cycle (days), Q6: general visual note, Q7: yield (kg ha^{-1}), Q8: insertion height of the first pod (cm), Q9: number of nodes, Q10: number o locules per pod, Q11: number of grains per pod, Q12: number of pods per plant, and Q13: number of grains per plant.

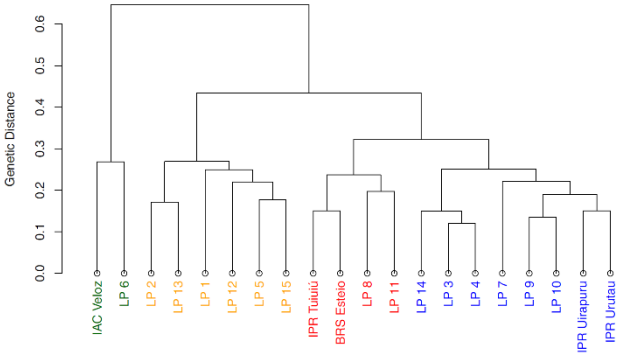

Figure 2. Dendrogram generated from Gower's dissimilarity matrix and Ward's grouping method of 20 black bean genotypes through qualitative and quantitative variables.

CONCLUSIONS

The evaluated traits enabled the discrimination and the diversity study between the cultivars and breeding lines. It was possible to observe the superiority of some breeding lines for desirable characteristics (e.g. yield) in relation to the cultivars present in this study.

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ACKNOWLEDGEMENTS

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GENETIC ARCHITECTURE OF IDEOTYPE-RELATED AGRONOMIC TRAITS IN COMMON BEAN (*PHASEOLUS VULGARIS* L.) THROUGH SINGLE-, MULTI-TRAIT GWAS AND EPISTASIS ANALYSIS

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INTRODUCTION

Several studies have identified quantitative trait loci (QTL) involved in the control of agronomic traits in common bean populations (Hoyos-Villegas et al., 2017; Moghaddam et al., 2016). Depending on the location, new validations lead to the discovery of additional loci or genes that may contribute to the observed variation in the traits (Sahito et al., 2024). Despite the enormous contributions and benefits of GWAS in QTL mapping, some limitations need to be overcome. Improved models, Multi-trait, and genome-wide epistasis analyses offer new opportunities for the study of the genetic architecture of these traits and future applications in breeding programs.

MATERIALS AND METHODS

The common bean Mesoamerican Diversity Panel (MDP) from BeanCap (Moghaddam et al., 2016) was evaluated during three growing seasons between 2021 to 2023 at McGill University, Montreal, Quebec, Canada with standard agronomic practices. Days to flowering (DTF), days to maturity (DTM), Lodging, and yield were evaluated.

With publicly available genotypic data, single-trait GWAS was carried out using FarmCPU in GAPIT v3. To detect SNPs with pleiotropic effects, multi-trait GWAS was conducted based on the multi-trait mixed model (MTMM) approach by Korte et al. (2012). A three-variance-component multi-locus random-SNP-effect mixed linear model (3VmrMLM) approach (Li et al., 2022) was used to identify quantitative trait nucleotides (QTNs) with epistatic effects (QTN-by-QTN interactions – QQIs).

For both the st-GWA and mt-GWA, the threshold for declaring a significant association was corrected for multiple testing using the False Discovery Rate (FDR) criterion. For epistasis analysis, significant interactions were declared based on the Bonferroni-corrected threshold for multiple testing. Candidate genes were analyzed in a 100 Kbp SNP-centered window using the *Phaseolus vulgaris* reference genome v2.1.

RESULTS

Single-year and multi-year analyses were conducted. For days to flowering (DTF), in the combined analysis, four significant loci were detected on Chr01, Chr02, Chr08 and Chr11, where the SNP S11_50734871 was only 4 bp away from the SNP S11_50734875 identified in the analysis of the third year. For days to maturity (DTM), a total of 18 significant SNPs were identified. In the combined analysis, the SNP S01_34529676 was common with year two and had a maximum estimated effect of 5.4 days (16% PV). In lodging, 19 SNPs were detected for the three analyses with similar percentages of PVE (48% for each SNP). For the combined analysis, S04_4185944 and S06_18742429 colocalized with S04_4185943 and S06_18744301 from year 2 and year 1,

respectively. For yield, 10 significant SNPs were identified in the first year, 5 in the third year and 7 in the multi-year analysis. S04_47063565 explained 13% PV and had an effect of -254.94 units, followed by S02_37292928 with an effect of 80.24 units.

In the multi-trait analysis, the SNPs S04_29597528 and S02_26051524 had marginal (MAF=0.04) but significant interaction effects between flowering and yield. An interaction indicates that the SNP has an opposite effect in each of the traits. Between maturity and lodging, a locus led by the S04_4185944 showed a common effect increasing or decreasing both traits at the same time.

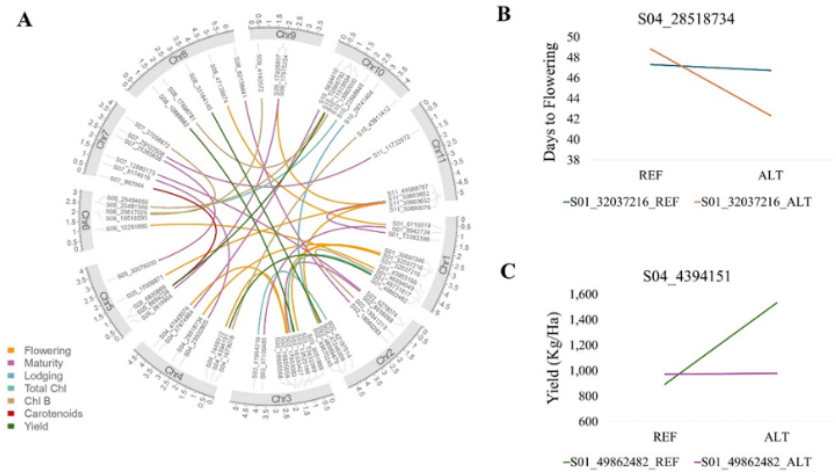

Figure 1. QTN-by-QTN interactions for the agronomic traits (A). Example of epistasis for flowering time (B) and yield (C). REF denotes the reference allele and ALT is the alternative allele in the variant calling against the common bean reference genome v2.1.

For flowering, 10 significant QQIs were detected across the genome with PVE by the interaction between 0.04 to 10.5% and a total of 20.8% when adding the significant interactions. Days to maturity had similar behavior with 10 epistatic interactions in different chromosomes and PVE between 1.49 and 6.98% (total 32.5%). Lodging had one significant QQI between Chr02 and Chr10 with a PVE of 2.65%. In yield, the QQI between S01_49862482 on Chr01 and S04_4394151 on Chr04 explained the 5.19% of the phenotypic variance. When S04_4394151 is considered alone, the observed yield remains at 975 Kg/Ha independent of the allelic state. Nevertheless, when both SNPs are present in the alternative allele, the average yield increases to 1,533 Kg/Ha. Multiple candidate genes were identified for each of the significant markers in the QQI (Figure 1).

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EXTENSIVE ADOPTION OF PINTO SALTILLO COMMON BEAN IMPROVED CULTIVAR IN THE SEMIARID HIGHLANDS OF MÉXICO

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INTRODUCTION. In the semiarid highlands of México, common bean (*Phaseolus vulgaris* L.) is considered the second most important staple crop, used by farmers for family´s food supply and to obtain economic income along the year. Problems were observed for grain marketing in traditional cultivars showing accelerated seed coat darkening. The Pinto Saltillo improved common bean cultivar increased farmers income through higher yield potential and an extended time for negotiation with brokers, related to slow darkening grains. Pinto Saltillo showed high adoption level in North-Central México, mainly in the states of Durango and Chihuahua (Ávila *et al*., 2011) and then its acceptance was expanded to other states and production regions. The objective was to analyze the adoption area reached for Pinto Saltillo improved cultivar in the Semiarid Highlands and other common bean productive areas of México.

MATERIALS AND METHODS. The total area planted with Pinto Saltillo cultivar was consulted at the Statistical Yearbook of Agricultural Production (SIAP, 2025). The database was constructed from 2012 to 2023 including planted area, seed yield and total grain production considering rainfed and irrigated conditions. Differentiated statistics were obtained for Pinto Saltillo showing higher marketing importance. Data was analyzed using descriptive statistics, and the results were displayed by using graphics obtained with the Excel® computer program.

RESULTS AND DISCUSSION. An accelerated adoption process was observed for Pinto Saltillo common bean cultivar mainly in Chihuahua and Durango (Ávila *et al*., 2011). Adoption process resulted in extensive plantings mainly under rainfed conditions, reaching accumulated values from 833,478 ha to 1,668,040 ha from 2012 to 2023 (Table 1). Accumulated values for planted area reached 4.6 million of hectares and 2.6 million of metric tons of grain produced for Pinto Saltillo (Figure 1). Results overpassing previous estimations including reductions in the total area planted with Pinto Saltillo and receding predictions starting in 2020 (González *et al*., 2009). That response was related to an improved acceptance and significant advance for Pinto Saltillo planting area under irrigation in Zacatecas and other states in the Highlands of México.

High areas planted with Pinto Saltillo under irrigation were detected in Zacatecas, Chihuahua and Nayarit, related to off-season refreshing process of the seed used in Durango and other states of the Mexican Highlands. Under irrigation high seed yield were registered for Pinto Saltillo in Guanajuato and Zacatecas (> 2 t/ha). High yield was also reported in Nayarit under rainfed conditions (1.48 t/ha), related to deep soils and high values for rainwater and irrigation, compared to the Semiarid Highlands showing yield variation from 0.41 t/ha to 0.74 t/ha (2012-2023).

In the tropical wet-and-dry region (Nayarit and Sinaloa) Pinto Saltillo was used by local farmers for off-season certified seed multiplication, also adopting this cultivar for grain production, mainly due to the domestic and international market demand. In 2023, Pinto Saltillo was planted in 11 states of México reaching 18,341 ha under irrigation with an average yield of 1.79 t/ha. Under rainfed conditions 281,162 ha were planted using Pinto Saltillo obtaining an average yield of 0.64

t/ha. Traits related to Pinto Saltillo acceptance included disease tolerance, higher seed yield, and slow darkening grains with shorter cooking time. Higher values for consume acceptance and grain prices also influenced the adoption of Pinto Saltillo, improved cultivar developed in Durango. A new commercial class named as 'Pinto Saltillo' was included in government statistics for agricultural production and food market.

Table 1. Accumulated values (2012-2023) for variables related to Pinto Saltillo common bean cultivar adoption and its effects on yield average in several states of México.

State	Irrigation			Rainfed		
	Planted Area (ha)	Total Production (t)	Yield (t/ha)	Planted Area (ha)	Total Production (t)	Yield (t/ha)
Chihuahua	30,840	57,406	1.96	833,478	556,607	0.73
Durango	18,166	28,416	1.62	1,668,040	726,172	0.44
Guanajuato	5,300	12,515	2.38	35,394	17,007	0.52
Nayarit	19,180	29,855	1.48	70,212	96,342	1.32
SLP	4,958	9,498	1.90	428,427	134,306	0.44
Sinaloa	697	1,108	1.54	540	395	0.74
Sonora	8,911	17,806	1.96	--	--	--
Zacatecas	47,838	102,390	2.13	1,387,218	798,917	0.64

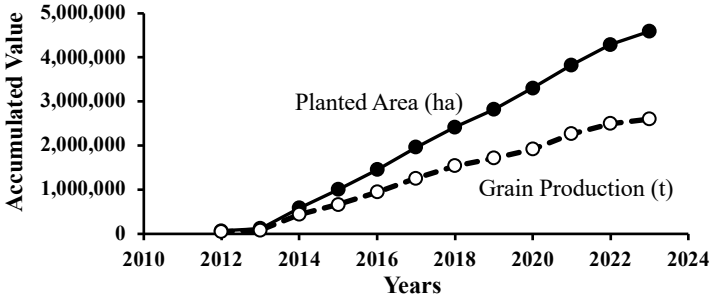

Figure 1. Accumulated value for total area planted and grain production obtained with Pinto Saltillo common bean cultivar in México. 2012-2023.

CONCLUSIONS. Pinto Saltillo improved common bean cultivar showed extensive adoption reaching more than 4.6 million hectares planted in several states of México. More than 2.6 million of tons of grain were obtained with Pinto Saltillo to supply the domestic and international markets. Additional benefits included improved seed yield, grain quality and reduction of the marketing problems registered for traditional pinto common bean cultivars.

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AGROCLIMATIC ZONES SUITABLE FOR GROWING TROPICAL BLACK BEANS IN CONDITIONS OF RESIDUAL HUMIDITY AND ACIDIC SOILS IN SOUTHEASTERN MEXICO

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INTRODUCTION. In southeastern Mexico, terminal drought, together with soil acidity, are the main environmental factors that reduce bean yield (Tosquy *et al*., 2018). This production decline has increased as a result of climate change, so the average yield obtained in the states across this region is less than 800 kg ha^{-1} (SIAP, 2022). In the INIFAP Bean Program for southeastern Mexico, improved cultivars adapted to drought and acidic soil conditions have been developed (Villar and López, 1993; Tosquy *et al*., 2016). However, it is necessary to identify optimal alternative areas that allow maximizing the productive potential of this technology.

MATERIALS AND METHODS. To identify suitable areas, ecological niche models were used with data on the presence of geo-located experimental sites from 2000 to date, generated by the INIFAP Bean Program for southeastern Mexico. These data were obtained from 30 bean genotype yield trials that were conducted under residual moisture conditions with and without terminal drought, and in soils with pH ranging from neutral to strongly acidic, in which grain yields greater than 1,000 kg ha-1 were obtained. This information was used to delimit the calibration area of the model, which was intersected with the terrestrial ecoregions proposed by Olson *et al.* (2001) in order to guarantee that the modeling area included environmental characteristics similar to those of the experimental sites. From the WorldClim (elevation) and Chelsa databases, 19 bioclimatic variables, combining temperature and precipitation, were utilized. Relevant edaphic variables for the crop were obtained from the SoilGrids database, including texture, pH, organic matter, and the content of sand, silt, and clay at depths of 5 and 10 cm. To prevent multicollinearity among all edaphoclimatic layers, a Pearson correlation analysis was conducted ($r > 0.85$). The modeling algorithm used was the minimum volume ellipsoid algorithm. The parameterization and selection of the final model was carried out with the ntbox package in R, following the methodology described by Fadda *et al*. (2024). The model generated environmental suitability values between 0 and 1, which were classified into six categories ranging from 0 - 0.10 (unsuitable), 0.10 - 0.15 (very low suitable), 0.15 - 0.25 (low suitable), 0.25 - 0.50 (moderately suitable), 0.50 - 0.75 (suitable) and 0.75 - 1 (optimal), in each of which the surface area was estimated.

RESULTS AND DISCUSSION. The selected model identified as key variables the clay content, soil pH, annual precipitation, and annual thermal range of greatest ecological and practical relevance for the bean crop. The results indicated that the optimal (2,333 km²) and suitable (9,345 km²) categories for bean cultivation are located in the states of Veracruz (Central, Sotavento, Papaloapan, and Los Tuxtlas sub regions), Tabasco (Central zone and La Chontalpa sub regions), and Chiapas (Los Valles and La Frailesca sub regions) (Figure 1). The moderately suitable areas with 21,848 km² included the central region of Veracruz and the states of Oaxaca and Chiapas, where it is convenient to investigate crop management strategies applicable to specific environmental conditions. The low and null categories (205,814 km²) are found in coastal and lowland areas of southeastern Mexico.

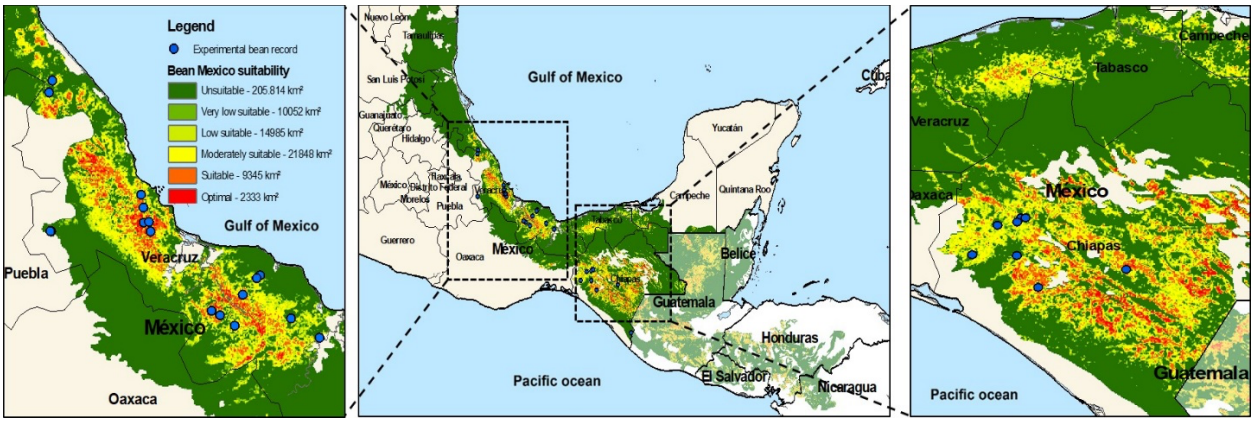

Figure 1. Potential distribution of bean cultivars improved for tolerance to terminal drought and acidic soils. Areas with warmer colors represent higher environmental suitability for crop production.

CONCLUSIONS

A surface area of 11,678 km² with high potential for bean production (including both the optimal and adequate categories) and 21,848 km² of moderately suitable land were identified in the south-southeast region of Mexico, where cultivating the varieties developed by INIFAP is feasible.

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IMPORTANCE OF THE DURANGO COMMON BEAN BREEDING PROGRAM ON SEED YIELD, PRODUCTION AND QUALITY IN MÉXICO

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INTRODUCTION. Common bean (*Phaseolus vulgaris* L.) is considered one of the most important crops in the state of Durango, northern México. Several studies have demonstrated the critical role of common bean breeding programs in increasing agricultural production related to economic development, seed quality, and food availability. A common bean breeding program developed in the state of Durango released several cultivars that have shown high adoption levels by farmers since 1970, showing an important impact on economic and social development, as well as on market quality and human nutrition in several states of México. The objective was to analyze the productive impact of the common bean genetic improvement program on the yield and total grain production and quality in Durango, México.

MATERIALS AND METHODS. Mexican national databases provided the common bean statistics for Durango, Méx. (SIAP, 2025). In Durango, common bean is grown in most of the state's 39 municipalities. The study area was defined by considering state-level data for the most important variables, such as the annual number of planted hectares, seed yield, and total grain production from 1975 to 2023. The planted area was separated, considering water availability, into rainfed (< 95%) and irrigated (5%) conditions. Graphics and trendlines were created using the Excel® computer program.

RESULTS AND DISCUSSION. The results show a gradual reduction of common bean planted area in both water conditions (Figure 1a), reaching -39.2% under rainfed conditions and -63.0% in irrigation area, where forage maize (*Zea mays*) and sorghum (*Sorghum bicolor*) cultivation is preferred. Under rainfed conditions, the reduction in planting area, from 347,000 to 150,000 ha, was also related to forage crops, such as maize and oats (*Avena sativa*), mainly used for cattle feeding. Perennial and industrial crops such as Gregg´s pine (*Pinus greggii*) and agave (*Agave* spp.) are also alternatives for agricultural soils in drought-prone areas. No straightforward advances were observed for seed yield and quality under rainfed conditions due to drought, high disease pressure, and low temperatures causing freeze in some years due to late plantings. Significant yield variations were observed during the study period under rainfed conditions, from 0.15 t/ha in 2011 to 0.82 t/ha registered in 2006 (Figure 1b). For this reason, the common bean breeding program has been criticized in México, showing poor results compared to breeding programs for wheat and maize, mainly produced under favorable conditions (deep soils, fertilizer use, high rain accumulation and irrigation). Significant progress in the common bean breeding program was observed for seed yield under irrigation, showing increments from 10.3 to 31.7%. Commercial and grain cooking quality was also obtained in cultivars such as Pinto Saltillo, PID 1, and PID 2, resulting in high productive volume and improved market acceptance.

Significant reduction for total common bean grain production was registered during the study period (Figure 2), as a result of a diminishing plantation area combined with irregular rain patterns, reducing seed yield and quality. Significant reduction and variable data were observed under rainfed conditions, registering the lowest value in 2023 (13,619 t), considering that a requirement of 18,000 t to supply the domestic demand in Durango. Nearly 200,000 t for total production values were observed in 1996 and 2006, registering commercialization problems due to grain darkening of some pinto cultivars such as Pinto Villa. These problems have been reduced since 2006 by using slow darkening cultivars, including Pinto Saltillo and Pinto Centauro, which have strengthened negotiations between farmers and grain brokers.

CONCLUSIONS. Significant advances (impacts) were obtained for seed yield, drought and disease tolerance, and seed quality by common bean breeding program in Durango, México. Genetic advances are mainly observed under favorable water conditions and permitted the maintenance of common bean production despite the drastic reduction in the planting area. Cultivation of common bean is recommended in areas with irrigation and higher rain accumulation also showing regular distribution to increase seed yield and quality.

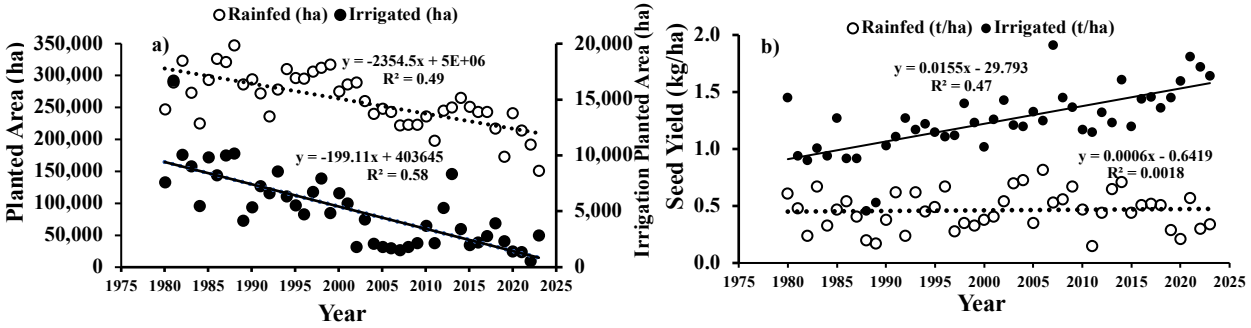

Figure 1. Total planted area (a) and seed yield (b) obtained with common bean in Durango during a 50-year period and two water conditions.

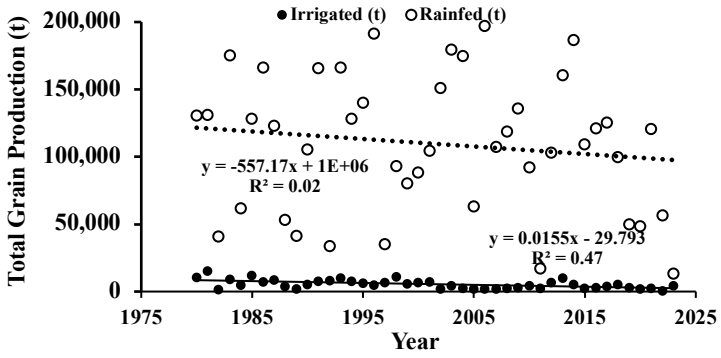

Figure 2. Total grain production for common bean in Durango during a 50-year period and two water conditions.

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EFFECT OF DIFFERENT LIMESTONE SOURCES ON SNAP BEAN (PHASEOLUS VULGARIS) GROWTH AND YIELD

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INTRODUCTION

Snap bean (*Phaseolus vulgaris*) is an important vegetable crop widely cultivated for its high nutritional value and economic significance. However, its growth and productivity are significantly influenced by soil conditions, particularly soil acidity, which can limit nutrient availability and root development. Liming is a fundamental agricultural practice used to correct soil pH, enhance nutrient uptake, and improve overall soil health. Among the various liming materials available, marine shell limestone has gained attention as a sustainable alternative to conventional limestone. Derived from natural shell deposits, this material is known for its high reactivity and potential to supply additional minerals, such as calcium and magnesium, essential for plant growth. This study aims to evaluate the effects of different sources and formulations of marine shell limestone, in comparison to common limestone and an untreated control, on the growth and yield of snap beans.

MATERIAL AND METHODS

The study was conducted from April to June 2024 in a greenhouse at the State University of Londrina, Paraná, Brazil (23°18'S, 51°09'W), using snap bean (Phaseolus vulgaris L. 'Macarrão') with indeterminate growth habit (Type IV) and 90-day cycle. Plants were grown in 10-L pots filled with Rhodic Nitisol soil (62% clay, pH 5.2 in $CaCl_2$, 2.1% organic matter, 35% base saturation) collected from the 0-20 cm layer. The completely randomized design included five treatments with four replications: (1) F - NPK (20-10-15 formulation) + 2.8 t/ha powdered marine shell lime (<0.5 mm, 85% $CaCO_3$ eq.); (2) S+B - granulated shell lime (2-4 mm) + 1.5 kg B/ha as borax; (3) S - granulated shell lime; (4) G - powdered shell lime; and (5) C - unamended control. Lime doses were calculated to raise base saturation to 50% using the SMP buffer method and incorporated 30 days before sowing. All pots received basal fertilization of 180 kg/ha 8-28-16 (N-P_2O_5-K_2O) at planting, with treatment F receiving additional topdressing of 40 kg N/ha + 30 kg K_2O/ha at V4 stage. Three seeds were sown per pot on 5 April 2024 and thinned to one plant at 10 days after sowing (DAS). Greenhouse conditions were maintained at 18-30°C (automated ventilation), 70±5% relative humidity (digital hygrometer), and natural photoperiod supplemented with 400W LED lighting. Plants were drip-irrigated to maintain 100% ETc (measured by tensiometers) and received weekly preventive neem oil applications (0.5%) for pest control. At physiological maturity (R8 stage, 95 DAS), we measured plant height (cotyledon scar to apical meristem), fresh shoot weight (65°C dried to constant weight), pod number (counting commercial pods >8 cm), and fresh pod weight (digital scale ±0.01g). Data were analyzed in R v4.3.1 using Shapiro-Wilk normality test, Levene's homoscedasticity test, ANOVA ($p \leq 0.05$), and Tukey's HSD post-hoc comparisons, with results presented as mean ± standard error.

RESULTS AND DISCUSSION

The NPK + shell lime treatment (F) significantly outperformed other treatments, producing taller plants (158.25 cm), greater biomass (52.14 g), and higher pod yields (9.85 g, 6.5 pods) compared to the control (C) and other lime formulations ($p < 0.05$). This aligns with Fageria & Stone (2008), who demonstrated that balanced NPK fertilization combined with lime maximizes legume growth in moderately acidic soils (pH 5.5–6.5). The superior performance of F suggests that shell lime enhances nutrient availability from NPK fertilizers, possibly by mitigating aluminum toxicity or improving phosphorus solubility, as observed by Caires et al. (2020) in tropical soils. Despite theoretical benefits, standalone lime treatments (S+B, S, G) failed to improve yields relative to the control (C), contradicting Silva et al. (2021) but supporting Alvarez et al. (2022)'s finding that lime alone is ineffective in near-neutral soils (pH ≥6.0). The lack of boron response (S+B) implies either sufficient native boron or inadequate uptake timing, consistent with Gupta (2021)'s threshold of >0.5 mg/dm³ for bean crops.

Table 1. Plant height (cm), Fresh plant mass (g), pod mass per pot (g), and pods per pot.

Treatment	Plant height (cm)		Fresh Weight (g)		Pod weight (g)		Pod Number	
F	158.25	a	52.14	a	9.85	a	6.5	a
S+B	50.00	b	9.39	b	3.51	b	2.0	b
S	60.75	b	12.10	b	5.00	b	3.2	b
G	52.75	b	7.12	b	1.89	b	1.0	b
C	64.25	b	5.58	b	1.22	b	1.8	b
CV%	36.2		58.7		72.3		68.4	

* Different letters differ according to Tukey's test at a 5% probability level.

CONCLUSION:

NPK + shell lime (F) significantly boosted yield, while lime alone showed no effect. Always combine lime with NPK in moderately acidic soils.

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EFFECT OF DOLOMITIC LIMESTONE APPLICATION ON GROWTH AND YIELD OF SNAP BEAN

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INTRODUCTION: Snap bean (Phaseolus vulgaris L.) is an economically and nutritionally important legume. Soil acidity is a common issue that can limit crop productivity. The application of dolomitic limestone is a common practice to correct soil acidity and improve soil fertility. This study aimed to evaluate the effect of superficial and incorporated dolomitic limestone application on the growth and yield of snap bean cultivated in a greenhouse.

MATERIAL AND METHODS: The experiment was conducted from April to June 2024, in the greenhouse of the State University of Londrina (UEL). Nine-liter pots containing nitossolo were used, with the following chemical characteristics: pH ($CaCl_2$) of 5.5, organic matter of 3.2%, P of 15 mg/dm³, K of 3.5 mmolc/dm³, Ca of 40 mmolc/dm³, Mg of 10 mmolc/dm³, Al of 0.5 mmolc/dm³, H+Al of 30 mmolc/dm³, CEC of 84 mmolc/dm³, and base saturation of 64%. During the experimental period, the average temperature in the greenhouse ranged from 18°C to 30°C, with relative humidity maintained around 70%. The snap bean variety used was 'Macarrão', known for its determinate growth habit and 65-day pod harvest cycle. The experimental design was completely randomized, with three treatments and four replications: control (no lime), superficial application of dolomitic limestone, and incorporation of dolomitic limestone to a depth of 10 cm. The lime requirement was calculated using the formula: NC = (V2 - V1) * T / PRNT, where V2 is the desired base saturation (70%), V1 is the current base saturation (64%), T is the cation exchange capacity (84 mmolc/dm³), and PRNT is the relative neutralizing power of the lime (80%). The calculated lime requirement was applied one month before sowing, and the pots were watered daily. Three seeds were sown per pot, and after 10 days, thinning was performed to leave only one plant per pot. Sowing was carried out in April 2024. Initial fertilization in the sowing furrows was calculated based on soil analysis, following the recommendations of Pauletti and Motta (2019), with a specific formulation of 180 kg/ha of the 8-28-16 formula applied. Weed control, 25 days after emergence, involved the application of the herbicide fluazifop-p-butyl + fomesafen (200 + 250 g/ha 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. At 65 days, the plants and pods were harvested at the beginning of the R8 stage. Evaluations included height, stem diameter at the first node, pod diameter and average length, number of pods per plant, and yield. Data were subjected to analysis of variance, and means were compared by Tukey's test at 5% significance.

RESULTS AND DISCUSSION: The results showed that the application of dolomitic limestone had a significant effect on the analyzed variables. The data are presented in Tables 1 and 2.

Table 1. Effect of dolomitic limestone application on height, stem diameter, and pod diameter of snap bean

Treatment	Height (cm)	Stem Diameter (mm)	Pod Diameter (mm)
Control	45.2 c	5.8 c	8.2 c
Superficial Application	50.1 b	6.5 b	9.0 b
Incorporated Application	55.3 a	7.2 a	9.5 a
CV (%)	5.2	4.8	3.9

Table 2. Effect of dolomitic limestone application on pod length, number of pods per plant, and yield of snap bean

Treatment	Pod Length (cm)	Pods per Plant	Yield (g/plant)
Control	12.5 c	15.3 c	150.3 c
Superficial Application	13.8 b	18.5 b	170.5 b
Incorporated Application	14.5 a	20.2 a	185.7 a
CV (%)	4.5	6.3	6.1

Plants treated with incorporated dolomitic limestone showed significant improvements in all measured variables. Specifically, these plants exhibited significantly higher height (55.3 cm), stem diameter (7.2 mm), and pod diameter (9.5 mm) compared to the control, which had values of 45.2 cm, 5.8 mm, and 8.2 mm, respectively. These findings are consistent with previous studies highlighting the benefits of lime application on soil pH and nutrient availability. In terms of pod length, number of pods per plant, and yield, the incorporated limestone treatment also performed better, with values of 14.5 cm, 20.2 pods per plant, and a yield of 185.7 g/plant. The superficial application of limestone also showed significant improvements compared to the control but was less effective than the incorporated method. This enhanced performance is likely due to the better distribution and availability of calcium and magnesium in the soil, which are essential for plant growth and development. This is consistent with the observation that plants treated with incorporated dolomitic limestone showed the greatest improvements in height, stem diameter, and pod diameter (Souza et al., 2020).

CONCLUSION: The application of dolomitic limestone, either superficially or incorporated, significantly improved the growth and yield of snap bean. The incorporated application was the most efficient, highlighting its potential as a promising practice for improving soil fertility and crop productivity.

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BREEDING SNAP BEAN FOR POD QUALITY TRAITS AND YIELD IN EASTERN AFRICA

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INTRODUCTION

Snap bean (*Phaseolus vulgaris L.*) is an important vegetable crop in Kenya employing over 500,000 smallholder farmers, and more than 1 million people along its value chain (Odero et al., 2013). It is also important in other eastern Africa countries such as Uganda, Tanzania, Rwanda, Zambia, Zimbabwe and Burundi. Pod traits such as colour, firmness, stringiness, pod shape, pod length, turgidity and yield potential strongly influence acceptability of a new variety by farmers, exporters, processors and consumers (Kimani et al., 2009). Yield of snap beans in smallholder farms varies from 2 to 8 t ha^{-1} compared with large scale production attaining over 14 t ha^{-1} (Wahome et al., 2011). Breeding locally adapted snap bean varieties with market demanded traits has received relatively little research attention despite its economic importance in the region. In 2001, a regional market-led snap bean programme based at the University of Nairobi, was initiated to develop improved snap bean varieties with high pod quality, yield potential and resistance to biotic stresses for smallholder farmers, exporters and processors (Kimani, 2006; Wahome et al., 2011). The objective of this study was to evaluate new locally bred snap bean lines for pod quality traits, yield potential, shelf life and other organoleptic characteristics.

MATERIALS AND METHODS

Thirty six F_9 snap bean lines and five commercial varieties were evaluated in advanced yield trials at Thika (1548 masl) and Mwea (1159masl) in the central Highlands of Kenya during the short rain season (Sep-Dec). These lines were previously selected for resistance to rust, angular leaf spot and anthracnose, pod traits and pod yield from breeding populations at Thika and Mwea (Wahome et al, 2011). Forty pods were randomly taken from each plot and used to determine pod length, pod shape, pod curvature, shelf life and cooking time and other organoleptic (flavor and texture) characteristics. Pod yield was determined as the cumulative weight from all harvests until physiological maturity was reached. Pods from each harvest were graded as extra fine, fine and bobby using standard commercial criteria. Shelf-life was based on moisture loss, wilting, colour changes and loss of moisture over an eight day period. A 1 to 9 wilting scores were used. Pods with scores of 7 and 9 were considered acceptable, 5 and 7 as slightly acceptable, and 1 to 3 as not acceptable. The duration of time during storage which the pods maintained score of 7 or better was considered its shelf life. To determine cooking time, flavor and texture, pods from each plot were cut into 1 cm pieces and placed in boiling water. Pods were sampled after 3, 5, 7 and 9 minutes. A panel of 20 evaluators assessed the pods of each variety on a scale of 1 to 7. Scores of 1 to 4 were considered as acceptable cooking time for both texture and flavor. Texture was determined using a CT3-100 0.1-10g/0.01g texture analyzer at Kenya Industrial Development Institute (KIRDI), Nairobi, Kenya.

RESULTS AND DISCUSSION

Results showed significant differences in pod yield, pod length, shelf-life and cooking time, and colour retention during storage. Five lines had significantly higher pod yield than the checks (Fig

1). All the new lines had a pod length of >10cm required by the markets. Pod length of elite lines varied 15.3 to 17cm, compared to 14 to 17 cm for the checks. They also had better grade distribution (51.2% fine and extra fine) than checks (35%). Eleven lines had better storage characteristics and maintained more than 80% moisture and pod colour after 7 days of storage in room conditions (21-25°C). The lines produced marketable pods for 6 weeks (18 harvests, 3 harvests per week). They had the market preferred straight, thin (< 6mm diameter) and round pods. However, peak harvest occurred during the second and third weeks. Cooking time varied from 3 to 7 minutes. New lines were comparable or better than existing commercial varieties in all key attributes. They also possess good texture and flavour, and were preferred than checks by the tasting panel.

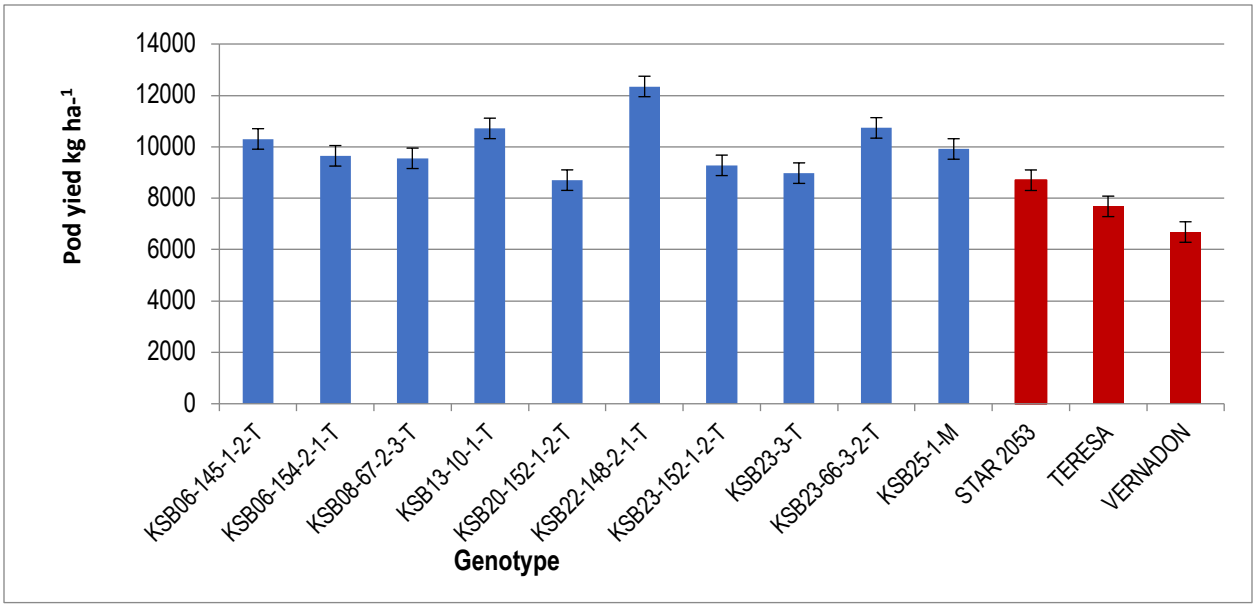

Figure 1. Pod yield of F_9 snap bean lines at Mwea,Kenya. Star 2053, Teresa and Vernadon were the checks.

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NATURAL DARKENING OF BAYO BEAN VARIETIES DURING THE FIRST POSTHARVEST DAYS

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INTRODUCTION

Darkening of light-colored bean kernels reduces their commercial value, as it is associated with an old and possibly hard to cook. Therefore, genetic improvement seeks slow-aging varieties. To select genotypes with this characteristic, accelerated aging tests with ultraviolet light or high temperature and humidity, as well as natural darkening at room temperature, are used. Previous studies indicate that reflectance spectrophotometry can detect early changes in grain color. The objective of this study was to determine the minimum aging time in environmental conditions necessary to select genotypes in Bayo type beans, using the color parameters of the CIE Lab system.

MATERIALS AND METHODS

Five genotypes of light-colored beans were evaluated, Bayo Azteca, Altiplanomex, Bayo Mecentral and Bayomex, planted in Santa Lucía de Prías, Texcoco, state of Mexico. The experimental plot was one 4 m- long row. Upon reaching maturity, eight plants from each plot were hand threshed.

A control sample was taken from each variety while another was exposed to natural light at room temperature.

The first day after threshing color was measured using a CM-5 spectrophotometer (Konica Minolta, Inc., Osaka, Japan). Color reflectance was recorded in the CIE Lab color coordinate system, with D65 Illuminant and 10º observer.

The grain color was determined in the control samples and, subsequently, they were kept refrigerated at 5 °C. The experimental samples, with four replicates per variety, were placed on tables inside the laboratory, exposed to natural light, each in a 15 cm diameter Petri dish. Daily, the grains were moved to ensure uniform exposure to natural light across their surface. Color determination was carried out every seven days until completing 70 days. In both the control and experimental samples, color measurements were carried out in triplicate.

The data were processed using an analysis of variance and a correlation matrix (Pearson)

RESULTS AND DISCUSSION

Altiplanomex, Bayo Azteca, Bayo Mecentral and Bayomex, the genotypes showed L* values from 59.8 in Bayomex, to 67.9 in Altiplanomex, which indicates that the Altiplanomex seedcoat was lighthest. While the values of a* were from 3.3 in Bayomex, to 5.8 in Bayo Mecentral; which indicates that this last variety showed more red tones. And finally, the b* values were from 21.5 in Bayo Mecentral to 31.2 in Bayo Azteca; which indicates that this last variety showed more yellow tones.

Table 1.Color data L* a* b*, of four recently harvested Bayo bean varieties			
Variedad	L*	a*	b*
Bayo Azteca	65.3	3.9	31.2
Altiplanomex	67.9	4.5	24.8
Bayo Mecentral	63.1	5.8	21.5
Bayomex	59.8	3.3	25.9

L* measures whether the sample is light (high L*) or dark (low L*)
+a* indicates colors in the red direction, -a* indicates colors in the green direction
+b indicates colors in the yellow direction -b* indicates colors in the blue direction

Statistical analyzes indicated highly significant differences ($P<0.01$) between varieties in L* a* b* values in control samples, as well as after 70 days of exposure to natural light. It was observed that when the seedcoat darkened, the values of a* (red tones) increased and those of L* decreased.
It was noticeable that from 21 days post-harvest, some changes < 1.0 were detected in the color, loss of lightness (decrease in the L* value), increase in red tones (increase in the a* value), while in yellow tones no defined trend was observed.
After 35 days, it was observed that in the varieties L* decreased from 2.1 to 4.2 units, while red tones (a*) increased between 0.31 to 1.84 units, while yellow tones (b*) did not show a defined trend.
After 70 days, the L* value decreased between 4.7 and 6.9 units, while the a* value increased between 2.2 and 3.0 units. The b* value as at 35 days, while in three varieties it decreased, in one it increased.
Bayo Azteca was the slowest variety to darken, while Bayomex was the one that showed the greatest darkening.
A highly significant and positive correlation was observed between the values of L* a* and b* between 35 and 70 days ($r=0.99$, $r=0.98$, $r= 0.94$ respectively), which suggests that for bayo beans it would be reliable to use the change in color as an indicator of propensity for darkening after 35 days of natural aging.

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NAVY BEAN CANNING QUALITY: BRINE GELLING PREVALENCE

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Introduction: Navy beans are the second most important dry bean market class grown in Michigan and they have a long history as a canned bean product. Substantial research and breeding efforts have been focused on ensuring that navy bean canning quality meets processor and consumer expectations. Canning quality is a measure of how well a bean withstands the canning process. The assessment includes hydration and texture measurements and a rating of how appealing the canned bean appearance is for customers (Wang et al., 2022). A favorable navy canning quality is fully hydrated beans, soft enough texture to be edible, but firm enough that the beans stay intact with few split seed coats. Further, canned bean brine should be clear and of fluid consistency, not viscous, cloudy or with free starch from the beans. Canning quality evaluation is an integral part of the MSU and USDA-ARS programs in Michigan as it is a major factor of value for the crop.

Observation of Brine Gelling Problem: Recently (2023 and 2024 crops) within the MSU Dry Bean Performance trials and the Dry Bean breeding nurseries, some navy beans have been exhibiting a canning quality concern that can be described as gelling of the brine. In this case, the brine is highly viscous, with a jelly-like consistency. Interestingly, it does not necessarily appear to be caused by broken beans or starch leaching from the beans into the brine. In many cases, with gelling of the brine, the brine is not cloudy, but relatively clear, and the canned bean samples are fully intact and there is no sign of starch leaching. There is no clear current explanation of what is causing the observed gelling. While it is known that during thermal processing free starch swells and thickens, proteins undergo denaturation and gelation, and various hydrocolloids (pectin and soluble fiber) can serve as a matrix for this gel structure, excessive starch or pectin/hydrocolloids leaching through intact seedcoats have not previously been observed. Thus, there is a need for directed studies and objective brine viscosity measurements and compositional analyses to better understand the nature of the problem and potential causes. The prevalence of the problem was document in two navy bean trials conducted in Michigan in 2024: 1) MSU Bean Performance trials, 30 entries, two on-farm locations, Bay and Huron; and 2) MSU advanced yield trials, 36 entries, two locations, Saginaw Valley Research Farm (SVREC)-Tuscola, and Huron, on-farm. The Huron location was the same site for both the Performance and Breeding trials. The canned samples were rated for prevalence of brine gelling as a 'yes' or 'no' (Table 1).

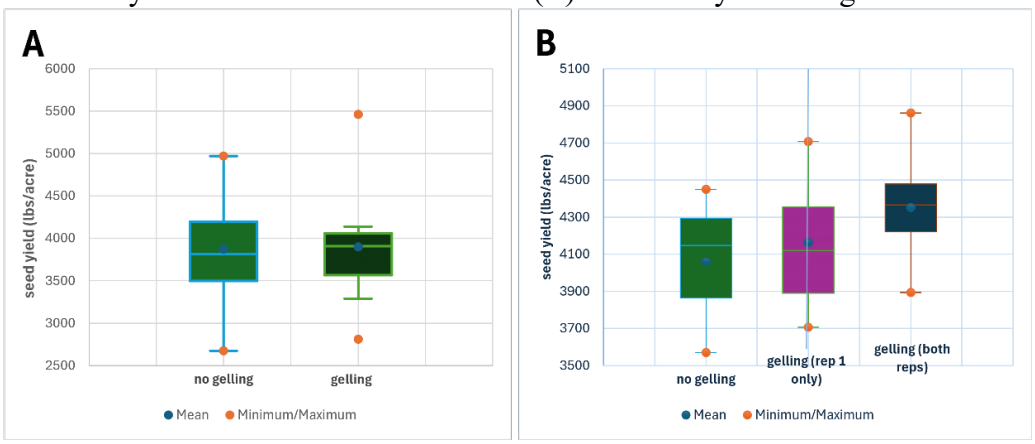
Table 1: Environmental characteristics, seed yield, harvest moisture, and canned navy bean brine gelling incidence across four Michigan field trials

	Performance Trial		**Breeding Advanced Yield Trial**	
County	**Bay**	**Huron**	**Tuscola**	**Huron**
Nitrogen Fertilizer (lbs/acre)	45	0 (Clover)	60	0 (Clover)
Growing Degree Days	1877	1882	2027	1882
Total precipitation (inches)	10.5	10.5	11.7	10.5
Seed Yield (lbs/acre)	2244	3996	2621	4211
Harvest Moisture (%)	14.3	18.4	17	15.4
Brine Gelling (%)	0% (0/30)	27% (8/30)	0% (0/72)	39% (28/72)

Table 2: Agronomic and Canning Quality Characteristics of Navy Bean breeding lines in the Huron trial grouped by Canned Bean Brine Gelling

Canned Bean Brine Gelling	Yield	Flowering	Maturity	Lodging	Plant Height	Can rating	Seed Harvest Moisture
	lbs/acre	days	days	(1-5)	(cm)	(1-5)	(%)
No	4054	42.9	96.2	2.4	46.3	2.6	15.4
Yes (one rep only)	4116	43.8	95.9	2.3	48.4	2.1	15.4
Yes	4357	44.1	96.8	2.2	48.5	2.2	15.5

Figure 1: Box Plots of Seed Yield across trials grouped by Canned Bean Brine Gelling: (A) MSU Navy Bean Performance Trials and (B) MSU Navy Breeding trials.



Results and Discussion: These preliminary observed brine gelling characteristics is county location specific (Bay and Tuscola =no gel; Huron=gelled). It is noted that not all samples in Huron have the problem, some genotypes don't have it at all, some on only one rep, and some on both reps (Table 1). In 2023, the problem was also observed in Huron Performance trials, in 100% of navy bean samples. The 2023 plot, while in the same county as the 2024 Performance trial, was 50 miles away, so the problem cannot be said to be specific to a single farm. In the Navy bean breeding trial in Huron, there is a trend that the samples with the most consistent gelling (i.e. both reps showed the effect) were higher yielding than those that did not show the gelling (Table 2 and Figure 1B). Nine navy bean entries were grown in both the Huron Performance and Breeding trials, 67% were consistent for their gelling response, while 34% do not follow similar patterns in the two trials, suggesting there is not a clear genetic component associated with brine gelling.

Future Plans: Continue to monitor the incidence of the problem. Collect objective measures of brine viscosity via a viscometer. Evaluate the gelled brine for compositional characteristics that may be causing the gelling.

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ORGANOMINERAL FERTILIZER AS TOPDRESSING IN GREENHOUSE-GROWN SNAP BEANS

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INTRODUCTION

Snap bean (*Phaseolus vulgaris* L.) is widely cultivated due to its high nutritional value and market acceptance. This leguminous crop is not only a vital source of protein, vitamins, and minerals for human consumption, but also plays a significant role in sustainable agriculture through its ability to fix atmospheric nitrogen, enriching soil fertility. Proper fertilization management is essential to maximize yield and pod quality, especially in greenhouse cultivation where environmental conditions can be closely controlled to achieve optimal plant growth. This study aimed to evaluate the effect of different doses of organomineral fertilizer on the growth and production of snap beans, providing insights into the best fertilization practices to enhance both the quantity and quality of the yield. The findings of this research could have important implications for improving agricultural productivity and sustainability, aligning with global efforts to ensure food security and environmental conservation.

MATERIAL AND METHODS

The experiment was conducted from April to June 2024 in a greenhouse at the State University of Londrina (UEL), Paraná. The snap bean variety 'Macarrão' was used, characterized by an indeterminate growth habit (type 4) and an approximate pod harvest cycle of 65 days. The soil used was a Nitisol, previously characterized chemically. Ten-liter pots filled with this soil were used. The soil analysis revealed the following characteristics: pH ($CaCl_2$) of 5.5, pH in water of 5.8, organic matter of 3.5%, phosphorus (P) of 15 mg/dm³, potassium (K) of 0.35 cmolc/dm³, calcium (Ca) of 4.5 cmolc/dm³, magnesium (Mg) of 1.2 cmolc/dm³, aluminum (Al) of 0.1 cmolc/dm³, H+Al of 30 mmolc/dm³, cation exchange capacity (CEC) of 7.0 cmolc/dm³, and base saturation (V%) of 70%. The data indicated well-structured and fertile soil, suitable for snap bean cultivation. The experimental design was completely randomized, with five treatments and four replications, totaling 20 experimental units. The treatments consisted of different doses of organomineral fertilizer applied as topdressing: control (0 g/pot), 150 g/pot, 300 g/pot, 600 g/pot, and 900 g/pot. Sowing was carried out in April 2024. Three seeds were sown per pot, and after 10 days, thinning was performed, leaving only one plant per pot. Initial fertilization followed the recommendations of Pauletti and Motta (2019), using 180 kg/ha of the 8-28-16 formula. The organomineral fertilizer was later applied as a topdressing. During the experimental period, the greenhouse temperature ranged from 18°C to 30°C, with relative humidity maintained at around 70%. The remaining cultural practices followed general recommendations for the crop, with supplemental irrigation as needed. At 95 days after sowing, the plants were harvested at the beginning of the R8 stage. The following parameters were evaluated: plant height (cm), fresh plant mass (g), number of pods per plant, fresh pod mass per plant (g), and yield (g/plant). Data were subjected to analysis of variance (ANOVA), and means were compared using Duncan's test at a 5% significance level.

RESULTS AND DISCUSSION

The results of the experiment on fertilization with organomineral fertilizer in green beans indicate that the dose of 900 g was the most effective, resulting in the highest fresh plant mass (89.35 g), pod mass (43.2225 g), and the number of pods per pot (16.25). The dose of 600 g also showed good results, especially in plant height (170.25 cm), but was inferior to the 900 g dose in the other variables. The smaller doses (300 g, 150 g, and 0 g) resulted in lower values for all analyzed variables, highlighting the importance of adequate fertilization for the development of green beans. Comparing these results with other studies, Silva et al. (2020) also observed a significant increase in green bean production with the use of organomineral fertilizers, corroborating the effectiveness of this practice. Oliveira et al. (2019) emphasized the importance of adequate fertilizer doses to maximize plant growth and production, which is in line with the results obtained in the present experiment. These studies reinforce the recommendation to use organomineral fertilizers to improve green bean productivity. The data are presented in Tables 1

Table 1. Plant height (cm), dry plant mass (g), pod mass per pot (g), and pods per pot.

Treatment	Height of Plants	Plant Fresh Mass	Pod Mass	Pods per Pot
900	151.25 a	89.35 a	43.2225 a	16.25 a
600	170.25 a	61.345 b	31.455 ab	12.75 ab
300	127.5 ab	52.97 b	23.0375 b	10.5 bc
150	94.5 bc	19.385 c	7.6725 c	6.25 cd
0	64.25 c	5.58 c	1.215 c	1.75 d
C.V.%	22.46	30.24	36.39	35.15

* Different letters differ according to Duncan's test at a 5% probability level.

CONCLUSION:

Based on the experimental results and comparisons with other studies, the application of organomineral fertilizer significantly enhances the growth and productivity of green beans. Among the doses tested, 900 g per pot was the most effective, yielding the highest values in fresh plant mass, pod mass, and the number of pods. The lower doses (300 g, 150 g, and 0 g) were insufficient to support optimal growth and development. These findings advocate for the use of adequate organomineral fertilizer dosages to achieve improved green bean productivity, thereby contributing to better agricultural practices and food security.

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SUMMARY OF ACTIVITIES ONGOING IN THE UC DAVIS PLANT SCIENCES BEAN/GRAIN LEGUME BREEDING PROGRAM

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Our lab was established in 2019 with a focus on improving crop nutritional quality and tolerance to drought and high temperatures, and took on the UC Davis Plant Sciences bean/grain legume breeding program in early 2023 upon the retirement of Distinguished Professor Emeritus Paul Gepts. Our lab is breeding large- and baby-seeded dry lima beans (*Phaseolus lunatus* L.) and large-seeded kabuli-type garbanzo beans (*Cicer arietinum* L.), with pre-breeding/genetics work in those species as well as common bean (*Phaseolus vulgaris* L.), tepary bean (*Phaseolus acutifolius* A. Gray), common bean/tepary bean interspecific lines, and cowpea (*Vigna unguiculata* [L.] Walp). The goal of this note is to describe ongoing research activities in our lab and collaborative teams, as we would look forward to connecting on topics of mutual interest.

Key workstreams

The long-time focus of the lima breeding program is to develop high-yielding, *Lygus hesperus* tolerant white-seeded cultivars for dry-seed production (i.e., harvested from mature pods). We are breeding for both large- and baby-seeded market classes, as well as bush and vine growth habits to suit the specific rotational and market needs of California growers and processors. In the lima pre-breeding context, we are working on agronomic/adaptation, nutritional quality, and sensory/culinary traits including in diverse materials in collaboration with several institutions including the USDA National Plant Germplasm System. We have also been testing use of a winter nursery location for lima beans in southern California in collaboration with UC Riverside for seed increase and evaluation of photoperiod-sensitive materials.

We are also working on the pairwise and three-way integration of AI-enabled sensing, 3-D biophysical modeling, and pre-breeding/genomics, again with several collaborative partners (projectgemini.ucdavis.edu). We have been testing (and sensing and modeling) germplasm across phylogenetic and environmental gradients, as we develop an integrated analytical framework and apply it to dissect photothermal response and other factors impacting productivity and quality.

Finally, to investigate levels of bioaccessible nutrients in common bean samples with diverse seed coat patterning, we are using robotic stomach chambers to dynamically simulate the digestive process (for instance, wavelike contractions among other physico-chemical aspects) and measure starch and protein hydrolysis alongside phenolics.

Key methodologies

Field evaluation: Across our projects, we have been evaluating a few grain legume species across sites and years, and (in certain site-years when feasible) within the same field block to enable comparative analyses (Figure 1). We have assessed agronomic traits such as days to anthesis, growth habit, yield, and hundred-seed weight, and traits/features such as canopy area/biomass and leaf, flower, and pod counts from aerial and rover-based sensing platforms.

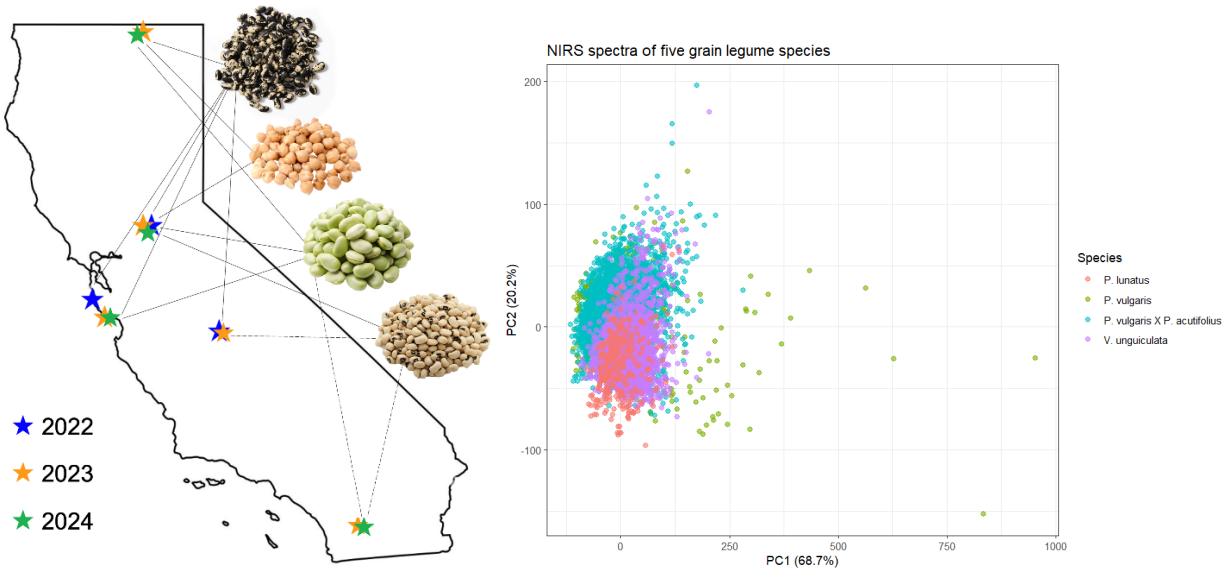

Figure 1a (*left*). Planting locations across California for common/tepary bean, garbanzo bean, lima bean, and cowpea (images from top to bottom) in 2022, 2023, and 2024. Dashed lines indicate that a trial was conducted in collaboration with UC Riverside.

Figure 1b (*right*). Principal components 1 vs. 2 for NIR spectral data on common bean (green), common bean x tepary bean interspecific lines (teal), lima bean (red), and cowpea (purple).

Near-infrared spectroscopy (NIRS): We are scanning ground samples from diverse genotypes and environment types on a FOSS DS2500 benchtop NIRS instrument (400 to 2500 nm; i.e., including visible wavelengths). Initial predictions were made using a pre-existing Vegetal Protein Meals calibration, which predicts protein, starch, fat, ash (total mineral), crude fiber, and moisture percentages but is largely based on oilseeds. A subset of the samples (selected via Kennard-Stone algorithm, based on a modified Mahalanobis distance) additionally underwent wet-chemistry reference analyses, and we have developed a script base to develop custom calibrations for multiple species. We are also testing and preparing to routinely implement a kit-based assay for quantification of phytate, to also be used in calibration development.

Finally, we are conducting genetic mapping and genomic prediction for agronomic and quality traits, including genome-wide association studies in lima and common bean x tepary bean interspecific populations and QTL mapping in a cowpea multi-parent advanced-generation intercross population; QTLxE analyses are also planned in the near term. The results of these analyses are being used to develop tools/technologies and information for use in breeding.

Acknowledgments: We gratefully acknowledge Drs. Paul Gepts and Steve Temple for their contributions to bean breeding/genetics, including germplasm and genetic resources. We gratefully acknowledge all members of the GEMINI/FFAR, LIMA! SCRI, and USDA Pulse Crop Health Initiative collaborative teams and sponsors, as in Adaskaveg et al. 2024 and Lo & Berlingeri et al. 2024 in the *Annual Report of the Bean Improvement Cooperative* (BIC) vol. 67, pages 3-5 and 19-20, and in Bolt et al. *preprint* (doi: 10.1101/2024.05.09.592089); we apologize that all were not able to be listed here by name due to space limitations. Finally, we would acknowledge the BIC and USDA *Phaseolus* Multi-State Hatch project communities for facilitating this and other venues.

PRELIMINARY CHARACTERIZATION AND GENETIC MAPPING OF NUTRITIONAL COMPOUNDS IN TEPARY BEAN (*PHASEOLUS ACUTIFOLIUS*)

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INTRODUCTION

Heat and drought are two abiotic stressors that greatly impact plant productivity and can also reduce the nutritional quality of crops[1]. Tepary bean (*Phaseolus acutifolius* A. Gray) is a dry bean species endemic to the Southwestern US, Mexico, and Central America that exhibits high levels of tolerance to the hot, arid conditions prevalent in these regions[2]. Given tepary's natural ability to thrive under these conditions, it could serve as a key source of plant-based protein in areas threatened by changing climatic conditions and specifically, increased prevalence of heat and drought stress conditions[3]. To this end, we characterized cultivated entries from the Tepary Diversity Panel for micronutrient levels, protein content, and total antioxidant capacity to investigate phenotypic variability for these traits, as well as conduct preliminary genetic mapping.

MATERIALS AND METHODS

A total of 214 entries from the Tepary Diversity Panel (TDP) along with a common check were planted on May 8, 2023, at the Maricopa Agricultural Center in Maricopa, AZ in an unreplicated, randomized incomplete block design. Plots were 4.17 meters in length with interrow spacing of 1.01 meters and were managed using standard cultivation practices for the low altitude, Southwest desert region. At the end of the season, plots were hand-harvested and threshed. Approximately four grams of dried seeds were ground into powder for compositional analysis. Micronutrient analyses (Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, Se, Mo, Cd, and Ba reported as μg/g) were performed by performing an acid digestion of samples and then analyzing the solution with inductively coupled plasma mass spectrometry (ICP-MS) at the Arizona Laboratory for Emerging Contaminants. Total protein (μg/mg fresh weight) was determined via the Bradford method[4] and total non-enzymatic antioxidant capacity (nmol/mg fresh weight) was determined as in [5], with minor modifications. Phenotypic data were used in conjunction with genotyping data (2,367,572 SNP makers) for genome-wide association studies to identify marker-trait associations at a false discovery rate significance threshold of $4.63e^{-5}$.

RESULTS AND DISCUSSION

The panel exhibited extensive variation for each of the individual compounds analyzed. Protein concentration ranged from 92.42 to 170.26 mg/g, while iron and zinc concentrations had ranges of 36.63 to 98.49 and 26.01 to 49.91 μg/g, respectively. Trait correlations among iron and zinc with total protein content were found to be 0.12 (NS) and -0.16 ($p = 0.018$), respectively. Principal component analysis (PCA) was performed on the entire data set, and entries from the TDP were split into two distinct subpopulations (Figure 1A). The two subpopulations were labeled positive and negative based on PC1 scores. The mean difference in zinc concentration between the two groups was found to be 8.61 μg/g ($p < 0.001$, Figure 1B). There was a significant marker-trait association identified for zinc concentration on chromosome seven (Figure 1C); however, no

significant hits were found for protein or iron. This was likely due to the unreplicated nature of the data set as there were several peaks indicative of causative loci for each trait, but they were below the significance threshold. The zinc-associated peak identified on chromosome seven displayed increased allelic diversity within the U.S.-Mexico subpopulation relative to the Mexico only and non-U.S. subpopulations (Figure 1D). In summary, the TDP exhibits significant phenotypic and genotypic variation that can be leveraged for investigating the genetic architecture of nutritional composition in tepary bean with a focus on providing a climate resilient source of plant protein.

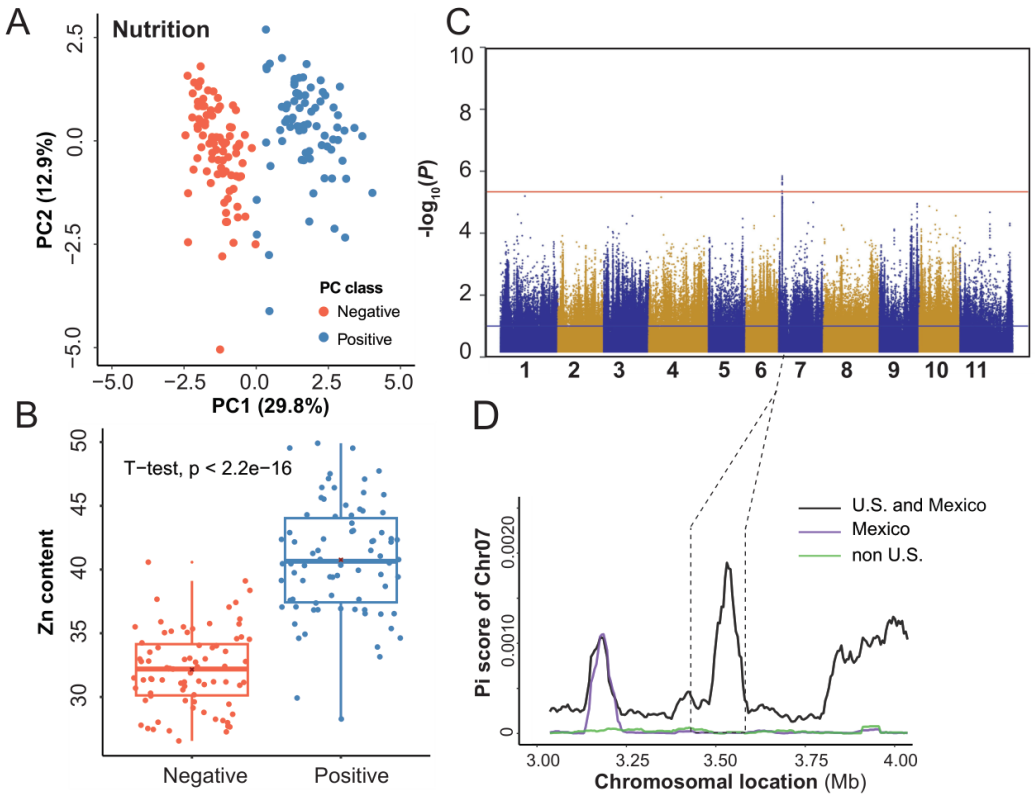

Figure 1. Characterization and genetic mapping of zinc concentration (µg/g) in tepary seed from the Tepary Diversity Panel (TDP). A) Principal component analysis of nutritional compounds for the TDP reveals two subpopulations identified as negative or positive based on the PC1 score. B) Difference in zinc concentration between the two subpopulations. C) Manhattan plot identifying significant marker trait association on chromosome seven, and D) allelic diversity underlying the significant association, highlighting the elevated allelic diversity in the U.S.-Mexico subpopulation relative to the others.

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IDENTIFYING KEY TRAITS FOR BEAN ADOPTION IN UGANDA: A MULTI-DISTRICT SURVEY

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INTRODUCTION

Common bean is the third most widely grown food crop and is the most important source of dietary protein in Uganda [1]. Bean breeding efforts need to reflect the preferences and needs of consumers so that improved bean varieties will be adopted. This study sought to identify consumers' priority traits in geographically distinct areas across Uganda via a house-hold survey.

MATERIALS AND METHODS

Fifty-one participants were selected each from Hoima, Kamuli, and Rakai districts who had an interest in growing and consuming beans. In total, 153 participants were interviewed about their age, gender, bean types they grow, and the factors that influence their decision on which type to grow, and bean cooking practices. Multiple choices were offered to participants except for age. This study was deemed exempt under Exempt Category 6 by Michigan State University Institutional Review Board (IRB#: x14-920e). Participants gave consent to participate in the study. Statistical analyses were conducted in R. For all the categorical variables (survey questions), a cross-table was generated that shows counts for each level for each district. If one or more cells had a count <10, then Fisher's exact test was used to test differences in proportions among districts; else, chi-square test was used. A p-value < 0.05 was considered significant, and in that case, pairwise comparisons of proportions between districts were performed. The fisher.multicomp function of the RVAideMemoire package v0.9-83-7 was used for the variables that were tested by Fisher's exact test; and the pairwise.prop.test function of the multcompView package v1.4-18 was used for the variables that were tested by chi-square test. For variables with two choices (Yes/No or Female/Male), the proportions of "Yes" or "Female" among districts were compared. The Hochberg method was used to adjust for multiple pairwise comparisons.

RESULTS AND DISCUSSION

The average age of the participants was 41.8 years old, and 75% was female. The mean ages and the percentages of female participants were not different among districts (p>0.05 by ANOVA and Fisher's exact test, respectively) In all the three districts, 90% or more participants said they grew red mottled type (**Figure 1A**). More participants in Hoima grew cranberry, yellow, and mix types than other districts, though the proportion of participants who grew yellow beans was also high (78%) in Kamuli. In all districts, 92% or more of the participants indicated early maturity was important in deciding which market class to grow (**Figure 1B**). 78% of participants in Hoima said drought tolerance was important, higher than in the other two districts, and same for disease resistance (76%), indicating that drought, diseases, and pests may be an urgent problem that more growers in Hoima are facing. This coincides with a higher percentage of "Mix" grown in Hoima (**Figure 1A**). "Mix" refers to a mixture of varieties with the same morphological traits but with diversified levels of stress resistance. Across the three districts, 75-92% of participants said cooking time was important. Moreover, 39% of the participants in Kamuli reported a long

cooking time of 180 min (unsoaked), and over 90% of participants in all districts indicated they used firewood and/or charcoal for cooking beans. It showed the need for fast-cooking bean varieties, which can save time and energy used in cooking beans. Analyses will be continued on other variables to characterize important traits that consumers seek in new bean varieties in the three districts of Uganda.

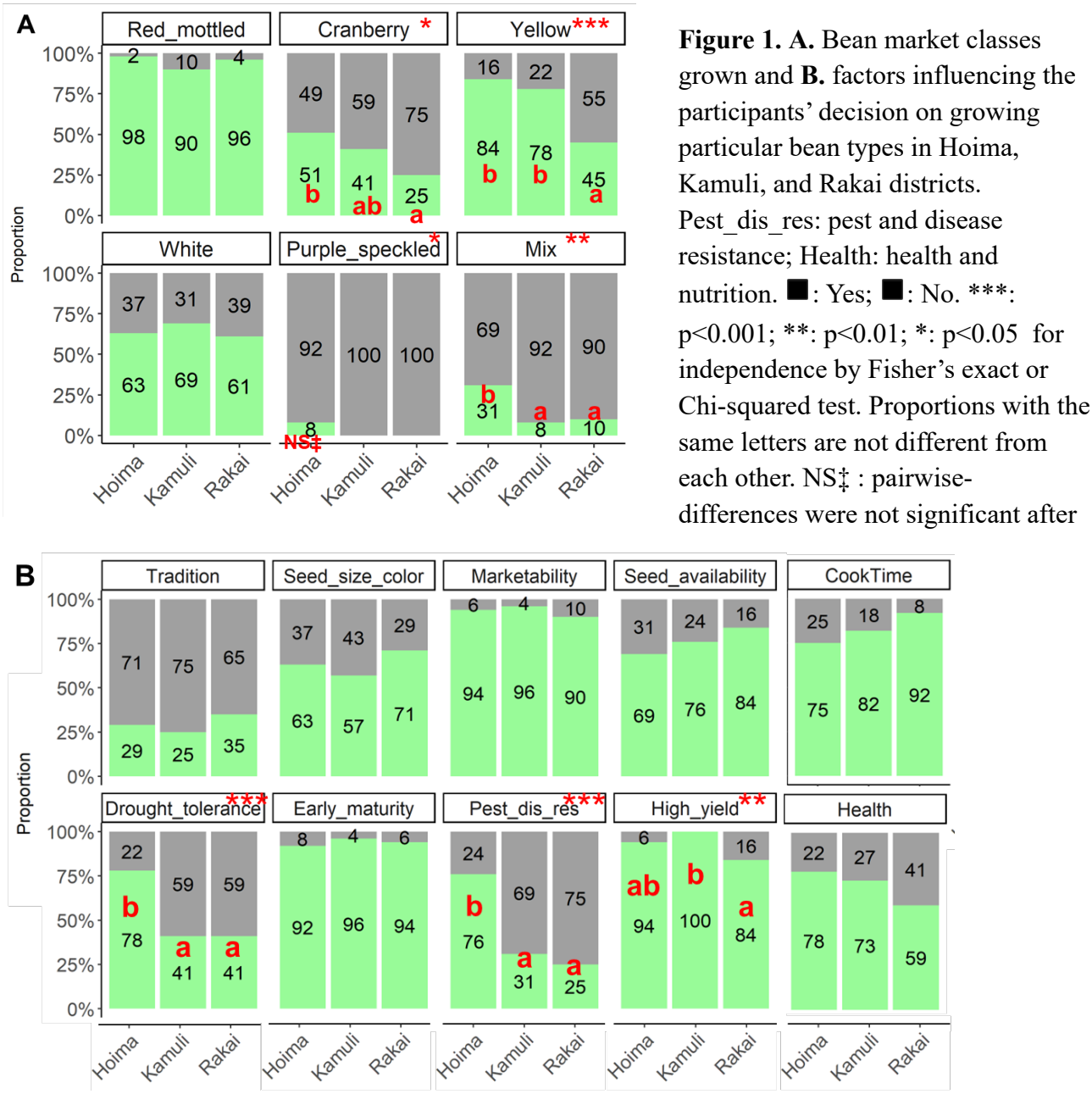

Figure 1. A. Bean market classes grown and **B.** factors influencing the participants' decision on growing particular bean types in Hoima, Kamuli, and Rakai districts. Pest_dis_res: pest and disease resistance; Health: health and nutrition. ■: Yes; ■: No. ***: $p<0.001$; **: $p<0.01$; *: $p<0.05$ for independence by Fisher's exact or Chi-squared test. Proportions with the same letters are not different from each other. NS‡ : pairwise-differences were not significant after

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GRAIN YIELD, ADAPTABILITY AND STABILITY OF COMMON BEAN LINES IN MULTI-ENVIRONMENT TRIALS IN PARANÁ, BRAZIL

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Introduction

Common bean (*Phaseolus vulgaris* L.) is a crucial crop for global food security and nutrition, especially in developing countries. In Brazil, it is a staple food, particularly among low-income populations, due to its high nutritional value and affordability (Petry et al., 2015; Vaz Patto et al., 2015). Breeding efforts focus on the developing cultivars that with improved grain yield, adaptability, stability across varying environmental conditions, and enhanced resistance to both biotic and abiotic stresses (Ramalho et al., 2012). This study evaluated the performance of common bean lines across different environments in the State of Paraná, aiming to identify genotypes that combine high productivity, stability, and adaptability. Additionally, the lines were evaluated for resistance to major diseases and other key agronomic traits.

Materials and Methods

This study evaluated the common bean lines CHC 04-233-2, CHP 12-355-02, LEC 03-16, LEP 01-16, LP 08-186, and LP 09-180, along with the control cultivars IPR Sabiá, Pérola, BRS Esteio, and IPR Urutau. The experiments were conducted in six municipalities in the State of Paraná, Brazil: Guarapuava, Ponta Grossa, Santa Tereza do Oeste, Pato Branco, Maringá, and Campo Mourão (2021 only)-during the 2020, 2021, and 2022 agricultural years. The experimental design, a randomized block design with three replications, included 10 common bean treatments across 16 different environments. Each experimental unit comprised four rows, 5.0 meters long and spaced 0.50 meters apart, with an effective area of 5 m². The evaluation of productivity, adaptability, and stability across theses environments followed methodologies established by Eberhart and Russell (1966), Lin and Binns (1988) modified by Carneiro (1998), Annicchiarico (1992), and WAASB (Olivoto et al., 2019), enabling a thorough analysis of productivity patterns across varying environmental conditions.

Results

ANDEV results revealed significant effects for both the genotypes and the GxA interaction ($p<0.01$), underscoring the genetic variability among the genotypes and their differential responses across environments. The genotype CHP 12-355-02 stood out for its superior performance in productivity, adaptability, and stability, consistently ranking high in all analysis methods. Lines LP 09-180 and LP 08-186 also showed promising performance in specific environments, though with greater variability. These findings emphasize the value of GxA analysis in identifying robust genotypes suited for diverse agricultural conditions.

Table 1. Rankings of 10 common bean lines based on average grain yield (kg.ha^{-1}) and stability and adaptability parameters from various analysis methods, evaluated across sixteen environments in Paraná, Brazil, from 2020 to 2022.

Lines / Cultivars	Yeild[1]	E&R[2]	L&B[3]	Annicchiarico	WAASB
CHP 12-355-02	4°	1°	6°	5°	1°
BRS Esteio	6°	2°	4°	3°	2°
Pérola	3°	4°	3°	2°	3°
IPR Sabiá	7°	9°	7°	4°	4°
LP 08-186	5°	3°	5°	6°	5°
IPR Urutau	1°	5°	1°	1°	6°
LEP 01-16	9°	6°	9°	8°	7°
LEC 03-16	10°	7°	10°	10°	8°
LP 09-180	2°	8°	2°	7°	9°
CHC 04-233-2	8°	10°	8°	9°	10°

Yeild[1]: average grain yield; E&R[2]: classification according to the method of Eberhart and Russell (1966); L&B[3]: classification according to the method of Lin and Binns (1988) modified by Carneiro (1998).

Conclusion

Identifying genotypes with high adaptability and stability, like CHP 12-355-02, is crucial for increasing productivity and ensuring the sustainability of common bean cultivation in Brazil. These findings highlight the importance of integrating GxA analysis into breeding programs to select genotypes that perform consistently across diverse environments, thereby supporting food security and enhancing agricultural resilience.

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LINKING HERBICIDE CROSS-TOLERANCE TO AGRONOMIC AND SEED VIGOR TRAITS: A MULTI-TRAIT GWAS IN SNAP BEAN

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Introduction

Snap bean (Phaseolus vulgaris L.) is a major vegetable crop, and weed control is essential for maintaining high yields. Herbicide tolerance is necessary to prevent crop injury, yet little is known about the genetic factors influencing this trait. This study explored the genomic basis of agronomic traits, herbicide cross-tolerance, and seed vigor through a multi-trait genome-wide association study (GWAS). The findings provide insights into plant resilience mechanisms, aiding future breeding efforts for improved snap bean cultivars.

Materials and Methods

A panel of 377 snap bean genotypes from the Snap Bean Association Panel (SnAP) was evaluated in field trials from 2019 to 2024. Agronomic traits, including seed weight (SeedWt), plant density (PD), shoot biomass (BP), and at-flowering vigor, were assessed. Herbicide tolerance was measured across eight herbicide treatments. GWAS was conducted using 20,619 SNP markers, employing multi-trait statistical models to detect genetic associations.

Results and Discussion

Significant correlations between seed vigor traits and herbicide cross-tolerance were identified, suggesting common genetic mechanisms. Large-seeded cultivars exhibited stronger early growth, which enhanced tolerance to pre-emergence herbicides. GWAS identified 179 marker-trait associations (MTA), with 28 regions showing pleiotropic effects on multiple traits. Candidate genes associated with cell wall biosynthesis, DNA repair, and oxidative stress response were linked to herbicide tolerance. Four genomic regions consistently influence both PD and BP under control and multiple herbicides stress. Notably, favorable alleles for stress resilience were more prevalent in Middle American snap bean genotypes.

Conclusion

This study provides strong evidence that herbicide cross-tolerance and seed vigor are genetically linked. Identified loci can serve as targets for breeding snap bean cultivars with improved tolerance to herbicides and environmental stresses.

Figure 1. Relationship among the PD (A) and BP (B) values under each of the herbicide treatments and control conditions for the SnAP. Pearson's coefficient of correlation was calculated using the cultivars' all-trials average for each the traits. The cultivars values for each herbicide treatment are the mean of two individual trials. The size and color of the circle indicates the strength and sign of the correlation. Treatment abbreviations. Aceto1: Acetochlor. Aceto2: Acetochlor micro-encapsulated. Pyro: Pyroxasulfone. Metri: Metribuzin. Lacto: Lactofen. Saflu: Saflufenacil. Sulfen: Sulfentrazone. Flumio: Flumioxazin. Control: no herbicide treatment.

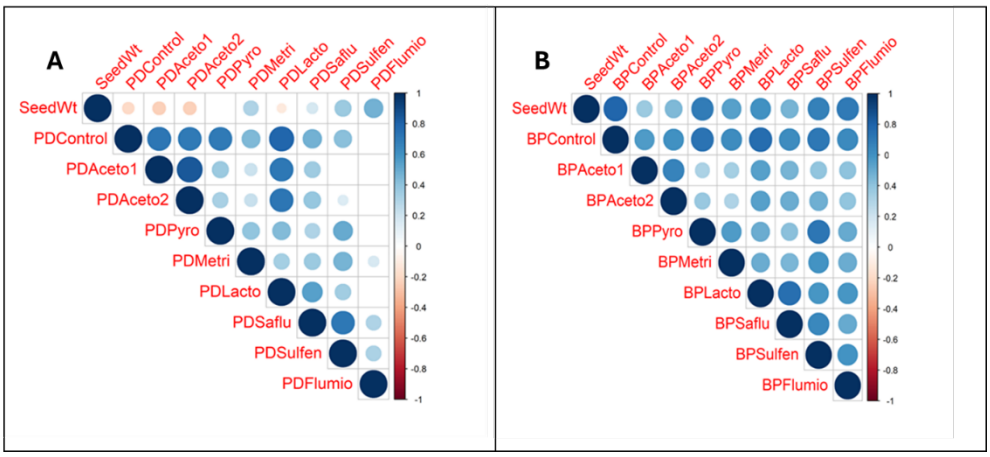

Table1. Multi-trait GWAS results for traits measured in snap bean association panel from 2019-2024 at Urbana, IL. Negative Log10(p) values are presented for the trait groups for which significant SNP were detected. The GWAS method for which the SNP was significant is indicated by an "x". PDcon: Values of PD in control treatment for each of the trials. PDcon-herb: Values of PD in control and herbicide treatment for each of the trials. PDherb: Values of PD in herbicide treatment for each of the trials. BPcon: Values of BP in control treatment for each of the trials. BPcon-herb: Values of BP in control and herbicide treatment for each of the trials. BPherb: Values of BP in herbicide treatment for each of the trials. PD: Plant density. BP: Biomass per plant

Region	PDcon - LOG10(p)	PDcon-herb - LOG10(p)	PDherb - LOG10(p)	BPcon - LOG10(p)	BPcon-herb - LOG10(p)	BPherb - LOG10(p)	Method	
							PC-MLM	MSTEP
Chr01_43.7-44.7			9.40	3.32-18.19	3.64	3.17-3.50	x	x
Chr02_46.9-48.4	4.50-5.23	4.61-9.19	4.66-13.4	3.83	3.32	3.63	x	x
Chr 04_2.4-2.7			13.36	3.27-3.93	3.72-26.60	3.25-24.49	x	x
Chr 05_37.99	4.78		5.30				x	
Chr 07_34.6-34.9				3.25	3.20	3.22	x	
Chr 08_52.8-54.8	4.01-7.14	4.09-4.43	4.32-4.38	4.50	3.49-4.30	3.15-3.24	x	x

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CANNING QUALITY OF ADVANCED SNAP BEAN LINES IN EASTERN AFRICA

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INTRODUCTION

Demand for canned snap beans in eastern Africa and other regions is growing due to changing consumer's preferences, urbanization and changing eating habits. Canned snap beans are becoming a major form of vegetable consumption especially in urban areas for its convenience, distinctive flavour and excellent consumer value. Vegetable quality traits related to end-user preferences are of utmost importance for success of new snap bean varieties. Breeding snap bean varieties suitable for processing industry has received very limited research attention in eastern Africa. In Kenya, the food processing industry depends on low yielding and disease susceptible commercial varieties. Nearly all snap beans for fresh market and processing have pods with some shade of green (Myers and Baggett, 1999). Fresh market cultivars traditionally had lighter green colours compared to the processing types, but the gap in colour differences is narrowing. While not true in every case, many processors would prefer dark green pods for canning and freezing. Several snap bean lines with superior agronomic traits were recently developed at the University of Nairobi (Wahome et al, 2013). However, their potential for use by the processing industry is not known. The objective of this study was to evaluate the canning quality of the new breeding lines and to identify the lines that combine most of the canning quality traits.

MATERIALS AND METHODS

Twenty seven advanced snap bean lines, and one canning check variety (Julia) were grown at Kirogo Research Station, Mwea (Kirinyaga County) of the Kenya Agricultural and Livestock Research Organisation (KALRO) and evaluated for canning quality. Evaluation for canning quality was conducted in partnership with a major canning factory in Kenya. The factory started operations in 1938 and now produces a wide range of food products such as frozen vegetables, canned vegetables, dehydrated vegetables, pickles and relishes, jams and marmalades, sauces, spices and desserts.Canned snap beans are one of the most important production lines in the factory. At early pod formation, pods were harvested, cleaned soaked, snipped, sorted, blanched, canned in brine and incubated for seven days, and subsequently evaluated for canning quality attributes including hydration coefficient (HC), washed drained weight (WDWT) and percentage washed drained weight (PWDWT), fiber content, water uptake after soaking and blanching, and percent waste after sorting using a modification of procedures described by Warsame and Kimani (2014) and those used by two major canning factories. Analysis of variance was performed and means separated using Fisher's protected least significant difference (LSD) method at 5 and 1 percent probability levels.

RESULTS AND DISCUSSION

Results showed significant ($p<0.05$, 0.01) differences in all traits evaluated apart from HC and fiber content (Table 1). Twenty lines met the industrial canning standards. Among the best performers were KSB22-147-2M/1, KSB22-147-2M/2 and KSB52-2M. Lines that showed poor canning quality attributes such as low HC and WDWT included KSB69-1-1MR1, KSB14-1-1MR1 and KNSB79-1R1/1. Industry reference variety, Julia had low HC (1.1) and high fiber content (20%). Pod fiber content varied from 0 to 35%. Twenty lines had lower fiber content than the check (Julia, 20%). These improved lines of snap bean will provide the bean processing industry

with raw materials that meet consumer's preferences while increasing production of processed products.

Table1. Canning quality attributes of new snap bean lines bred in Kenya.

Genotype	%Water uptake after soaking	Water uptake after blanching (%)	Waste after sorting (%)	Fibre content (%)	HC	Brine pH	Percent PWDWT	WDWT (g)
KNSB13-90-188	5.1	6.7	28.0	5.0	1.2	5.3	58.0	233.0
KNSB79-1R1/1	3.7	2.3	43.0	0.0	1.1	5.5	57.0	223.5
KNSB90-59-R1	3.9	4.7	46.0	0.0	1.1	5.5	56.0	225.5
KSB06-1-1-2M	4.6	9.4	29.5	5.0	1.2	5.3	58.0	231.0
KSB13-14-218/2	4.2	6.3	20.5	15.0	1.2	5.3	57.0	228.0
KSB13-20-208	6.1	8.7	30.5	5.0	1.2	5.3	55.0	223.5
KSB13-23-239/1	5.8	3.7	31.0	20.0	1.1	5.4	56.0	230.5
KSB13-23-248/2	6.2	3.4	20.0	25.0	1.1	5.3	57.0	231.0
KSB13-30-26	4.3	3.8	19.0	5.0	1.1	5.4	57.0	229.0
KSB13-30-27/1	3.5	6.7	20.0	5.0	1.2	5.4	56.0	227.5
KSB13-30-27/2	7.7	9.6	39.0	10.0	1.2	5.4	55.0	228.5
KSB13-39-121	3.4	8.2	35.5	10.0	1.2	5.3	59.0	234.0
KSB13-39-169/1	2.9	4.4	29.5	15.0	1.1	5.4	58.0	228.0
KSB14-1-1MR1	4.7	2.3	37.5	5.0	1.1	5.4	54.0	223.0
KSB20-146-2-1-4MR1/1	5.9	9.2	38.5	20.0	1.2	5.4	57.0	226.0
KSB20-146-2-1-4MR1/2	4.0	3.5	35.5	25.0	1.1	5.4	57.0	225.0
KSB22-147-2M/1	9.2	7.7	17.5	0.0	1.2	5.4	55.0	228.0
KSB22-147-2M/2	7.6	6.6	22.0	20.0	1.2	5.4	57.0	231.0
KSB22-3-1T	5.7	7.8	41.0	35.0	1.2	5.4	56.0	224.0
KSB23-66-2-2M/A	4.1	4.9	23.5	0.0	1.1	5.5	55.0	226.0
KSB27-2-2M	2.5	8.5	10.0	10.0	1.2	5.4	55.0	219.0
KSB33-1-2M	5.2	5.2	37.0	25.0	1.2	5.3	56.0	225.0
KSB33-3-1M	5.6	8.9	24.5	15.0	1.2	5.4	56.0	224.0
KSB39-1-4M	5.8	3.0	30.5	15.0	1.1	5.4	57.0	226.5
KSB39-3M	6.1	3.1	33.5	5.0	1.1	5.4	57.0	227.5
KSB52-2M	5.4	4.0	21.5	15.0	1.1	5.5	60.0	238.0
KSB69-1-1MRI	2.5	3.2	17.0	10.0	1.1	5.5	55.0	224.0
Check								
Julia	2.8	3.8	29.0	20.0	1.1	5.6	57.0	228.5
Mean	4.9	5.7	28.9	12.1	1.2	5.4	56.0	227.5
LSD $_{0.05}$	0.3	2.9	8.9	20.0	0.0	0.1	1.4	4.1
CV (%)	2.5	8.4	15.0	8.3	6.1	0.5	1.2	0.9

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FIELD-GROWN TEPARY BEAN (*PHASEOLUS ACUTIFOLIUS*) REVEALS SYMBIOTIC COMPETENCE UNDER ARID-LAND AGRICULTURE CONDITIONS

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INTRODUCTION

In a rapidly changing world, drought and heat threaten reliable production of nutritious calories and protein for the global population[1]. Studies of plants in arid agriculture conditions can presage the onset of environmental stressors in other agricultural regions and provide a natural laboratory for understanding how plants survive abiotic challenges. Legume crops such as beans are important sources of protein, iron, and zinc under emerging environmental stresses[2]. Though widely cultivated for its nutritional profile and broad acceptance by consumers, common bean (*Phaseolus vulgaris* L.) is susceptible to water stress[3]. Tepary bean (*P. acutifolius* A. Gray) is a drought- and heat-resilient alternative suitable to cultivation in hot and dry regions, including the southwestern USA. To complement studies of constitutive aspects of tepary bean that confer its tolerance to abiotic stress, we characterized the microbiome of selected, cultivated types from the Tepary Diversity Panel (TDP) under field conditions in Arizona. Using amplicon sequencing on the Illumina platform, we compared endophytic microbes occurring in roots, leaves, and beans; distinguished communities of root endophytic microbes in higher- vs. lower-performing lines; and evaluated how root microbiomes shifted under water limitation and performance class. In doing so we identify symbiotic competence as a desirable phenotype for high-performing tepary bean.

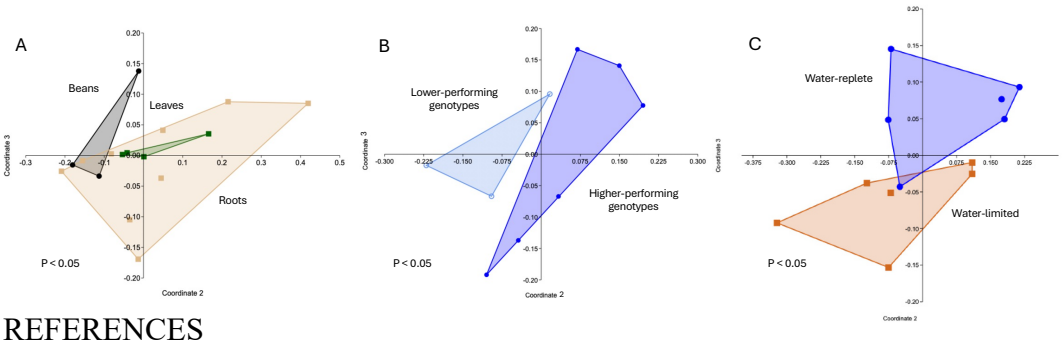
MATERIALS AND METHODS

As described by Yu et al. [5], 214 entries from the TDP were planted in May 2023 at the Maricopa Agricultural Center (Maricopa, AZ, USA; for a site description, see [6]). Seeds were planted at a depth of 5 cm with a mechanical row planter in a randomized incomplete block design with 4.17 m plots spaced at 1.01 m (see [5]). Two treatments were applied: standard watering for cultivation of tepary bean in the desert southwest (i.e., pre-irrigation, irrigation at 14 days after planting [DAP], and irrigation every 14-18 days thereafter), or water limitation (dry-down events at 45 and 60 DAP). When plots were hand-harvested and threshed (August 2023), we collected leaves, roots, and beans from three plants of representative genotypes grown under water-replete and water-limited conditions. Genotypes were rated as lower- or higher performing based on yield. We surface-sterilized tissues, extracted genomic DNA from roots (2 cm^2), leaves (2 cm^2), and beans, and sequenced the V4 region of 16S rRNA, including positive and negative controls[6]. Raw reads were processed, trimmed, filtered, and clustered into operational taxonomic units (OTU) at 97% sequence similarity[6]. A minimum read number of 10 was used to filter spurious OTU, false positives were excluded, and sequences representing chloroplasts or mitochondria were removed prior to analyses in PAST[7]. More than 1.47 million reads were obtained before quality control, with 603,097 bacterial sequences and 657 OTU passing quality control and filtering.

RESULTS AND DISCUSSION

Here we report four main characteristics of the microbiome of field-grown tepary bean. First, we characterized taxonomic composition of the microbiome associated with the interior of healthy tissues. Common bacteria included Proteobacteria (e.g., Rhizobiales, Xanthomonadales , Sphingimonadales, Steroidobacterales), Bacteriodota (Flavobacteriales, Cytophagales, Chitinophagales), and Actinobacteria (Streptomycetales, Micrococcales, Micromonosporales). Several of these lineages have been reported in other legume crops[8,9], but in >80% of cases, a distinctive strain was detected in tepary bean relative to microbiomes in other members of the Fabaceae, consistent with a geographically or environmentally distinctive microbiome in this arid land system. Second, we detected distinctive bacterial communities in healthy roots, leaves, and seeds of tepary bean (Figure 1), consistent with previous work showing that microbial communities differ among plant tissues[6]. Third, root endophytes differed between higher- and lower-performing genotypes of tepary bean, regardless of position in the field (Figure 1). Such performance-specific microbiomes are compelling because those associated with higher-performing plants may be used in synthetic communities that, when applied to lower-performing genotypes, enhance field performance considerably[9]. Fourth, higher-performing genotypes shifted their root endophytic microbiome under water limitation, whereas lower-performing genotypes did not (Figure 1). We use the term 'symbiotic competence' to highlight this stress-associated shift in microbiome composition in higher-performing genotypes and the absence thereof in lower-performing genotypes, and thus conceptualize a trait of interest for tepary bean and other plants in arid agriculture systems. Ultimately, our work outlines the biodiversity, alignment with plant phenotypes, and potential functional aspects of the tepary bean microbiome in a setting with drought and heat that exceed conditions found in most agricultural areas today, but where tepary bean can thrive.

Figure 1. Characterization of the tepary bean microbiome reveals differences as a function of tissue type (A), performance class (B), and water limitation in higher-performing genotypes of tepary bean (C). Non-metric multidimensional scaling based on Bray Curtis distances; P-values reflect permutational analyses of variance; stress values ≤ 0.15.



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EARLY PLANTING OF MICHIGAN DRY BEANS

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Introduction:

In the last decade the varietal landscape of Michigan dry beans has transformed rapidly. We have documented high levels of variety improvement and adoption in both black and navy beans, this accounts for over 80% of total Michigan acres. With this transition we have also observed a few trends: 1.) longer average maturities are providing additional yield potential and are being accepted by growers 2.) Improved plant vigor with newly adopted varieties 3.) higher levels of fungicide use in crop 4.) more focus on winter wheat planting dates. With these trends it has provided the opportunity to reanalyze the traditional dry bean production system. Growers have responded to trend #1 and have adopted varieties that have long term average maturities that are longer than 100 days, a historical upper limit. While this has provided yield to offset the opportunity cost of delayed harvest it has also pushed back wheat planting dates in the fall. In the past this was not a major concern unless fall weather became inclement. However, recent MSU research documented a yield loss of 0.6 bushel per day for seedings planted after October 1 in Michigan (Pennington & Singh 2022) and has driven trend #4. This direct economic cost is in addition to the unquantifiable quality risk of harvesting dry beans in October rather than September. The risk of late harvest has appeared to be greater in the last decade with variable rainfall and temperature patterns, and also helped growers understand trend #2. With early season waterlogging (wet feet) becoming commonplace it has become clear that modern dry bean varieties are much more tolerant of this type of stress than previous generations. Anecdotally, areas of water damage would get larger over time in the field after the initial wet period. However, it has been much more common for these areas to shrink as beans on the margins are able to recover from injury and provide a harvestable yield by the end of the season. This overall improvement in the system has increased total yield potential and made room in the balance sheet for a higher management system that includes inputs such as fungicide during the reproduction stages of development. Survey data from Michigan producers estimated more than 50% of all acres receive at least one fungicide application. While this has documented a positive return on investment for growers, it can contribute to longer yet maturities as leaf retention and 'green stem' can be more likely to persist after fungicide applications. With these challenges come opportunities and growers have adapted. It has become more commonplace to plant dry beans earlier in the spring than was traditionally the norm. In 2025 (weather allowing) more beans will be planted in the third week of May than the third week of June. Historically this was not true. Questions arise about what pressures do we put on the system as we look at adjusting this planting date. To research this question multiple projects were implemented in 2024, including a variety trial with the added factor of planting date that is the focus of this report.

Table 1. Varieties tested, market class, and average maturity in 2024.

Variety	Market Class	Maturity [a]
Black Beard	Black	95
Kona	Black	95
Spectre	Black	99
HMS Bounty	Navy	96
AuSable	Navy	90
Viper	Small Red	99

[a] average maturities for 2024 were 1-4 days earlier than normal due to high levels of GDD accumulation and late season dryness

Methods:

Dry beans were seeded in four-row plots that measured 6.6′ wide by 25′ long, with 20″ rows at five separate timings: May 6th, 13th, 20th, 27th and June 13th. Planting population was 130,000 seeds per acre for all entries. Entries included three black beans, two navy beans and one small red (Table 1.) 60 lb. of nitrogen was applied at plating utilizing 2x2 placement of 28-0-0 fertilizer. Trial was designed as a Split-Plot design. Main plot effect was planting date with **Four replications** per planting date. Subplot effect was variety planted with four replications per planting date. Care was taken to select commercial varieties at each end of the maturity spectrum when possible (short vs. long). Trials received industry standard seed treatments, fertilization, and weed control applications at labeled rates. PPI herbicide treatments and tillage were applied directly ahead of planting for each planting date. Yield data was obtained by direct harvest. Following harvest, samples were cleaned, weighed, and moisture tested. All yield data is adjusted to a standard 18% moisture for standardization.

Results:

Overall trial quality was poor in 2024. Early season heavy and repeated rain followed by a relatively dry reproductive period led to areas of waterlogging and low overall dry bean yields regardless of planting date or variety. Main plot effects (Planting Date) were significant. The subplot (variety) effects were also significant in this testing. There was not a significant interaction between factors. **Planting Date:** When averaged across all entries the mean results from planting dates ranged from 9.5 cwt.- 15.7 cwt./acre (Table 2.). May 20th was the only date that was significantly different (Pr>F= 0.01). On May 21st more than 2 inches of rainfall was received on the freshly planted beans greatly impacting early season root health. **Variety:** When average across all panting dates entries yielded from 10.9 cwt.- 17.8 cwt./acre (Table 3.). Black Beard significantly outyielded all other entries *(Pr>F= 0.007)* with an average performance of 17.8 cwt./acre when averaged across all planting dates.

Table 2. Dry bean response to planting date averaged over varieties tested (Main plot)

Planting Date	Yield [ab]
6-May	1266 a
13-May	1463 a
20-May	955 b
27-May	1397 a
13-Jun	1574 a

Table 3. Dry bean yield by variety averaged over planting dates (Sub plot)

Variety	Yield [ab]
Black Beard	1780 a
Kona	1417 b
Viper	1237 b
HMS Bounty	1233 b
Ausable	1105 b
Spectre	1094 b

[a] Yields followed by the same letter are not considered to be different P<0.05.

[b] Yield is in pounds per acre obtained by direct harvest, adjusted to 18% moisture.

Discussion:

As previously stated, no significant interactions between factors existed in 2024. Meaning one variety was not superior at an individual planting date. This data need repetition over years where early season waterlogging is not such a significant factor on trial quality and yield potential is not limited to such a great extent. However, these preliminary results do support the working grower hypothesis that the black bean variety 'Black Beard' handles stress better than some other varieties in the marketplace. The development of a viable data set on a complex trait such as response planting date will take years of continued trialing to grasp the scale of which genetics * environment * management= yield. We would like to thank the Michigan Bean Commission for supporting this work in 2024.

UNCOVERING IMPORTANT GENOTYPES AND KEY GENOMIC REGIONS CONFERRING WATERLOGGING TOLERANCE IN WILD AND CULTIVATED PHASEOLUS SPECIES

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Introduction

Common bean (*Phaseolus vulgaris* L.) is highly susceptible to waterlogging stress, particularly at early developmental stages (Soltani et al., 2018). On average, waterlogging decreases crop productivity by 32.9% (Tian et al., 2021), with yield losses in common bean ranging from 40% to 80%, depending on the severity of the stress. Despite its vulnerability, research on common bean's response to waterlogging is limited. Soltani et al. (2017, 2018) explored the Middle-America and Andean Diversity Panels, identifying some genomic regions linked to non-flooded conditions and flooded conditions. This study aims to examine genetic variation and identifying key genomic regions associated with waterlogging tolerance in wild and related Phaseolus species, providing valuable insights for breeding more resilient cultivars.

Materials and Methods

A total of 235 accessions from the USDA-ARS germplasm bank, including *P. coccineus* (118), *P. acutifolius* (9), wild *P. vulgaris* (100), and domesticated *P. vulgaris* (8), were evaluated in a randomized complete block design under flooded (4–5 cm water above soil, starting at V2 stage) and non-flooded greenhouse conditions following the protocol from Soltani et al.(2017) Phenotypic measurements included root, shoot, and total dry weights, chlorophyll content and photosynthetic efficiency, and adventitious root formation scored visually. Genotypes were sequenced using Genotyping-by-sequencing technology to identify single nucleotide polymorphisms (SNPs), and genome-wide association studies (GWAS) were performed to detect trait-associated genomic regions.

Results and Discussion

Significant genotype-by-treatment interactions ($P < 0.01$) were observed among all genotypes for all measured traits. Flooding reduced biomass by an average of 78%, but certain genotypes such as TARS-TEP32 and PI535447 retained higher biomass under stress suggesting adaptive mechanisms, while *P. coccineus* accessions exhibited notable adventitious root formation (up to 3.4 on a 0–5 scale). The correlation between adventitious root formation and biomass retention suggests that root plasticity is a key adaptive trait. Chlorophyll content and photosynthetic efficiency declined, 15 SPAD units and 0.3 Fv/Fm respectively, under flooding conditions, though tolerant accessions such as PI358088 (*P. coccineus* form North Macedonia*)* and Royalty (positive check), maintained similar values, indicating functional photosystems and sustained photosynthetic activity. Maintaining higher chlorophyll content and photosynthetic efficiency under stress suggests enhanced physiological resilience in tolerant genotypes.

Table 1: Response of the top tolerant and susceptible accessions for shoot and total weight under flooded conditions

Genotype	Species	Shoot Weight Flooded (g)	Shoot Weight Non-Flooded (g)	Total Weight Flooded (g)	Total Weight Non-Flooded (g)
Tolerant Accessions					
PI358088	*P. coccineus*	1.18	1.32	1.22	1.75
PI494068	*P. coccineus*	0.89	2.46	0.89	3.02
PI2301	*P. coccineus*	0.79	2.18	0.83	2.73
PI311185	*P. coccineus*	0.74	1.34	0.77	1.66
PI201309	*P. coccineus*	0.74	1.77	0.75	2.47
PI430178	*P. coccineus*	0.67	1.00	0.68	1.12
PI433239	*P. coccineus*	0.66	1.21	0.66	1.58
PI311176	*P. coccineus*	0.65	0.89	0.67	0.98
Royalty	*P. vulgaris*	0.40	1.01	0.40	1.14
Susceptible Accessions					
PI535406	*P. vulgaris* L. wild	0.02	0.29	0.02	0.40
PI535416	*P. vulgaris* L. wild	0.03	0.15	0.03	0.19
PI417784	*P. vulgaris* L. wild	0.03	0.16	0.03	0.20
Medalist	*P. vulgaris* L.	0.25	0.62	0.26	0.79
Huron	*P. vulgaris* L.	0.16	0.41	0.17	0.53

GWAS analysis identified several genomic regions associated with waterlogging tolerance. A significant locus on Pv01 (32.4 Mb) was linked to chlorophyll content, shoot weight, and total weight under flooded conditions, aligning with previous findings by Soltani et al. (2017). Additionally, three novel genomic regions were discovered: Pv07 (48.3 Mb), associated with root weight under non-flooded conditions and shoot/total weights under flooding; Pv09 (42.6 Mb), linked to biomass traits under non-flooded conditions; and Pv11 (20.6 Mb), associated with root weight in non-flooded conditions and shoot/total weights under flooding. Candidate genes analysis revealed multiple loci related to hypoxia response and stress adaptation. Notably, genes near to significant SNPs on **Pv01** and **Pv07** are involved in ethylene-mediated root growth and adventitious root initiation. Genes near **Pv09** are implicated in biomass accumulation, while loci on **Pv11** include regulators of chlorophyll degradation and photosystem II stabilization.

Conclusion

This study demonstrated significant genetic variation for waterlogging tolerance among *Phaseolus* spp. accessions. Genotypes with superior tolerance traits and associated genomic regions were identified, offering potential opportunities for developing resilient common bean cultivars. Further research should focus on validating candidate genes and integrating tolerance traits into breeding pipelines.

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PHYSIOLOGICAL RESPONSES OF COMMON BEAN GENOTYPES TO WATER DEFICIT

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INTRODUCTION: Common bean (*Phaseolus vulgaris* L.) is an important source of protein, fiber, and essential nutrients, playing a fundamental role in global food security. However, water deficit affects common bean yield, especially when it occurs during the plant's reproductive stage. Water deficit disrupts plant physiological processes, affecting water balance and photosynthesis, leading to reduced crop productivity (Zamani et al., 2024). Developing bean cultivars with high productivity under drought conditions is essential to ensure food security in the face of climate change. Thus, the evaluation of physiological traits helps to understand the mechanisms of drought tolerance in plants. The aim of this study was to analyze the physiological responses of common beans breeding lines and cultivars subjected to drought during the reproductive period.

MATERIAL AND METHODS: The experiment was conducted from May to August of 2021 under protected cultivation at the Experimental Station of IDR-Paraná, Londrina, Paraná State, Brazil (Latitude: 23°17' S, Longitude: 51°10' W, and altitude 550 m). It was arranged in a randomized blocks design with time split plot with two replications: water deficit (WD) and well watered (WW). Four commercial cultivars (IAPAR 81, IPR Campos Gerais, IPR Sabiá, and BRS Estilo), and two breeding lines (BAT 477 and SEA 5) were evaluated. The plants were submitted to WD since the R5 until R7 stage, with a 30% of field capacity and the irrigation was monitored by 20 TDR (Time domain reflectometry) probes. The water deficit period was 37 days and the traits evaluated every four days during the stress were: rate of photosynthesis (*A*) and stomatal conductance (*gs*), using the infrared gas analyzer (IRGA) instrument. Data were subject to Scott-Knottt test ($p<0,05$) for means grouping.

RESULTS AND DISCUSSION: For the rate of photosynthesis (*A*) and stomatal conductance (g_s), a greater differentiation between environmental conditions (WD and WW) was observed starting on the 12th day, remaining throughout the entire stress period (Figures 1 and 2). However, SEA 5 and BAT 477 breeding lines stood out with similar net photosynthesis values in both environments, indicating drought tolerance. The average g_s values varied up to Day 8. However, from Day 12 onward, the values in the WD environment were lower than those in the IRR environment. The IPR Sabiá cultivar showed the lowest g_s values from Day 12 onwards in both environments. Under WD, the SEA 5 breeding line showed the highest g_s values, indicating a drought tolerance mechanism. Water deficit reduces leaf stomatal conductance, decreasing water and nutrient absorption and the plant's photosynthetic activity. When the plants were subjected to drought, the evaluated physiological characteristics were reduced by 54% and 41% for *gs* and *A*, respectively.

CONCLUSIONS: The results demonstrated the influence of water deficit on the physiological responses of the evaluated genotypes, assisting in the selection of genotypes that can be used in breeding programs for the development of drought-tolerant bean cultivars.

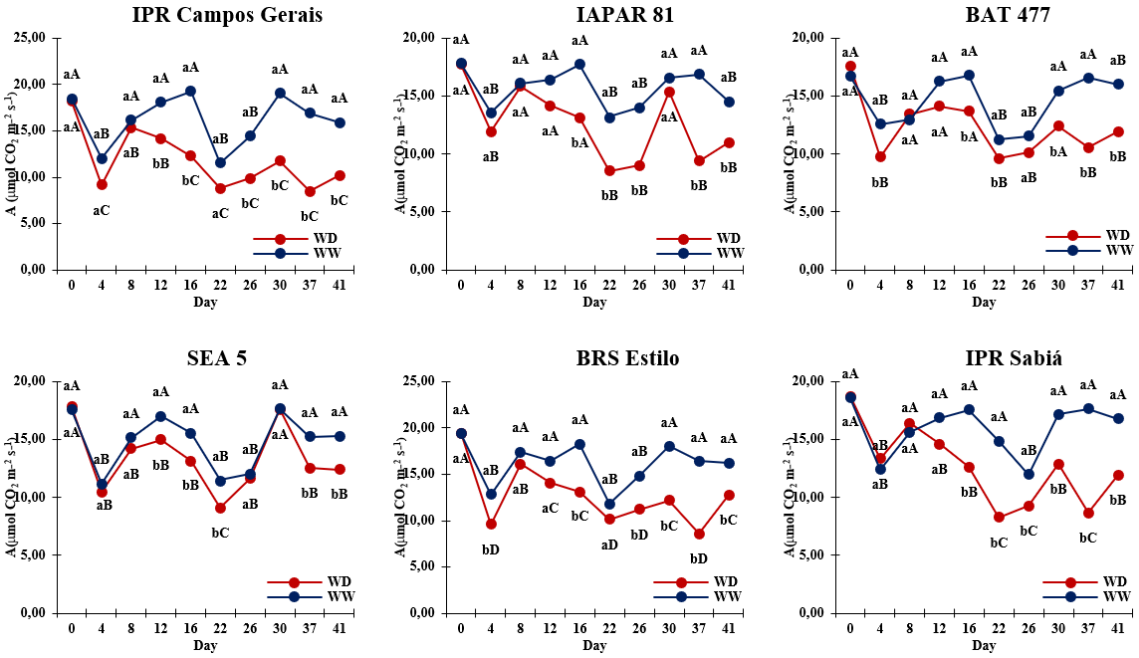

Figure 1. Monitoring of net photosynthesis of six common bean genotypes subjected for 37 days to two water regimes: well-watered (80% of field capacity) and water deficit (30% of field capacity).

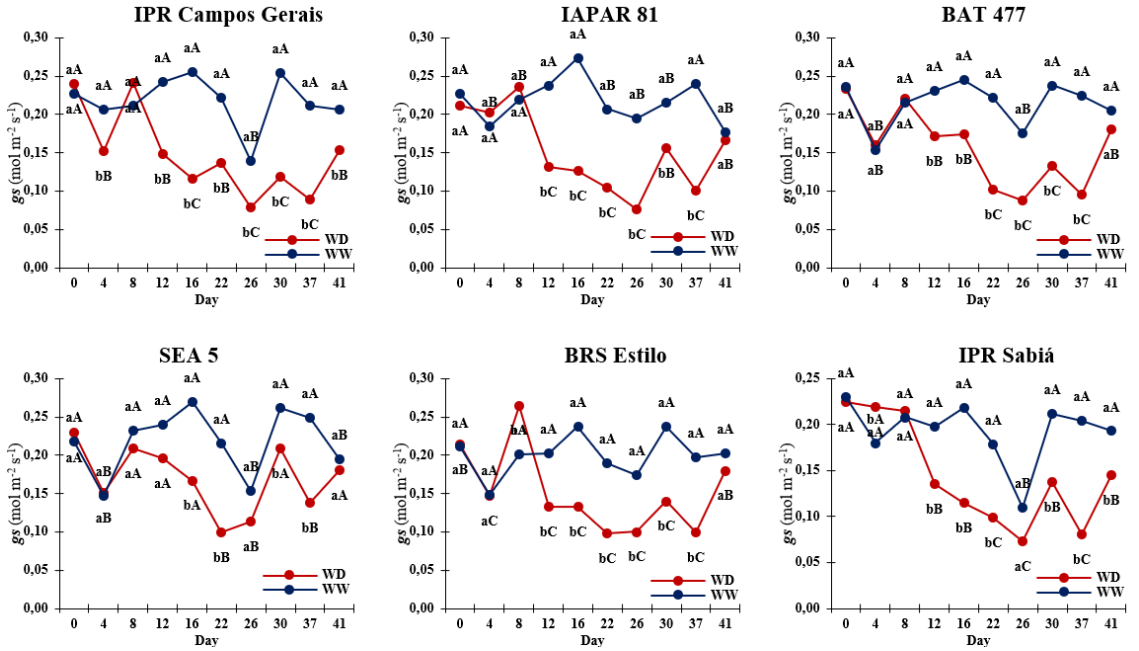

Figure 2. Monitoring of stomatal conductance of six common bean genotypes subjected for 37 days to two water regimes: well-watered (80% of field capacity) and water deficit (30% of field capacity).

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RELATIVE YIELD EFFICIENCY OF MESOAMERICAN BLACK BEAN GENOTYPES GROWN IN ACID SOIL AND TERMINAL DROUGHT

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INTRODUCTION. Acid soils and terminal drought are the main environmental factors that reduce bean yield in southern Veracruz, Mexico. Although dolomite liming can correct soil acidity and increase grain yield by more than 40% (Tosquy-Valle *et al.*, 2008), this technology has not been adopted in bean cultivation, mainly due to the high cost of the liming material and its application, which increases the total cost of production by 30%. An economically viable and practical alternative for producers is to have black bean varieties adapted to soil acidity stress and soil moisture deficiencies caused by the occurrence of terminal drought. The objective of this research was to identify Mesoamerican black bean genotypes with higher yield efficiency in acid soils and terminal drought than the Negro Jamapa improved cultivar commonly used in Veracruz.

MATERIALS AND METHODS. Two field trials were conducted in the fall-winter cropping season, 2023-24 on an acidic soil (with an initial pH= 4.6) in Juan Rodríguez Clara, Veracruz, Mexico. One trial was conducted under natural soil acidity conditions and the other with dolomite lime applied 46 days before sowing at a rate of 2.72 t ha^{-1}. Eleven advanced lines and five improved cultivars, including Negro Jamapa used as a regional control, were evaluated in a RCBD with three replicates. Rainfall was recorded during the entire cropping season, soil pH was measured across the growing season and grain yield was quantified in kilograms per hectare. Yield data were analyzed individually and combined with and without dolomite, and the Least Significant Difference (LSD 0.05) statistical test was used to separate yield averages. The effect of soil acidity on the yield of each genotype was estimated using with the relative efficiency index (REIi) suggested by Graham, 1984.

RESULTS AND DISCUSSION. Total rainfall during the entire cropping season was 281 mm, with drought occurring during the reproductive phase of the crop. The average soil acidity from 61 days after liming until the bean crop reached maturity was pH=5.8, which is within the optimal range of 5.8 to 6.5 for the bean crop development (Kyomuhendo *et al.*, 2020). In contrast, in the trial without dolomite application, pH values recorded throughout the crop cycle were low (pH≤4.8), consequently bean genotypes grew and developed under conditions of severe soil acidity stress. In the trial with applied dolomite, a group of nine genotypes had similar and outstanding seed yields, among these Jamapa Plus/XRAV-187-3-4-4, Jamapa Plus/XRAV-187-3-4-1 and Negro Citlali/XRAV-187-3-1-5 breeding lines, together with Rincón Grande, Negro INIFAP and Rubí improved cultivars had also higher seed yields under conditions without application of dolomite (Table 1). With regard to the average seed yields calculated from both soil acidity conditions, results indicated that a group of five genotypes had the highest seed yields of which Jamapa Plus/XRAV-187-3-4-4 and Jamapa Plus/XRAV-187-3-4-1 breeding lines also had the highest REIi values (Table 1), indicating that these genotypes had great productivity efficiency

with and without the application of dolomite; in contrast, the control cultivar, Negro Jamapa, had low efficiency in both soil acidity conditions, with a REIi value less than 1.0 (Graham, 1984).

Table 1. Grain yield and estimated selection indices of black bean genotypes in acidic soil, with and without application of dolomite.

Genotype	Seed yield (kg ha^{-1})		Average (kg ha^{-1}) [§]	REIi
	+ Dolomite	- Dolomite		
Papaloapan/SEN 46-2-6	685.00bcde	438.33bcdefg	561.67cde	0.88
Papaloapan/SEN 46-3-2	581.67e	288.33g	435.00f	0.49
Papaloapan/SEN 46-7-7	720.00bcd	385.00efg	552.50de	0.82
Papaloapan/SEN 46-7-10	671.67cde	435.00bcdefg	553.33de	0.86
Papaloapan/SEN 46-7-12	620.00de	308.33fg	464.17ef	0.56
Negro Citlali/XRAV-187-3-1-5	725.00abcd	571.67ab	648.33abcd	1.22
Negro Citlali/XRAV-187-3-1-6	716.67bcd	415.00cdefg	565.83cde	0.88
Negro Citlali/XRAV-187-3-1-8	811.67ab	398.33defg	605.00bcd	0.95
Rincón Grande	768.33abc	561.67abc	665.00abc	1.27
Jamapa Plus/XRAV-187-3-4-1	795.00abc	635.00a	715.00ab	1.49
Jamapa Plus/XRAV-187-3-4-4	853.33a	658.33a	755.83a	1.66
CIAT-103-25	755.00abc	375.00efg	565.00cde	0.83
Negro Jamapa	690.00bcde	446.67bcdef	568.33cde	0.91
Verdín	750.00abc	461.67bcdef	605.83bcd	1.02
Negro INIFAP	775.00abc	550.00abcd	662.50abcd	1.26
Rubí	740.00abcd	515.00abcde	627.50bcd	1.12
Average[§§]	728.65a	465.21b	596.93	1.01
ANOVA	*	**		
LSD (0.05)	128.38	154.91	111.12	

[§]Average seed yield in the combined analysis of variance.
REIi= Relative efficiency index.
[§§]Average seed yield with and without application of dolomite.
Genotypes with different letters in each column are statistically different according to the LSD test (0.05).

CONCLUSIONS. The Jamapa Plus/XRAV-187-3-4-4 and Jamapa Plus/XRAV-187-3-4-1 breeding lines showed the best adaptation to the soil acidity stress and terminal drought conditions of southern Veracruz, Mexico. Negro Citlali/XRAV-187-3-1-5 breeding line together with improved cultivars Negro INIFAP and Rincón Grande also showed greater yield efficiency than the control cultivar Negro Jamapa.

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IRON CONCENTRATIONS AND IRON BIOAVAILABILITY OF GREAT NORTHERN BEANS PRODUCED ACROSS THE UNITED STATES

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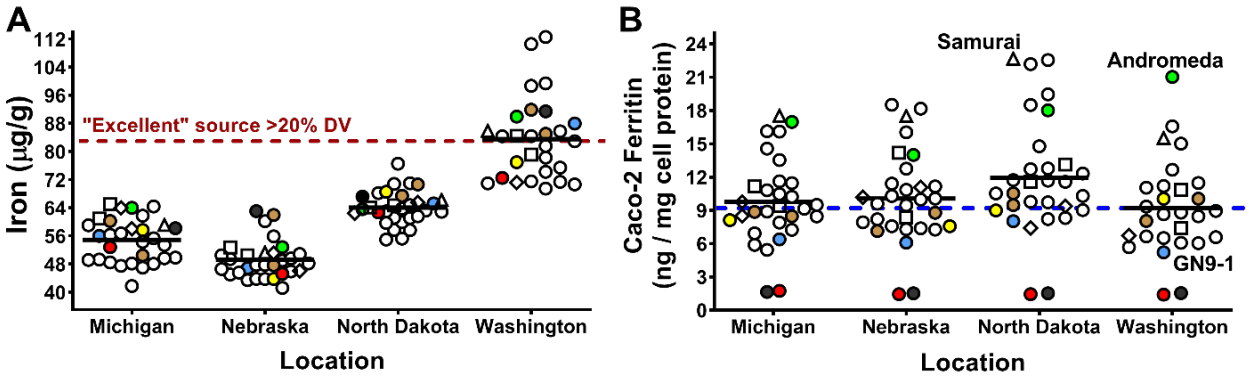
INTRODUCTION: Dry beans (*P. vulgaris*) are potentially a good source of iron, providing 10-15% of the Daily Value (DV) with each ½ cup serving. Public awareness of iron deficiency is increasing as anemia in the United States is on the rise [*JAMA Network Open* 2024 7(6) e2413967]. However, the consumption of dry beans and other pulses remains low. Promoting beans to consumers searching for iron can also be misleading, as many of the dark colored market classes have high concentrations of iron inhibiting flavonoids, such as proanthocyanidins in pinto beans, which can dramatically lower the absorption of iron during digestion. Iron must be absorbable (i.e., bioavailable) to be nutritionally beneficial. USDA-ARS and public breeders are currently identifying market classes of dry beans that can meet the iron needs of U.S. consumers by providing a reliable source of bioavailable iron. For example, slow-darkening (P^{sd}) pinto beans have higher iron bioavailability compared to regular darkening pintos due to the reductions of proanthocyanidins in their seed coats [*JFF* 2021 (8) 104444]. Great Northern beans lack pigmentation in their seed coats and have no iron inhibiting flavonoids. Therefore, a collection of Great Northern beans was assembled to assess the capacity of this market class to be a good source of bioavailable iron.

MATERIAL & METHODS: The Great Northern Collection (GNC) consists of 20 Great Northern dry bean varieties with some being identified as having different alleles in the master color *P* gene (p^{NEP-2} and $p^{Matterhorn}$). The GNC also includes ten non-great northern field checks, which are used to measure the iron nutrition of other white and colored bean market classes (w. kidney, navy, yellow, slow-darkening pinto, black and red) produced under the same field conditions and cooked in the same manner as the Great Northerns. The GNC was grown at research sites located in Michigan, Nebraska, North Dakota and Washington. Bean samples from each site were pre-soaked and cooked in distilled water using a Mattson pin drop device under standardized cooking conditions [*J. Sci. Food Agric.* 2005 (85) 1631-1635]. Cooked beans were stored at -80°C until freeze drying and milling into a fine powder using an analytical hammer mill. Iron concentrations were measured using ICP-AES. A phytate/total phosphorus kit (K-PHYT; Megazyme® International, Bray, Ireland) was used to measure phytate. Iron bioavailability was measured according to the established methods described in Glahn, 2022 [*JoVE* 2022 (182) e63859].

RESULTS AND DISCUSSION: Great Northern beans had cooking time ranges (17-38 min) similar to the field checks. The iron concentrations of the GNC after cooking are shown in **Figure 1A**. Iron concentrations ranged from 42-113 µg/g across all locations, but there were significant differences among locations, suggesting a high GxE interaction (**Figure 1A**). Beans produced in

Nebraska (soil pH 8) had the lowest iron concentrations, while the highest iron was measured in beans from Washington. In this location, over a dozen varieties had iron concentrations above the 84 µg/g threshold, equating to an "excellent" source (>20% DV) of dietary iron after cooking (**Figure 1A**). Despite the large differences in iron concentrations, the patterns of iron bioavailability for GNC were similar across the locations (**Figure 1B**). Several Great Northern varieties (Andromeda, Eiger, Panhandle Pride, Starlight, Marquis) produced Caco2 ferritin values (i.e., iron uptake values) similar to the Otebo bean (*cv*. Samurai), which is considered to have superior iron bioavailability among pulse crops (**Figure 1B**). Many of the Great Northerns had more bioavailable iron than the commercial white kidney bean (*cv.* Snowdon), as well as slow-darkening pintos (*cv*. ND Palomino and USDA Diamondback), which are used as reference standards for improved iron bioavailability (**Figure 1B**). In contrast, several Great Northern varieties (GN9-1, White Pearl, BerylR, JM-24, Matterhorn) had iron bioavailability scores lower than the white kidney standard but still higher than the red (*cv*. Merlot) and black bean (*cv*. Eclipse) field checks (**Figure 1B**). Interestingly, the high iron concentrations of the GNC from Washington did not result in more bioavailable iron (**Figure 1**). Beans produced in Washington had higher concentrations of all minerals (Cu, Zn, Ca, Mg, P), but the beans also had 15-20% more phytate when compared to beans produced in Michigan, Nebraska and North Dakota. Phytate can be an inhibitor of iron uptake in the small intestine. Furthermore, there was a significant (r = -0.314, *P* = 0.002) relationship between phytate concentrations and iron bioavailability of the GNC across locations. These findings suggest genotypic differences in the delivery of iron from Great Northern beans, with some varieties demonstrating superior iron bioavailability across all locations, independent of their iron concentrations. These varieties warrant further consideration and investigation as opportunities for producing bean crops with high iron bioavailability across the United States.

Figure 1. Dot plots depicting the (**A**) iron concentrations and (**B**) iron bioavailability of 30 dry bean entries from the



Great Northern Collection produced in different locations across the United States. Each dot represents the mean value of two field replicates grown over one field season at each production site. Hyphenated lines indicate target values for improved iron concentrations (A; red) and iron bioavailability (B; blue) in soaked and cooked beans. Green and blue dots highlight Great Northern beans with high (Andromeda) and low (GN9-1) iron bioavailability across all locations. Field controls: Δ Otebo (*cv*. Samurai); □ white kidney; ⋄ navy; yellow – *Mayocoba*; brown – *pinto (slow-darkening)*; red - *small red* (*cv.* Merlot) and black (*cv.* Eclipse) are represented as colored dots on each graph.

DIFFERENTIAL EXPRESSION OF GENES IN RESPONSE TO BEAN RUST INFECTION

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INTRODUCTION

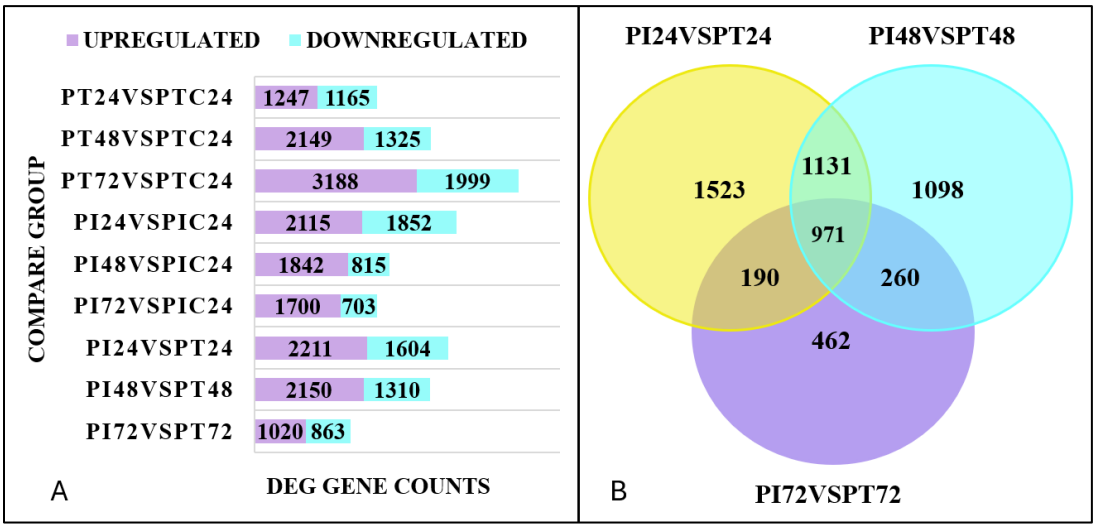
The bean rust disease, caused by the fungus *Uromyces appendiculatus*, often causes significant seed yield losses, especially in regions with cooler temperatures and high moisture conditions that favor the development of the rust disease (Stavely and Pastor-Corrales, 1989). Planting rust-resistance common bean cultivars is an effective strategy to manage the bean rust pathogen. Transcriptome sequencing (RNA-seq), which has been used to study resistance-related genes in common bean, provides significant insights into the early stages of infections (Jurado et al. 2022; Chang et al. 2024). PI181996, common bean cultivar containing the *Ur-11* gene that confers resistance to all but one of 90 races of *U. appendiculatus* maintained at the Beltsville Agricultural Research Center. Conversely, the common bean Pinto 114 is susceptible to most of the same races. In this study, we used RNA-seq to investigate differentially expressed genes (DEGs) elicited by the response of the PI181996 and Pinto 114 cultivars inoculated with race 53 of *U. appendiculatus*.

MATERIAL AND METHODS

This experiment was performed using 15 plants of the resistant PI181996 and the susceptible Pinto 114 cultivars. Twelve plants from each cultivar were inoculated with race 53 of *U. appendiculatus*. Ten days after seeding, the abaxial side of their primary leaves were inoculated with a water solution containing 2×10^4 spores of race 53 of *U. appendiculatus*. The inoculated plants were placed in a mist chamber maintained at 20 ± 1 ºC with relative humidity >95% and kept in darkness for 18 hours. Then, the plants were moved to a greenhouse bench. We collected the primary leaves from three plants of each cultivar (three replicates) 24, 48, and 72 hours after inoculation. Additionally, leaves from three uninoculated plants (controls) of each cultivar were also collected 24-hours after inoculation. The collected leaf tissues were placed in 50 mL Falcon tubes and immediately submerged in liquid nitrogen and then stored in a -80 ºC freezer. Samples were labeled as follows: PI24, PI48, and PI72 for the inoculated plants and PIC24 for the uninoculated plants (controls) of PI181996; PT24, PT48, and PT72 for the inoculated plants and PTC24 for the uninoculated plants (controls) of Pinto 114. In summary, we had a total of 12 collected samples per cultivar. The remaining three inoculated plants from each cultivar were maintained in the greenhouse during-10 days after inoculation to evaluate their rust symptoms. In total, we had 15 plants per cultivar. The leaf tissues were grounded using a Spex SamplePrep 6875D grinder. Total RNA was extracted using the Qiagen RNeasy Mini Kit for Plants. Messenger RNA was purified from total RNA, and cDNA was synthesized to prepare libraries for sequencing. The Illumina sequencing on NovaSeq X Plus platform with 150 bp paired end reads (> 6 G raw data per sample) was performed by Novogene, USA. The reads were mapped on the reference genome of common bean using Hisat2 v2.0.5. We measured the fragments per kilobase of transcript sequence per millions of base pairs of each gene, and Gene Ontology (GO) enrichment analysis of significant DEGs for each pairwise comparisons were performed using R software.

RESULTS AND DISCUSSION

To identify the DEG potentially involved in the resistance response in common bean to the rust pathogen, we performed transcriptome comparisons before and after inoculation of PI181996 and Pinto 114 with race 53 of *U. appendiculatus*. We also compared the significant DEGs between the resistant cultivar PI181996 and the susceptible cultivar Pinto 114 inoculated with race 53. The DEGs were identified based on a significance threshold of a fold change >2 and a p-value <0.05. When comparing the number of DEGs before and after inoculation, we observed that for the resistant PI181996 cultivar, the number of DEGs upregulated and downregulated was highest at 24 hours after inoculation (PI24VSPIC24) and decreased as time progressed (PI48VSPIC24 and PI72VSPIC24). In contrast, for the susceptible cultivar Pinto 114, the number of DEGs was highest at 72 hours after inoculation (PI72VSPIC24), indicating a progressive increase in DEGs upregulated and downregulated over time after inoculation (Fig. A). A similar observation was reported in the anthracnose resistance study conducted by Chang et al. (2024). GO analysis separated the DEGs into three groups: Biological Processes (BP), Molecular Functions (MF), and Cellular Components. Within the BP category, 31 genes related to plant defense were identified in PI181996, compared to 24 genes in Pinto 114 when analyzed 24 hours before and 24 hours after inoculation. Valentini et al. (2022) fine-mapped a resistance gene in PI181996 and identified PHAVU_011G200900 as a candidate gene. In our study, this gene was among the 971 DEGs detected when comparing the resistant and susceptible cultivars at 24, 48, and 72 hours after inoculation (Fig. B). The PHAVU_011G200900 candidate gene was classified as having an ADP-binding function and was included in the MF group in our analysis. These results will be used in future investigations, and we believe they could provide genetic resources for rust resistance breeding in common bean.



A) Number of differentially expressed genes in different combinations; B) Venn diagram of the DEGs between the resistant PI181996 and susceptible Pinto 114 common bean cultivars.

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REACTIONS OF WILD ACCESSIONS OF COMMON BEAN TO RACE 20-3 OF *UROMYCES APPENDICULATUS*

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INTRODUCTION

The common bean is a vital human food particularly in countries of the Americas and Eastern and southern Africa. Common bean production is often diminished by multiple pathogens including the fungus *Uromyces appendiculatus* that causes the rust disease of common bean. Host resistance is the most practical and effective way to manage the rust disease. However, the capacity of this pathogen to recurrently produce new virulent strains that often infect common beans that previously were resistant, complicates the effectiveness of host resistance. Developing common bean cultivars with two or more resistance genes is an effective solution to this problem. However, this strategy requires multiple rust resistance genes. In 2008, the rust disease was widespread in common bean fields in North Dakota. Isolates of the rust pathogen were collected, purified, and inoculated on a set of common bean differential cultivars with the purpose of characterizing their virulence. Many of these isolates had identical virulence that was characterized as race 20-3 of *U. appendiculatus* in 2008 (Markell et al., 2009). The objective of this study was the characterization of the reaction of 65 wild common bean accessions to race 20-3, for discovering new sources of resistance to the bean rust pathogen. Perhaps these accessions contain new rust resistance genes that would contribute to broadening the genetic base of the common bean and for the defense of this crop from the hyper-virulent bean rust pathogen.

MATERIAL AND METHODS

A total of 65 wild common bean accessions obtained from the germplasm bank of the International Center of Tropical Agriculture (CIAT), were evaluated for their reaction to race 20-3 of *U. appendiculatus*. The cultivars Montcalm, Pompadour Checa 50, PI181996, and Aurora were included as controls. The seed coats of eight seeds from each accession were scratched and planted in Promix soil, two seeds per pot. Ten days after seeding, we prepared a spore solution at a concentration of 2×10^4 urediniospores per mL of water. The primary leaves of each plant were inoculated by brushing the abaxial side with the spore solution. Then, the plants were transferred to a mist chamber maintained at 20 ± 1ºC with over 95% relative humidity and kept in darkness for 18 hours. Then, the plants were placed in a greenhouse bench. Ten days after inoculation we evaluated the reactions of these plants to race 20-3 using a rust severity scale from 1 to 6. Plants with no or few rust symptoms (scores of 1 to 3) were considered resistant, while those with scores of 4 to 6 were classified as susceptible (Stavely and Pastor-Corrales, 1989).

RESULTS AND DISCUSSION

Among the 65 common bean wild accessions inoculated with race 20-3 of *U. appendiculatus*, 20 (31%) were resistant. Seven of these accessions did not have any visible rust symptoms, four

exhibited necrotic spots without sporulation, and nine showed small sporulating pustules. The other 45 (69%) accessions were susceptible showing large sporulating pustules (Figure 1A; 1B). This study enables the important identification of new sources of rust resistance. The G10018A, G12852, G19893, G19895, G19907, G23444, and G50384 accessions appear to be highly resistant to race 20-3; they did not display any rust symptoms. Race 20-3 overcomes the resistance genes *Ur-3*, *Ur-6*, and *Ur-7*. Common bean breeding sometimes involves crosses among cultivars from the same gene pool that helps to preserve important agronomic and other traits. However, this practice contributes to the narrowing of the genetic base of common bean. This is why it is important to discover new disease resistance genes to broaden the genetic base of this very important human food.

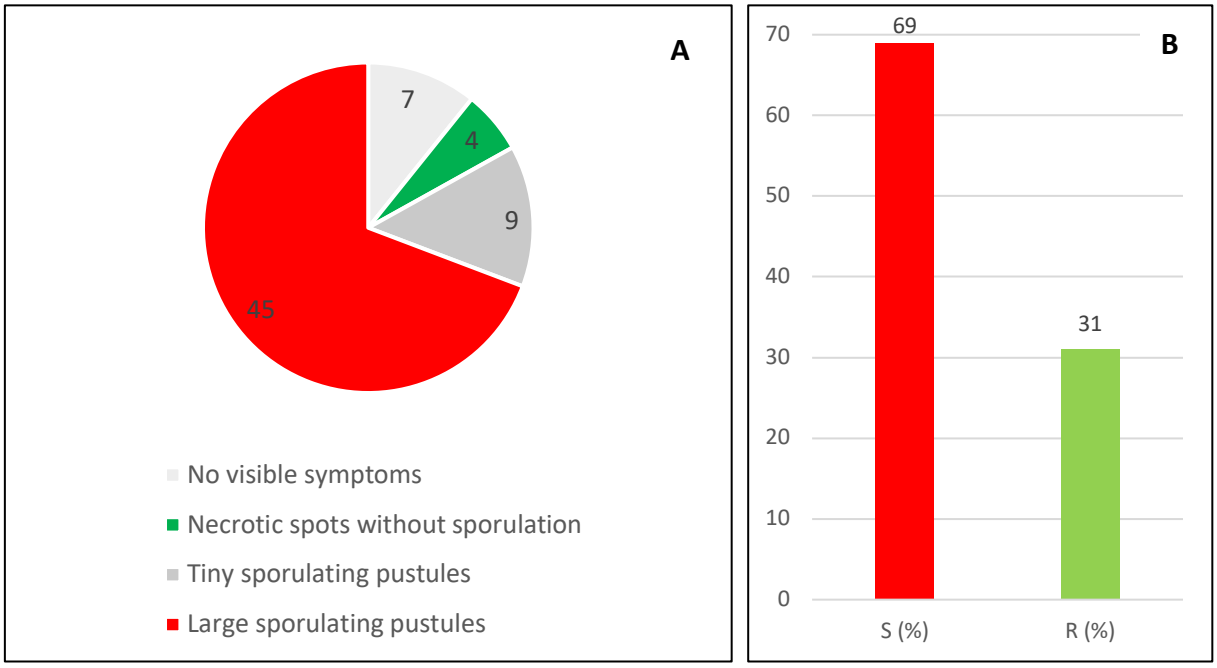

Figure 1. A) Number of wild accessions of common bean plants with their reactions to race 20-3 of *Uromyces. appendiculatus*; 45 were susceptible and 20 were resistant. **B)** Percent of susceptible (69%) and resistant (31%) wild accessions of common bean.

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MOLECULARLY MAPPING RUST RESISTANCE GENE IN THE COMMON BEAN CULTIVAR PI310762

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INTRODUCTION

The common bean rust pathogen, *Uromyces appendiculatus*, is known for its significant ability to create new virulent strains. Planting resistance cultivars is an effective strategy to manage the bean rust pathogen. Thus, identifying genes with broad spectrum resistance is needed for the development of common bean cultivars with rust resistance. The Middle American common bean cultivar PI310762 is resistant to 89 of 90 races of the rust pathogen maintained at the Beltsville Agricultural Research Center. PI310762 is susceptible only to race 85 from Guatemala (Pastor-Corrales et al., 2012). Shin et al. (2014) performed bulk segregant analyses using F_2 plants from the PI310762 × Pinto 114 cross. The Illumina BeadChip with 5,399 single-nucleotide polymorphism (SNP) markers were used for genotyping, followed by simple sequence repeat (SSR) markers development in the genomic region on Pv04 containing the rust resistance gene. Shin et al. (2014) mapped the rust resistance gene in PI310762 between the SSR1167 and SSR1170 markers. In this study, we mapped the rust resistance gene in PI310762 using SNP markers located on PV04, using Kompetitive Allele-Specific PCR (KASP) markers.

MATERIAL AND METHODS

A total of 149 F_2 plants from the PI310762 × Pinto 114 cross were phenotyped with races 67, 84, 105 and 108 of *U. appendiculatus* (Pastor-Corrales et al. 2012). DNA from the F_2 plants was used to perform KASP genotyping with nine SNPs markers located on PV04. The KASP markers were designed based on the SNP chip tables found in Song et al. (2015) and the whole genome sequence of PI310762 and Pinto 114. KASP genotyping was performed following the manufacturer's instructions (LGC Genomics). The data was analyzed using QuantStudio Real-Time PCR software. The genetic distances between KASP markers and the PI310762 gene were estimated using JoinMap 4.0. We then designed the linkage map using MapChart 2.3 software.

RESULTS AND DISCUSSION

PI310762 is an important source of resistance to the bean rust pathogen of common bean. In this study, we genotyped 149 F_2 plants from the PI310762 × Pinto 114 cross using nine KASP markers. Nine plants revealed recombination between the KASP markers SS502 and SS202, suggesting that the candidate gene in PI310762 was positioned in a region spanning 1.4 Mb (Table 1). The rust resistance gene in PI310762 was positioned at 0.4 cM from the SS502 and 0.3 cM from six KASP markers (SS122, SS505, SS509, SS515, SS123, and SS60) that were clustered together in the linkage map (Figure 1). Based on the genotyping results, we could observe a region with no recombination among KASP markers SS122, SS505, SS509, SS515, SS123, and SS60. These results suggested that increasing the number of plants could enable identification of additional recombination events in this region. We plan to genotype and phenotype the F_3 plants derived from the recombinants and the heterozygous $F_{2:3}$ families from the PI310762 × Pinto 114 cross to narrow the region containing the candidate resistance gene. Other rust resistance genes, including *Ur-5*,

Ur-14, and *Ur-G19833,* have also been mapped on PV04 (Valentini et al 2017; Valentini et al. 2021). Fine mapping of the rust resistance gene in PI310762 will be valuable for breeding programs aiming to develop rust-resistant common bean cultivars.

Table 1. Reaction of nine F_2 recombinant plants from the PI310762 × Pinto 114 cross to the rust pathogen races 67, 84, 105 and 108 and genotyped with nine KASP Markers.

Entry#	Rust Phen.	SS129 33,602	SS502 149,320	SS122 275,511	SS505 345,796	SS509 373,114	SS515 488,914	SS123 495,739	SS60 1,301,181	SS202 1,556,740
Pinto 114	S	AA	AA	AA	AA	AA	AA	AA	AA	AA
PI310762	R	BB	BB	BB	BB	BB	BB	BB	BB	BB
01	R	BB	BB	BB	BB	BB	BB	BB	BB	BB
04	R	AB	AB	BB	BB	BB	BB	BB	BB	BB
09	S	AA	AA	AA	AA	AA	AA	AA	AA	AB
16	R	AB	AB	AB	AB	AB	AB	AB	AB	AA
31	R	AA	AB	AB	AB	AB	AB	AB	AB	AB
32	R	AB	AB	AB	AB	AB	AB	AB	AB	BB
78	R	BB	BB	AB	AB	AB	AB	AB	AB	AB
134	R	BB	BB	BB	BB	BB	BB	BB	BB	AB
150	R	AB	AB	AB	AB	AB	AB	AB	AB	AA

S = susceptible; R = resistant; AA = Susceptible allele; AB = Heterozygous allele; BB = Resistant allele.

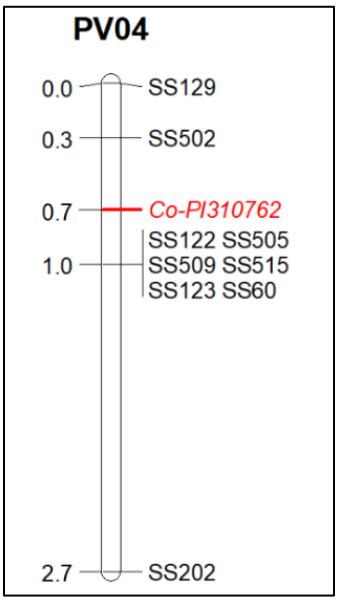

Figure 1. Genetic map of the rust resistance gene in PI310762.

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GENETIC ANALYSIS OF THE RUST RESISTANCE IN GOLDEN GATE WAX AGAINST FOUR RACES OF *Uromyces appendiculatus*

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INTRODUCTION

Rust, caused by *Uromyces appendiculatus*, is a major disease of common bean globally. The Andean common bean Golden Gate Wax (GGW) is one of the 12 rust differential cultivars. GGW carries the *Ur-6* gene, which is also present in the cultivar Olathe (*Ur-6*+). The *Ur-6* gene was first described in Olathe as Ur_a (Gafton et al., 1985). This gene was later renamed *Ur-6* (Kelly et al., 1996) and was mapped on chromosome Pv11 (Miklas et al., 2002; Park et al., 2004). The *Ur-6* gene was assigned to GGW because of its resistance response to several races of *U. appendiculatus*, and *Ur-6*+ was assigned to Olathe to identify that it possesses an additional gene besides *Ur-6*. More recently, Beerbower (2020) showed that the *Ur-6* gene in GGW was located on chromosome Pv07. Taking advantage of the unique capabilities of the common bean research laboratory at the SGIL, USDA-ARS in Beltsville, MD, which can use multiple races of the bean rust pathogen on a single plant, the objective of this study was to evaluate an F_2 population derived from the cross between the Pinto 144 and GGW cultivars with multiple races of *U. appendiculatus*, and to map the *Ur-6* gene in the genome of common bean.

MATERIAL AND METHODS

A total of 442 F_2 individuals from the Pinto 114 × GGW cross were inoculated with races 6-10 (44), 15-3 (47), 4-11 (63), and 6-15 (73) of *U. appendiculaus*. The parental line GGW shows a resistant reaction to the named four races. This resistance was observed as a hypersensitive reaction (HR), which is a necrotic post without sporulation (Table 1). Pinto 114 was susceptible (S) to all four races, with symptoms characterized by large sporulating pustules known as uredinia. Cultivars (with their rust resistance genes in parenthesis) Aurora (*Ur-3*), Early Gallatin (*Ur-4*), Mexico 309 (*Ur-5*), GN1140 (*Ur-7*), and PI 181996 (*Ur-11*) were used as internal controls of successful rust inoculations. The F_2 plants were simultaneously inoculated with a spore solution of the races 6-10 (44), 15-3 (47), 4-11 (63), and 6-15 (73). The spore solution was applied using a cotton swab, avoiding contamination between races. Symptom evaluation was performed 12 days after inoculation using a 1–6 scale proposed by Stavely et al. (1983). Newly emerged trifoliate leaves from each of the F_2 plants were collected for isolation of total genomic DNA. Five susceptible bulks were created. Each bulk consisted of DNA from eight F_2 susceptible plants. The DNA from the bulks and parents were genotyped with the Illumina BARCBEAN12K BeadChip. Any errors in allele calling were corrected manually. SNPs were considered to be associated with the *Ur-6* locus when they were polymorphic between parents and the three susceptible bulks were homozygous and clustered tightly with the susceptible parent (Pinto 114). KASP markers were developed in the genomic region identified during the BSA. First, the SNPs from the BeadChip were selected for KASP development. Some SNPs were also selected based on the whole genome sequencing of a group of common bean cultivars. KASP markers were used to genotype the 442

F_2 individuals from the Pinto 114 × GGW cross. The genetic distance between the KASP markers and the rust phenotype was estimated using the JoinMap 4.0. The genetic linkage map was drawn using MapChart. The positions of the SNPs in this study were based on the G19833 reference genome V2.1.

RESULTS AND DISCUSSION

Phenotypic evaluations of 442 F_2 plants from the Pinto 114 × GGW cross indicated that the rust reactions to races 6-10 (44), 15-3 (47), 4-11 (63), and 6-15 (73) co-segregated, suggesting that when an F_2 plant was resistant to one race, it also was resistant to all of the other races. Similarly, when an F_2 plant was susceptible to one race, it was also susceptible to all the other races. We observed a segregation of 337 resistant and 105 susceptible plants, fitting a ratio of 3 resistant to 1 susceptible (χ^2 = 0.365, P value = 0.5457), confirming that the rust resistance in GGW was conferred by a single gene (Table 2). Bulk segregant analysis identified SNPs associated with the rust resistance gene in GGW was positioned on chromosome Pv07 from 5.6 to 11.03 Mbp, on a 5.3 Mbp genomic region. In addition, eight KASP markers were developed, which were used to genotype the entire F_2 population from the Pinto 114 × GGW cross. The final mapping positioned the rust resistance gene between SS392 (6,748,703 bp) and SS365 (6,949,243 bp), in a genomic region of 200 kb on Pv07. The gene in GGW was mapped at 0.0 cM to the KASP markers SS394 and SS364 (Figure 1).

Table 1. Reaction of the GGW and Pinto 114 common bean parents and the six checks to four races of the bean rust pathogen used in this study

Line	6-10 (44)	15-3 (47)	4-11 (63)	6-15 (73)
Pinto 114	S	S	S	S
GGW (*Ur-6*)	HR	HR	HR	HR
Aurora (*Ur-3*)	S	S	S	S
Early G. (*Ur-4*)	HR	S	HR	HR
Mex. 309 (*Ur-5*)	TP	TP	TP	S
GN1140 (*Ur-7*)	TP	S	S	S
PI 181996 (*Ur-11*)	TP	TP	TP	TP

S-Susceptible, HR-Hypersensitive reaction, TP-Tiny pustules

Table 2. Inheritance test of the F_2 population from the Pinto 114 × GGW cross inoculated with races 6-10 (44), 15-3 (47), 4-11 (63), and 6-15 (73) of the bean rust pathogen

Phenotype	Observed	Expected	χ^2	P value
Resistant	337	331.5	0.365	0.5457
Susceptible	105	110.5		

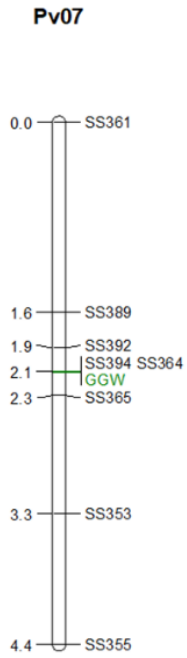

Figure 1. Mapping of the rust resistance gene on GGW

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MAPPING OF TWO GENES IN THE COMMON BEAN CULTIVAR OLATHE CONFERRING RESISTANCE TO RUST

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INTRODUCTION

Olathe is a Middle American common bean cultivar that contains the *Ur-6* gene that confers resistance to *Uromyces appendiculatus*, the fungal pathogen that causes rust disease. Olathe also contains an additional but not yet identified rust resistance gene. The *Ur-6* gene is also present in the Andean differential cultivar Golden Gate Wax (GGW). Based on a previous mapping, the *Ur-6* gene in Olathe and GGW was believed to be located on chromosome Pv11 (Miklas et al., 2002; Park et al., 2004). Olathe was used as the source of *Ur-6* in those studies. However, recent mapping has shown that the *Ur-6* gene in GGW is located on Pv07 (Beerbower, 2020). It is possible that the previous mapping of *Ur-6* on Pv11 was influenced by the limited mapping data available at the time. Alternatively, it may be that the gene mapped on Pv11 was actually the second resistance gene in Olathe. The objective of this study was to characterize and map the two rust resistance genes in Olathe.

MATERIALS AND METHODS

We inoculated 153 F_2 plants from the Pinto 114 × Olathe cross with races 6-15 (73) and 31-22 (67) of the bean rust pathogen. Pinto 114 is susceptible and Olathe is resistant to both races. Because race 6-15 (73) causes a hypersensitive reaction (HR) on Olathe; this race was used to identify the *Ur-6* gene. Similarly, race 31-22 (67) produces a resistant reaction characterized by tiny pustules (TP); thus, this race was used to identify the second rust resistance gene in Olathe. The F_2 plants were simultaneously inoculated with races 6-15 (73) and 31-22 (67). We used a cotton swab to apply the spore solution on the abaxial side of the primary leaves, which avoids contamination between races. The cultivars Aurora (*Ur-3*), Early Gallatin (*Ur-4*), GGW (*Ur-6*), GN1140 (*Ur-7*), and PI 181996 (*Ur-11*) were included in the study as internal controls. Bean rust symptom evaluation was performed 12 days after inoculation using a 1–6 scale proposed by Stavely et al. (1983). Total genomic DNA was isolated from each of the F_2 plants and genotyped with the Illumina BARCBEAN12K BeadChip. Errors in allele calling were manually corrected using GenomeStudio software. Monomorphic SNPs and those deviating from the 1:2:1 segregation ratio were excluded from the analysis. SNPs without a chromosome or a position assignment were also removed. The genetic distance between the SNP markers and the rust resistance genes was estimated using JoinMap 4.0, and the genetic linkage map was drawn using MapChart. The SNP positions in this study were based on the G19833 reference genome V2.1.

RESULTS AND DISCUSSION

The evaluation of the F_2 population from the Pinto 114 × Olathe cross revealed that two independent genes conferred rust resistance in Olathe. Some F_2 plants containing one gene exhibited an HR type of resistance reaction to race 6-15 (73) and S to race 31-22 (67). Other plants also containing a single but different rust resistance gene exhibited a TP type of resistance reaction to race 31-22 (67) and S to race 6-15 (73). Plants containing the two genes exhibited HR and TP for races 6-15 (73) and 31-22 (67), respectively. Plants with no genes were susceptible for the two

races. In the F_2 population, the segregation for race 6-15 (73) was 120 resistant plants showing an HR reaction and 33 susceptible plants, fitting a 3:1 ratio for a dominant single-gene (p-value = 0.3270). The HR reaction to race 6-15 (73) in Olathe is conditioned by *Ur-6*. The segregation for race 31-22 (67) was 106 resistant plants showing a TP reaction and 47 susceptible plants, fitting a 3:1 ratio for a dominant single-gene (p-value = 0.1023). The TP reaction to race 31-22 (67) in Olathe is conditioned by a gene distinct from *Ur-6*, referred to as Ur-?. The F_2 plants were further genotyped with the Illumina BARCBEAN12K BeadChip. The *Ur-6* gene in Olathe was mapped on the upper arm of chromosome Pv07 and is linked at 0.0 cM from the marker Chr07_6884444_A_G, which is in position 7,039,409 bp. The additional, unidentified gene Ur-? was mapped on the chromosome Pv11 at 0.0 cM of markers Chr11_41507454_G_A and Chr11_41555264_G_A which positions are 44,906,141 and 44,953,887 bp, respectively. Our data suggests the gene in Olathe previously mapped on Pv11 was not *Ur-6*, but the second gene Ur-?.

Table 1. Reaction of common bean parents and F_2 plants of the Pinto 114 × Olathe cross inoculated with races 6-15 (73) and 31-22 (67)

Genotype	Gene	Genes and races of *U. appendiculatus*	
		Ur-6	*Ur-?*
		6-15 (73)	31-22 (67)
Pinto 114	no gene	S	S
Olathe	*Ur-6+Ur-?*	HR	TP
# F_2 plants			
11	*no gene*	S	S
36	*Ur-6*	HR	S
22	*Ur-?*	S	TP
84	*Ur-6 + Ur-?*	HR	TP

S-Susceptible, HR-Hypersensitive reaction, TP-Tiny pustules

Table 2. Segregation of F_2 plants inoculated with races 6-15 (73) that identifies *Ur-6* and 31-22 (67) that recognizes the unknown Ur-? rust resistance gene

Race	Gene	Obs.	$\chi 2$	p-value
6-15 (73)	*Ur-6*	120:33	0.961	0.3270
31-22 (67)	*Ur-?*	106:47	2.669	0.1023

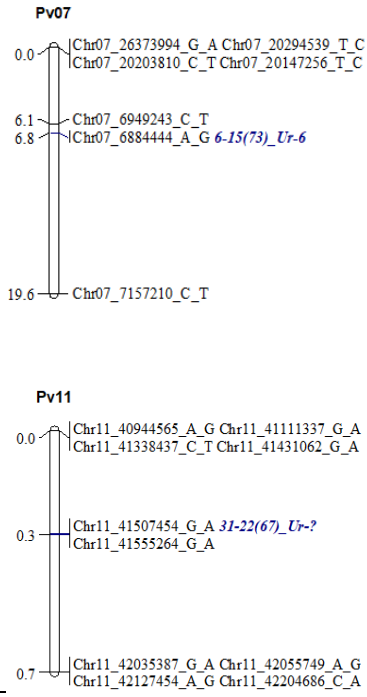

Figure 1. Mapping of *Ur-6* on Pv07 and *Ur-?* on Pv11 in Olathe cultiv

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PATHOGENIC RACE VARIABILITY OF *COLLETOTRICHUM LINDEMUTHIANUM* ISOLATES FROM THE MAJOR BEAN GROWING AGROECOLOGICAL ZONES IN UGANDA REVEALED THE DIFFERENTIAL BEAN CULTIVAR KABOON WITH RESISTANCE TO THE SEVEN MOST VIRULENT RACES

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INTRODUCTION

Colletotrichum lindemuthianum is a significant fungal disease of beans and a highly variable pathogen that easily breaks down resistance in cultivars with single-gene resistance (Kiryowa et al 2017). It is a seed borne pathogen that leads to substantial yield loss of 100% under favourable conditions, hence threatening food security. A total of 61 races have been previously reported in Uganda (Simbwa-Bunya, 1972; Nkalubo, 2006; Mwesigye, 2009; Kiryowa et al., 2017). Host plant resistance is the most suitable option for managing anthracnose disease among smallholder farmers. However, understanding pathogen race diversity is a pre-requisite for developing durable resistance in host plants. The objective of this study was to phenotypically identify races of *C. lindemuthianum* in Uganda and assess their virulence to the 12 CIAT bean anthracnose differential cultivars.

MATERIALS

The bean Anthracnose differential set consisting of 12 common bean cultivars (Michelite, Michigan Dark Red Kidney, Perry Marrow, Cornell 49-242, Widusa, Kaboon, Mexico 222, PI 207262, TO, TU, AB 136, G 2333) (Pastor-Corrales, 1991) was obtained from CIAT-Kawanda, Uganda. Fourteen-day-old bean seedlings of the 12 CIAT differential cultivars and the susceptible checks (CAL96 and NABE14) were grown in the screenhouse. One hundred and twelve isolates were used and for each isolate, four plants of each cultivar were inoculated by spraying on both leaf surfaces with a spore suspension containing 1.0 x10^6 spores/ml. Inoculated bean plants were then covered with transparent 2kg-capacity polythene bags to maintain high humidity (approximately 95%) in a cold chamber (21ºC) for 96hrs (Mwesigwa, 2009). After 96hrs, the polythene bags from plants were removed and plants transferred to the screenhouse for 4 days. Disease severity was assessed after 7 days of inoculation using a modified disease severity scale (1-9) (Pastor-Corrales, 1991).

RESULTS AND CONCLUSION

The interactions of 112 *C. lindemuthianum* isolates with the 12 differential cultivars resulted in the identification of 51 races. New races (8, 9, 15, 24, 26, 30, 38, 64, 66, 75, 78, 90, 94, 103, 111, 190, 207, 254, 335, 457, 512, 521, 557, 579, 587, 863, 985, 1803, 2087, 2624, 3141, 3593, 3609, 3656, 3663, 4039, 4041 and 4044) have been revealed for the first time in Uganda. Race 66 with 18 isolates was the most prevalent race and widely distributed throughout the agroecologies, followed by race 2 consisting of 13 isolates.

The most virulent races were determined by the numbers of resistant genes broken down and seven races (863, 985, 3663, 4033, 4039, 4041, and 4044) infected at-least 7 differential cultivars. Also Races 2621, 3141, 3593, 3609, 3656, 3663, 4039, 4041 and 4044 were reported as the most aggressive and were able to infect the most widely known anthracnose resistant genotype, G2333. In conclusion, whereas G2333 still remains an important source of the *Co-4*^{2} and *Co-5* anthracnose resistance genes, the findings from this study strongly suggest the need for the Uganda bean breeding program to also focus on exploiting the *Co - 1*^{2} in Kaboon for more durable resistance.

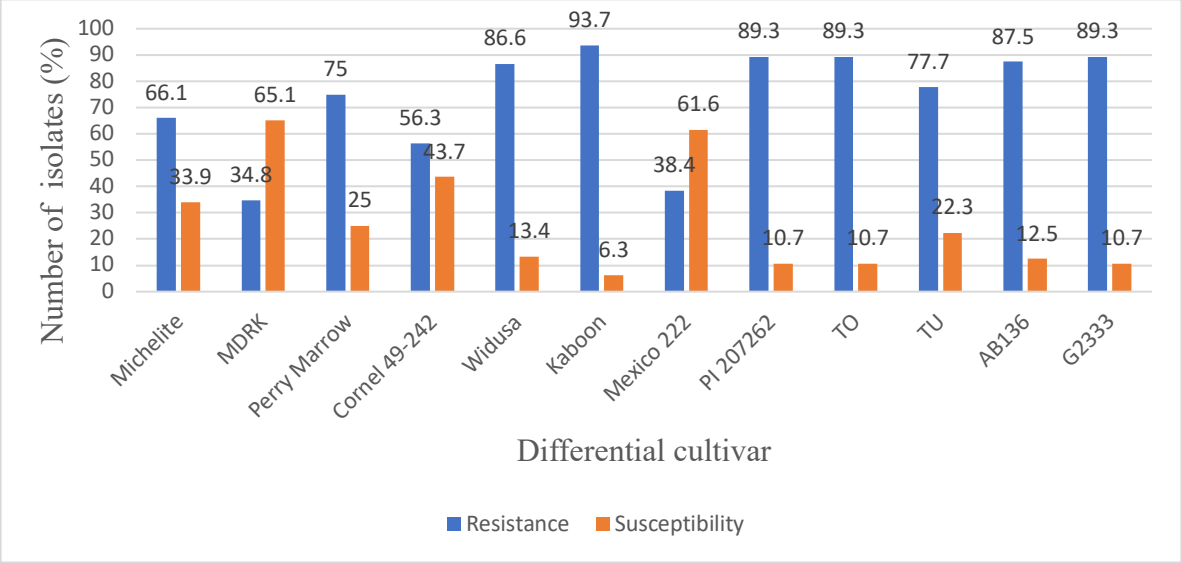

Figure 1: Resistance (R) and Susceptibility (S) of the bean anthracnose differential cultivars to 112 C. lindemuthianum isolates

ACKNOWLEDGMENTS

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ANALYSIS OF GENETIC GAIN FOR WHITE MOLD RESISTANCE IN DRY BEAN BREEDING

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Introduction

Understanding what progress has been made in breeding for a trait of interest is critical for breeders to be able to make informed decisions on how to best use germplasm resources. In order to quantify progress in breeding for white mold resistance in dry beans, historical data will be leveraged to calculate genetic gain for white mold resistance according to three metrics. The metrics are field white mold score, straw test white mold score, and accumulation of favorable alleles at the metaQTLs identified by Vasconcellos et al. in 2017. This analysis will be useful in revealing the rate of overall progress made in breeding for white mold resistance, elucidating which favorable alleles for white mold resistance are present in different populations and how they might best be combined. It may reveal lines with high value as donors for further resistance breeding. Differences in performance between field and straw tests will also allow determinations of whether physiological resistance or disease avoidance explain the white mold resistance of the lines examined.

Materials and Methods

Field and straw test scores in the form of ratings on 1-9 scales will be sourced from the multilocation collaborative Bean White Mold Nursery/National White Mold Trial data dating back as far as 2000. Because three constant checks (Bunsi, Beryl, and G122) have been maintained throughout this study, it will be possible to use these to standardize the test scores and account for location and year effects. Data will be cleaned and used to fit linear mixed effects models according to the formula for repeated checks experiments developed by Rutkoski et al. (2019). The methodology of McQueen et al. (2020) utilizing BLUPs will also be applied. Models will also be fitted to examine variety by location interactions for field test white mold score, as this trait encompasses disease avoidance, which may be subject to strong genotype by environment interactions.

Genotypic data will be sourced by sampling leaf tissue from seedlings of both BWMN/NWMT entries and varieties released in the period of interest (2000 to present). DNA will be extracted and SNP genotypes obtained using the BARCBean12K Infinium BeadChip. SNP genotype data will be processed to determine the allele state at the known QTLs for white mold resistance and used as a panel to search for new QTL signals. The prevalence of the positive allele at each locus in the varieties and BWMN/NWMT entries from each year will be plotted to analyze the accumulation of favorable alleles over time in the dry bean breeding population.

Preliminary Results

A preliminary dataset analyzed allowed the identification of significant and complex variety, location, year, and interaction effects. In terms of location, the Nebraska location showed a highly significant ($p<0.001$) reduction in white mold rating with an effect of -8.05 on the nine point scale. There were also significant effects of certain years on white mold rating. The year 2009 showed a significant ($p<0.01$) increase in white mold rating while 2015 showed a significant ($p<0.001$) reduction. The varieties A195 and VRW32 showed reductions in white mold rating of -3.59 and -4 respectively that were significant at a $p<0.05$ significance level. Twelve varieties had significantly increased white mold rating in Nebraska, the three checks Beryl, Bunsi, and G122 and the experimental lines 11A-39, ASS 1865, Cayenne, Mist, N 13140, PRP-153, USPT-WM-12, VCP-13, and WM31. The variety N19246 in Michigan and B07104 in Idaho had significantly reduced white mold rating. A more comprehensive analysis will follow once the full dataset has been obtained.

Discussion:

The identification of significant field resistance to white mold across locations in A195 and VRW32 suggests that they have high levels of genetic resistance to this disease and may be suitable donors for this trait in breeding programs. The strong effect of the Nebraska location in reducing white mold may be a result of a hotter and drier climate less suitable to *Sclerotinia* spore dispersal and germination if it is borne out by the final analysis.

A trend of genetic gain in field and greenhouse test among lines selected for white mold resistance and an accumulation of positive alleles are expected to be identified in the complete data.

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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: Field and greenhouse evaluations were conducted in 2024 to determine white mold disease tolerance in dry bean breeding lines from three breeding programs. Evaluations were conducted at sites located in five US states (MI, NE, ND, OR, WA), and Quebec, Canada. 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 *Sclerotinia sclerotiorum* pathogen populations that may differ across locations.

MATERIALS AND METHODS: Greenhouse evaluations were conducted at four 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 un-adapted bean germplasm. Eighteen entries were evaluated, including G122 (partial resistance), USPT-WM12 (partial resistance), Bunsi (mostly field resistance), and Beryl (susceptible) that were included as checks. Field tests were conducted at five locations. However, only two locations developed sufficient disease pressure to collect meaningful disease severity and yield 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 one entry EX2145-P from Wisconsin was significantly more tolerant to white mold than resistant check G122. Four additional entries were not significantly different than G122 with scores ranging from 3.8 to 4.6 (Table 1). These were pintos from Wisconsin, P3-032-2 from Washington, as well as USPT-WM-12, added this year as a WM tolerant check with better yield potential. It is interesting that all the most tolerant experimental pintos derive their resistance from interspecific introgression of *P. coccineus,* suggesting this breeding strategy has been effective at broadening the genetic base of white mold tolerance in *P. vulgaris*. The most susceptible entries were pinto WMM lines as well as black B22041 from MI. Small red and SDP breeding lines from ND, WA, and WMM-820 showed more moderate disease tolerance. 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. EX2141-P, WMM-1022, P3-032-2, and EX2145-P pintos all showed similar disease tolerance as G122 (Table 2). EX2141-P demonstrated the best combination of white mold tolerance and yield potential (3000 pounds/acre). Small red NDF151006-2 shows promise with a moderate disease score, and high yield potential. P2-124-1, WMM-820, and EX2146-P also had moderate disease scores but less yield. Three WMM lines had the highest disease scores, but WMM-750 also produced the highest overall yield, similar to 2023 results. This underscores the continued importance of selecting

simultaneously for high yield potential under white mold disease pressure rather than focusing solely on a low disease rating.

Table 1. Straw test results for eighteen dry bean lines evaluated in the greenhouse at four locations.

Name	Source	WA	MI	ND	OR	Mean	Group
WMM-975P	Osorno	8.5	7.8	7.8	5.6	7.5	A
B22041	Wright	1.9	7.16	6.6	5.9	7.0	AB
WMM-1022	Osorno	7.3	5.9	1.9	4.9	6.6	BC
Beryl	Check	7.5	5.9	6.1	5.2	6.3	CD
WMM-556	Osorno	7.3	4.9	7.3	4.9	6.2	CD
WMM-750	Osorno	1.9	6.0	5.0	3.4	5.7	DE
ND220917-2	Osorno	7.0	6.4	3.5	3.9	5.2	EF
ND222212	Osorno	6.4	5.0	4.4	3.7	5.0	FG
P2-124-1	Miklas	5.9	6.4	3.6	4.2	4.9	FG
NDF151006-2	Osorno	6.4	5.7	4.4	3.5	4.9	FG
Bunsi	Check	6.1	5.7	4.1	3.6	4.9	FG
WMM-820	Osorno	6.5	5.3	4.0	3.6	4.9	FG
USPT-WM-12	Check	5.5	4.9	3.9	3.4	4.6	GH
EX2141-P	Kmiecik	6.1	5.2	3.6	1.6	4.2	HI
G122	Check	4.1	5.4	4.1	3.3	4.1	HI
P3-032-2	Miklas	5.2	3.7	2.9	3.3	3.9	IJ
EX2146-P	Kmiecik	5.0	4.2	4.0	1.8	3.8	IJ
EX2145-P	Kmiecik	3.9	4.3	3.8	1.8	3.4	J

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

Name	MI Yield	ND Yield	MI WM Score	ND WM Score	Mean WM Score	Group
Beryl	394.0	1458.6	8.5	8.0	8.3	A
WMMS556	2926.0	2621.8	7.5	7.0	7.2	A
WMM975P	2339.0	1938.0	6.5	7.0	6.9	AB
WMM750	2943.0	3289.7	6.5	4.8	5.8	BC
EX2146-P	1320.0	2363.3	5.5	5.5	5.4	C
USPT-WM12	2218.0	2986.4	5.0	5.5	5.3	C
WMMS20	1788.0	3248.1	6.0	4.1	5.0	CDE
INDF151006-2	3006.0	3109.5	5.5	4.9	4.9	CDE
Bunsi	1871.0	2623.8	4.0	5.4	4.7	CDE
P2-124-1	1532.0	2456.1	2.5	6.7	4.6	CDE
EX2141P	2761.0	3149.6	3.5	4.4	3.9	DEF
WMM1022	2038.0	1979.0	4.0	3.5	3.8	DEF
P3-032-2	2525.0	1580.5	3.5	4.6	3.8	DEF
EX2145-P	1515.0	2362.4	3.5	4.0	3.8	EF
G122	1387.0	2537.0	3.5	3.1	3.2	F

IDENTIFYING GENOMIC DIVERSITY AMONGST CANADIAN SCLEROTINIA SCLEROTIORUM ISOLATES: A STEPPINGSTONE TOWARDS IMPROVED WHITE MOLD SCREENING

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Introduction: White mold, caused by the fungal pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary (Ss), is a devastating disease for common bean. Ss isolates vary in their aggressivity towards common bean thus inoculation with multiple, diverse isolates is important to better understand the true white mold resistance of breeding material (Willbur et al., 2017). In the past, a limited number of SSRs has been used to distinguish between Ss genotypes but the resolution of the genomic picture SSRs can create is limited (Sirjusingh & Kohn, 2001). Our objective is to better understand genomic diversity of white mold isolates collected from geographically distant locations using whole genome sequence data in order to identify an appropriate panel of Ss isolates to be used for comprehensive white mold screening.

Materials & Methods: The isolates used in this study were collected by collaborators in four Canadian provinces (Alberta, Ontario, Quebec and Prince Edward Island), spanning over 2000 miles. Whole genome sequencing of 63 Ss isolates was done using an Illumina NovaSeq6000 machine. Reads were trimmed with Fastp then mapped to the reference genome using BWA-mem2. Following alignment, Samtools was used to create consensus sequences as input for Andi, which output pairwise distance matrices. The distance matrices were used for neighbor joining, then the phylogenetic tree was visualized using SplitsTree. Known effector coding regions extracted from consensus sequences using Samtools were aligned and visualized using Geneius prime. Next, BCFtools was used to call SNPs and InDels. Following formatting of the merged variant files, the number of subpopulations was assessed using STRUCTURE.

Results & Discussion: Sequencing was successful with most samples having a mean depth of coverage >100x. STRUCTURE analysis showed a spike in Δk at 4 subpopulations. The predominant subpopulations in each isolate fit well with the taxa delimited by nodes from the phylogenetic analysis. One cluster of highly related samples was identified including isolates from Quebec, Ontario and PEI (Figure 1, dark blue). This is not surprising given the proximity of collection locations in Quebec and Ontario, but it supports the need for distantly collected Ss samples in white mold screening projects. When individual coding regions for effector proteins were inspected, broad trends of mutations matching clades were observed, as expected. Many mutations, including some missense mutations, were identified. While we cannot yet speak to the actual effect of these mutations on the isolates' aggressivity, one notable mutation in the necrosis-inducing effector protein SSINE3 was found previously in only a highly aggressive isolate.

Conclusions: A better understanding of the genomic and genetic causes of the variable aggressivity of *Sclerotinia sclerotiorum* is needed to thoroughly screen breeding material. To address this gap, we studied broad Ss population trends and fine mutations within effector coding regions. Whole genome sequencing has demonstrated genomic diversity between and amongst

Sclerotinia sclerotiorum isolates collected from four provinces. Using STRUCTURE, we found evidence of four subpopulations amongst the samples. Interesting missense mutations were found within regions known to code for necrosis inducing effectors. While we can recommend screening with multiple Ss isolates when conducting white mold inoculations, aggressivity data from seedling straw tests on common bean containing diverse sources of resistance to white mold infection are needed before we can recommend a standardized white mold screening isolate panel.

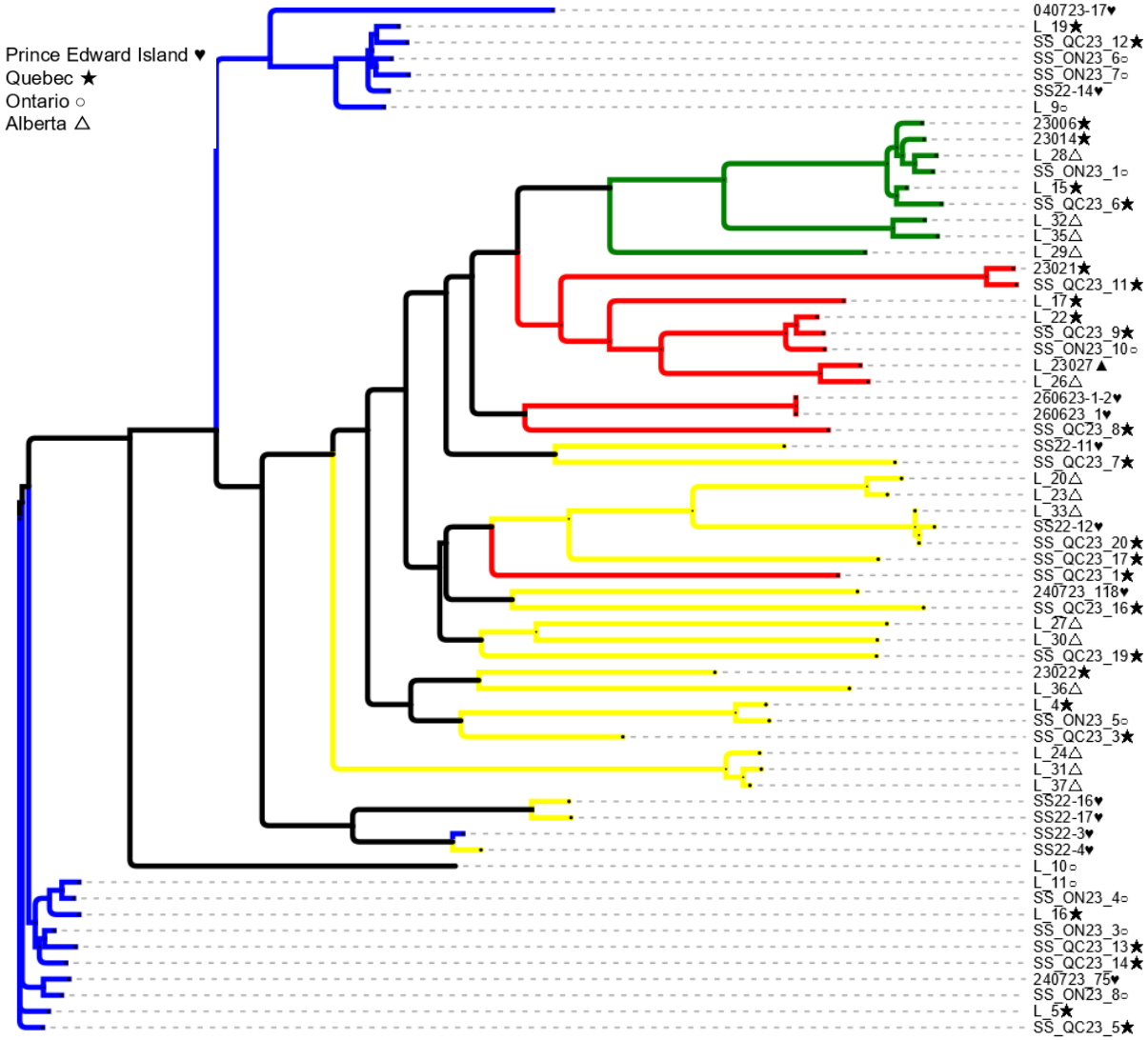

Figure 1. Tree constructed in SplitsTree with isolate names and symbols representing their province of origin (legend top left). The branch colors represent the isolates' most prominent subpopulation as determined by STRUCTURE.

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AN RNase H-LIKE GENE COMPLEMENTS RESISTANCE TO BEAN COMMON MOSAIC NECROSIS VIRUS IN PHASEOLUS VULGARIS

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INTRODUCTION: *Bean common mosaic virus* (BCMV) and *Bean common mosaic necrosis virus* (BCMNV) are related RNA potyviruses that affect common bean (*Phaseolus vulgaris* L.) worldwide. These viruses are transmitted by various aphid species and through infected seed, causing yield losses in bean fields of up to 80%. Host plant resistance is the primary disease control. Resistance is regulated by the dominant *I* gene and five recessive alleles (*bc-1*, *bc-2*, *bc-3*, *bc-u*^{d}, and *bc-u*^{r}) distributed across four loci (Drijfhout, 1978; Soler-Garzón et al., 2021a, 2021b, 2023).

Two receptor-like kinases (RLKs) on chromosome Pv03 were identified as candidate genes for the *bc-1* resistance locus in common bean, showing synteny with the soybean *Rsv4* gene that confers resistance to *Soybean mosaic virus* (SMV) (Soler-Garzón et al., 2021a). In soybean, the *GmMLRK1* haplotype and an RNase H-like protein were linked to the *Rsv4* locus, with *GmMLRK1* playing a major role in resistance by reducing viral accumulation and triggering a hypersensitive response (Che et al., 2023; Ishibashi et al., 2019).

We aimed to investigate the genes encoding similar RNase H-Like proteins in the *bc-1* genomic region of *Phaseolus vulgaris* for their potential resistance to BCMNV infection.

MATERIAL AND METHODS: RC (Rojo x CAL 143) RIL and Amanda/Blush F_2 populations were evaluated with BCMNV NL-3 strain under GH conditions. Linkage mapping and synteny analysis identified RNase-H genes on chromosome Pv03 near the *bc-1* gene, leading to the development of a specific SNP marker to track an RNase-H gene.

RESULTS AND DISCUSSION: Through a BLAST search against an RNase H family member associated with the *Rsv4* locus in soybean, three previously uncharacterized RNase H-Like genes (1, 2, and 3) were identified near the *bc-1* locus in *Phaseolus vulgaris*. The synteny between the *bc-1* and *Rsv4* loci in P. *vulgaris* and *Glycine max* had already been established (Soler-Garzón et al., 2021a), and the presence of orthologous RNase H-Like genes within the same genomic region in both species further supports this connection. The broad-spectrum resistance to related potyviruses conferred by these loci strengthens the argument for functional synteny. The *bc-1* locus provides resistance to BCMV, BCMNV, and *Peanut mottle virus* (PeMoV), while *Rsv4* confers resistance to SMV and BCMV, as well as transient resistance to PeMoV, Potato virus Y (PVY), and other potyviruses according to several studies.

Two distinct studies have proposed candidate genes for the *Rsv4* locus in *G. max*, an RNase H-Like gene (Ishibashi et al., 2019) with a 3.6 kb deletion and the malectin-like receptor kinase GmMLRK1 (Che et al., 2023). However, only the latter study examined both candidates, revealing that GmMLRK1 had a significant resistance effect against SMV, with a slight enhancement from

the RNase H-Like gene. A similar pattern was observed in *P. vulgaris*, where a receptor-like kinase (*PvRLK*) was identified as the candidate gene for *bc-1*.

CONCLUSION: In this study, we identified an RNase H-Like 1 gene, which was found to enhance resistance when combined with the *I*, *bc-1*, and *bc-u* genes, helping to limit the hypersensitive response levels triggered by the BCMNV NL-3 strain. Additionally, a SNP marker (G03_4166082) was developed to track the resistant and susceptible alleles for RNase H-Like 1 in breeding programs.

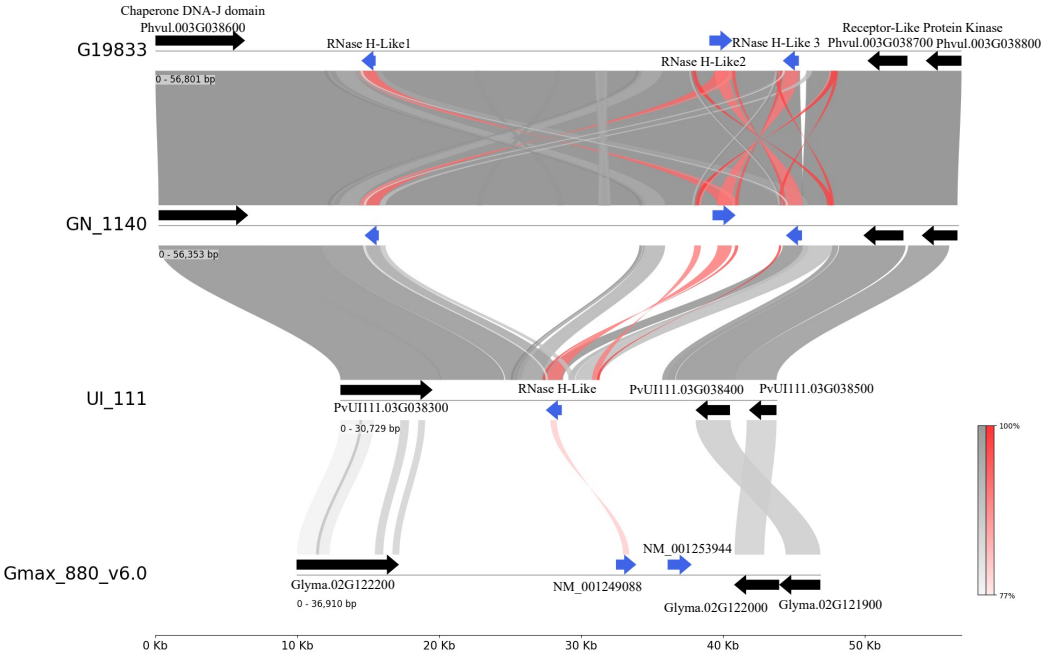

Figure 1. Comparison reference genomes for *bc-1* (*Phaseolus vulgaris*) and *Rsv4* (*Glycine max*) regions associated with resistance to BCMNV/BCMV and SMV, respectively. Gene models (black arrows) and unannotated *RNase H-Like 1* to *3* genes (blue arrows) with assembled spliced alignments obtained from Phytozome *v*13.

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COMMON BACTERIAL BLIGHT INOCULATION RESPONSE IN TEPARY (*PHASEOLUS ACUTIFOLIOUS* A. GRAY) BREEDING LINES

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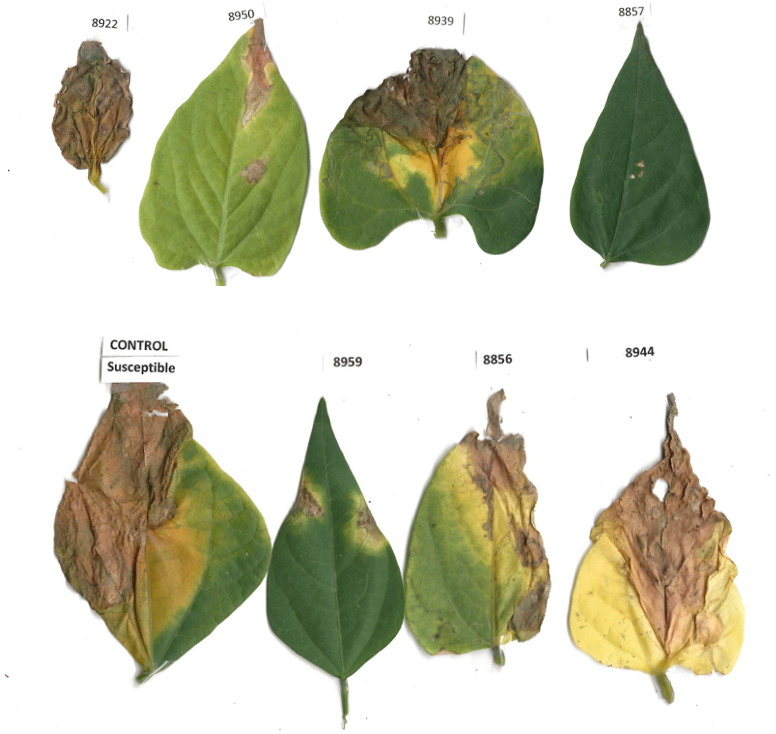
INTRODUCTION: Symptoms of bacterial blight (CBB) in common beans (*Phaseolus vulgaris* L.) were observed in Andean genotypes in Isabela, Puerto Rico. Yield losses by CBB can exceed 40% and pathogenic variation complicates breeding for resistance. Moreover, unseasonal rains and warm temperatures have increased incidence of CBB in growers' fields planted with traditional cultivars. In Isabela, symptomatic plants exhibited small light green spots dispersed in the leaves, that developed into water-soaked lesions with a necrotic center and chlorotic border. Under high relative humidity (>80%), leaves from affected plants became brown and appeared burned. In susceptible cultivar 'Beniquez', the disease caused water-soaked areas in the pods. In a nursery with Andean genotypes, symptoms observed were consistent with CBB caused by *Xanthomonas axonopodis* pv. *phaseoli* (Smith) Dye (Xap) (Beaver et al, 2020). Pathogenicity tests with four isolates isolated from Isabela confirmed the virulence of Xap. using the multiple-needle inoculation on PR 0443-151 and Beniquez under screenhouse conditions. This report presents the foliar response of 98 tepary lines and 2 common beans varieties to Xap. isolates from Isabela, Puerto Rico.

MATERIALS AND METHODOS: Isolations were made on nutrient agar media (NA), resulting in typical growth of a gram-negative, mucoid and yellow-pigmented *axonopodis* species of the genus *Xanthomonas*. Single colonies were purified in nutrient broth and individual Xanthomonas colonies were transferred to yeast dextrose calcium carbonate agar (YDC), and incubated for 24 hours at 28 ^{0}C. DNA extraction was completed from pure colonies using a Plant Dneasy kit (Qiagen, Germany). The polymerase chain reaction (PCR) assay with the universal primers 27F and 1492R were sequenced (Psomagen, MD). Multiple needle inoculation was conducted on 98 tepary lines and two controls (Zapata, et al. 1985).

RESULTS AND DISCUSSION: Isolates UPR-H1 amplified a 973 bp. band showing 99.8% identity with *Xanthomonas citri* pv. *glycines* (GenBank PQ415738.1) and 99.6% identity with *Xanthomonas phaseoli pv. phaseoli* (GenBank CPO94243.1), respectively. H0 and H3 isolates amplified a 930 bp band with 99.7 identity and 100% coverage for *Xanthomonas phaseoli pv. phaseoli*. Pathogenicity tests conducted using multiple needle inoculation on two-week-old plants of PR-0443-151 and Beniquez (10^5 CFU) developed brown lesions of various sizes 5 days after inoculation, covering 20-40% of the leaf surface. The lesions enlarged, and the leaves wilted and

abscised. In the screenhouse experiment, from a total of 89 lines evaluated, 46 lines and 6 TDP accessions did not exhibited symptoms. Fifty-one lines and four controls (Tep-23, Tep-215, G40035 and G-46178 developed symptoms with disease severity ranged from 1.5 - 4 (scale 1-9). The highest disease severity value was for 9028 (Susceptible control), PR-0443-151 and line 24IS-8846. Some tepary beans are reported to have high levels of resistance to CBB (Zapata and Vidaver, 1987), therefore continuous screening of breeding lines to select for high levels of resistance is important.

Figure 1. Symptoms of common bacterial blight in six tepary lines (8922, 8950, 8857,8859, 8856 and 8944) and two common beans (8939 and susceptible control).



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MOLECULAR CHARACTERIZATION OF *Xanthomonas* spp. ISOLATES FROM LEAVES IN COMMON BEAN

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INTRODUCTION

Common bacterial blight (CBB), caused by the bacteria *Xanthomonas citri* pv. *fuscans* (Xcf) and *Xanthomonas phaseoli* pv. *phaseoli* (Xpp), is the main bacterial disease affecting the production of common beans (*Phaseolus vulgaris* L.). Xcf can be phenotypically differentiated from Xpp by the in vitro melanin production (Constantin et al., 2016). The most effective strategy for controlling the disease in the field is the combination of resistant cultivars and appropriate management practices. Additionally, the identification of resistance sources and genetic diversity of pathogen populations is essential for the success of common bean breeding for CBB resistance. The morphological identification of *Xanthomonas* spp. has limitations and molecular characterization can add this identification. PCR technique, for example, has enabled the amplification of small samples of bacterial DNA using specific primers (Barrocas et al., 2009). Thus, this study aimed to molecularly characterize 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 UFLA 17, 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 19, UFLA 20, UFLA 21, UFLA 22 e UFLA 23) 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). Bacterial DNA extraction was performed according to the methodology described by Richards et al., (1994). The polymerase chain reaction (PCR) was conducted using the primer pair X4c (5' GGC AAC ACC CGA TCC CTA AAA CAG G 3') and X4e (5' CGC CGG AAG CAC GAT CCT CGA AG 3'), as described by Audy et al., (1994). The PCR reaction was carried out in a total volume of 25 µL, consisting of 12.5 µL of GoTaq® Green Master Mix (Promega Corporation, Madison, WI, USA), 1 µL of forward primer and 1 µL of reverse primer (10 mM), 1 µL of DNA obtained from the previous step (at an approximate concentration of 30 ng/µL), and ultrapure water added to complete the reaction volume. The positive control was represented by a pathogenic reference isolate (CFBP 6165), while sterile water was used as a negative control. Amplification was performed with an initial denaturation at 94°C for 3 minutes, followed by 35 cycles of 94°C for 45 seconds, 64°C for 30 seconds, and 72°C for 1 minute, with a final extension at 72°C for 10 minutes. The quality of the extracted DNA and the PCR product was assessed by electrophoresis

on a 0.7% agarose gel, stained with Gel Red Nucleic Acid Gel Stain (Biotium®), and visualized using a MiniBis Pro gel documentation system (DNR Bio-Imaging Systems®).

RESULTS AND DISCUSSION

The morphological characterization of these isolates was performed by Barros et al., (2024). All evaluated isolates presented band sizes ranging from 750 to 500 base pairs (Figure 1). The isolates UFLA 12, UFLA 08, UFLA 10, and UFLA 06 exhibited the same banding pattern as the reference isolate CFBP 6165 Xpp (Figure 1). The other isolates showed distinct banding profiles and have been classified as Xcf. The use of additional molecular methods is recommended for more accurate identification of the isolates such as the application of multiple sets of primers. Molecular characterization using specific primers has shown to be a promising approach to identifying Xpp and Xcf isolates and the most isolates evaluated have been classified as Xcf.

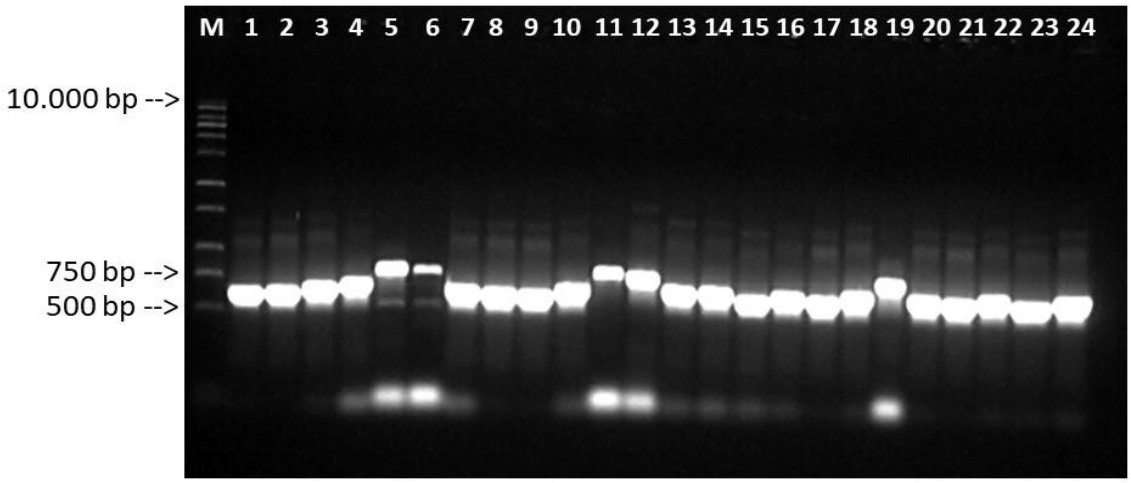

Figure 1. Identification of isolates using primers X4c and X4e.

Legend: (M) Marcador 1kb; (1) UFLA 09; (2) UFLA 19; (3) UFLA 15; (4) UFLA 17; (5) UFLA 08; (6) Referência CFBP 6165; (7) UFLA 07; (8) UFLA 21; (9) UFLA 04; (10) UFLA 02; (11) UFLA 12; (12) UFLA 06; (13) UFLA 13; (14) UFLA 20; (15) UFLA 05; (16) UFLA 11; (17) UFLA 22; (18) UFLA 03; (19) UFLA 10; (20) UFLA 23; (21) UFLA 16; (22) UFLA 72; (23) UFLA 01; (24) UFLA 14.

ACKNOWLEDGEMENTS

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OPTIMIZATION OF THE ASSESSMENT OF THE SEVERITY OF COMMON BACTERIAL BLIGHT IN BEAN PLANTS AT THE V2 STAGE

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INTRODUCTION

Among the bacterial diseases of common bean, common bacterial blight (CBB) stands out due to its severity, potentially causing significant losses in common bean production. CBB is caused by two species of bacteria, *Xanthomonas phaseoli* pv. *phaseoli* (Xpp) and *Xanthomonas citri* pv. *fuscans* (Xcf). The development of breeding programs aimed at increasing resistance to a specific disease requires prior knowledge of the pathogen's variability, using isolates with appropriate virulence levels. Therefore, the evaluation of genotypic resistance to diseases in various plants is commonly carried out through artificial inoculation of the pathogen). Several inoculation methods have been used to ensure the penetration of the bacterium responsible for common bacterial blight into the host's tissues (Pastor-Corrales et al., 1981). An efficient inoculation methodology is important for identifying resistance sources that can be used in breeding programs. Thus, the objective of this study was to test and assess the inoculation methods of scissor incision and multiple needles in assessing the severity of CBB in common bean plants at the V2 stage.

MATERIALS AND METHODS

Three cultivars of common bean (Pérola, BRSMG Amuleto, and BRSMG União) were assessed for their reaction to eight isolates of bacterial pathogens. The plants were inoculated at the V2 stage using two different methods: the scissor incision method (Schoonhoven and Pastor-Corrales, 1987) and the multiple needle method (Pompeu and Crowder, 1973). Two isolates of Xpp (UFLA 09 and UFLA 23) and six of Xcf (UFLA 05, UFLA 07, UFLA 11, UFLA 15, UFLA 20, and UFLA 22) obtained from the collection of the Plant Bacteriology Laboratory at the Departamento de Fitopatologia, Universidade Federal de Lavras (UFLA) were used. The pathogenicity test consisted of eight experiments, one for each isolate. Each trial was conducted in a completely randomized design in a 3 x 2 factorial scheme with three replicates and one plant per plot. Colonies of each pathogen isolate were cultured using the parallel streak method in MB1 culture medium (Kado and Heskett, 1970) and incubated at 28°C in a B.O.D. chamber for 48 hours. Bacterial suspensions were prepared in sterile saline solution (0.85% NaCl) with the concentration adjusted to A600nm = 0.1 (Monteiro et al., 2020). The common bean seedlings (V2 stage) inoculated were incubated in a moisture chamber for 14 days. **Scissor incision method:** three cuts were made on the edges on both sides of the leaf with scissors previously dipped in the bacterial suspension. **Multiple needle method:** a support containing several needles previously dipped in the bacterial suspension was pressed against the surface of the leaf using a sponge moistened with the same suspension, positioned under the leaf. The severity of CBB in plants was assessed seven days after inoculation using a diagrammatic scale with scores of 1 to 9 (Aggour et al., 1989). An analysis of variance (ANOVA) and the comparison of means test (Scott-Knott $P \leq 0.05$) were applied with the SISVAR statistical software (version 5.6).

RESULTS AND DISCUSSION

All sources of variation in the joint analysis of variance were significant, including all interactions to severity scores of CBB. According to the Scott-Knott test, the average severity scores were higher with the scissor method, especially in the Pérola cultivar. Additionally, for both methods, the isolates were grouped into three categories (Table 1). Overall, the UFLA 23 isolate was the most virulent in both methods and UFLA 05 showed the lowest virulence. UFLA 23 and UFLA 05 isolates and were the most and least virulent, respectively in all three cultivars assessed and lent. BRSMG Amuleto and BRSMG União cultivars were more susceptible and resistant, respectively. The inoculation method using a scissor incision was more efficient than the inoculation method using multiple needles in assessing the severity of common bacterial blight (CBB) in common beans. The superiority of this method may be attributed to the greater uniformity in introducing the pathogen into the leaves, resulting in a more consistent infection allowing an accurate assessment of the cultivars resistance. Therefore, in this study, the scissor incision method is a simple technique that can help breeders assess and identify CBB-resistant genotypes, particularly in the early stages of common breeding when many genotypes need to be assessed.

Table 1: Severity scores of common bacterial blight isolates within the methods and cultivars assessed.

Isolates	Methods		Cultivars		
	Needle	Scissor	Amuleto	Pérola	União
UFLA 05	2,44 A	4,11 A	4,0 A	3,3 A	2,5 A
UFLA 07	5,47 B	6,44 B	7,3 C	5,5 B	5,0 B
UFLA 09	6,44 B	7,44 C	8,5 C	5,8 B	6,5 C
UFLA 11	4,98 B	6,25 B	8,3 C	5,1 B	3,5 A
UFLA 15	3,66 A	4,33 B	5,2 B	4,2 A	2,7 A
UFLA 20	5,22 B	7,22 C	8,5 C	4,8 A	5,3 B
UFLA 22	6,66 B	7,22 C	7,8 C	8,6 C	4,3 B
UFLA 23	7,17 C	7,66 C	8,3 C	8,0 C	6,0 C

* Means followed by the same letter in the column belong to the same group (P<0.05) according to the Scott-Knott test.

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CAPES, CNPQ, and FAPEMIG for financial support.

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CO-SEGREGATION OF RESISTANCE GENES TO *COLLETOTRICHUM LINDEMUTHIANUM* AND *PSEUDOCERCOSPORA GRISEOLA* IN INBRED LINES OF COMMON BEAN (*Phaseolus vulgaris*)

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Introduction

Anthracnose, caused by *Colletotrichum lindemuthianum*, and angular leaf spot, caused by *Pseudocercospora griseola*, are two major diseases that severely impact common bean (*Phaseolus vulgaris* L.) production, often leading to significant yield losses. The most effective strategy for disease management is the deployment of cultivars carrying broad-spectrum resistance genes (Gonçalves-Vidigal et al., 2020). This study aimed to investigate the co-segregation of resistance genes against *C. lindemuthianum* race 73 and *P. griseola* race 63-39 by phenotyping F2:8 inbred lines derived from a cross between the cultivars Awauna UEM and IPR 88 Uirapuru.

Material and Methods

In this study, 56 recombinant inbred lines (RILs), derived from a cross between the cultivars Awauna UEM and IPR-88 Uirapuru, were evaluated for resistance to anthracnose (*Colletotrichum lindemuthianum*, race 73) and angular leaf spot (*Pseudocercospora griseola*, race 63-39). For inoculation, 10 seeds from each RIL, along with the parental cultivars, were sown, and plants were inoculated at the V3 phenological stage. The *C. lindemuthianum* race 73 inoculum was prepared at a concentration of 1.2×10^6 spores mL^{-1}, while the *P. griseola* race 63-39 spore suspension was adjusted to 2.0×10^4 spores mL^{-1}. Inoculated plants were maintained in a mist chamber, and disease severity was evaluated at different time points: 7 and 10 days post-inoculation for anthracnose and 15 days post-inoculation for angular leaf spot. Symptom severity was visually assessed using established severity scales: Pastor-Corrales et al. (1995) for anthracnose and Inglis et al. (1988) for angular leaf spot, with scores ranging from 1 to 9. Plants scoring between 1 and 3 were classified as resistant, while those scoring between 4 and 9 were classified as susceptible. To assess the segregation ratio (1:1) in the $F_{2:8}$ population, a chi-square (χ^2) test was performed on the phenotypic data using the Genes software (Cruz, 2013).

Results

The results of the phenotypic evaluations are summarized in Table 1, including observed and expected segregation ratios, chi-square (χ^2) values, and P-values. As anticipated, the $F_{2:8}$ lines exhibited a 1RR:1SS segregation ratio for anthracnose ($\chi^2 = 0.071$, *P*-value = 0.78), consistent with the inheritance of the single dominant resistance allele *Co-4*^{2} from Awauna UEM (RNC-MAPA, 2016, no. 35631). Likewise, the segregation pattern for angular leaf spot also followed the expected 1RR:1SS ratio ($\chi^2 = 0.64$, *P*-value = 0.42). Among the 56 lines evaluated, 50 exhibited consistent co-segregation for resistance or susceptibility to both pathogens, while six recombinant

lines were identified. This strong co-segregation pattern suggests a tight genetic linkage between the anthracnose resistance allele (*Co-4*^{2}) and the angular leaf spot resistance allele.

Table 1. Reaction of the 56 F2:8 inbred lines from the cross of the common bean cultivars Awauna UEM × IPR88 Uirapuru inoculated with *Colletotrichum lindemuthianum* race 73 and *Pseudocercospora griseola* race 63-39.

Parents and cross	Generation	Observed segregation (1RR:1SS) [a]	Expected segregation (1RR:1SS)	χ^2	*P*-value
Race 73 of *C. Lindemuthianum*					
Awauna UEM	R	10:0			
IPR88Uirapuru	S	0:10			
Awauna UEM x IPR88 Uirapuru	$F_{2:8}$	27:29	28:28	0.071	0.78
Race 63-39 of *P. Griseola*					
Awauna UEM	R	10:0			
IPR88 Uirapuru	S	0:10			
Awauna UEM x IPR88 Uirapuru	$F_{2:8}$	25:31	28:28	0.64	0,42

Chi-square *P*-values greater than 0.05 indicate that the observed values were not significantly different from the expected values; RR: Resistant; SS: Susceptible.

Conclusion

The co-segregation analysis of resistance genes for anthracnose and angular leaf spot is essential for common bean breeding programs, enabling the selection of cultivars with broad-spectrum resistance. By identifying and incorporating resistance genes against *Colletotrichum lindemuthianum* and *Pseudocercospora griseola*, breeders can develop more resilient cultivars, mitigating yield losses caused by these pathogens. Moreover, stacking multiple resistance genes enhances productivity while reducing reliance on chemical pesticides, contributing to sustainable agricultural practices. Thus, integrating co-segregation analysis into breeding strategies is a key step toward developing durable, disease-resistant common bean cultivars.

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COMPARATIVE GENOMICS OF VIRULENCE FACTORS AND CHARACTERIZING DIFFERENCES BETWEEN HIGHLY VIRULENT AND LESS VIRULENT STRAINS AND OF *P. SYRINGAE* PV. *SYRINGAE* PATHOGENIC TO ADZUKI BEAN

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INTRODUCTION: Canada the second-largest exporter of adzuki beans, generated 38 US$ million from exports of dried adzuki beans in 2023 (Tridge, 2023). *Pseudomonas syringae* pv. *syringae* (Pss), causes bacterial brown spot (BBS) in beans. Yield losses from BBS in beans range from benign to entire crop losses (Wally 2019). BBS is the second biggest threat to azuki bean cultivation after white mold (King, 2019). Cultural, physical, and chemical control measures have not been effective in managing BBS. The most effective control measure is cultivating resistant varieties (OMAFRA, 2023). No commercial adzuki variety grown in Ontario shows resistance to BBS (OMAFRA, 2023). Furthermore, there is no publication on the mechanisms of interactions between the pathogen and the adzuki host that confer resistance or susceptibility to the disease. Intra-pathovar strains show variation in pathogenicity. Comparative genomics of the array of virulence factors between intra-pathovar strains is a complementary strategy to characterize evolutionary dynamics vital for host-specific pathogenicity (Marroni *et al*., 2024). This work hypothesizes that differences in pathogenicity caused by the strains used in this study in the adzuki bean host are determined by the repertoire of virulence factors present in the strains.

MATERIALS AND METHODS: Pss27, Me2, Me3, and Li6 are the Pss strains used in this study. Pss27 was obtained from AAFC, Harrow Research Centre, Ontario, Canada courtesy of Dr. Owen Wally. Me2, Me3, and Li6 were isolated from adzuki seed lots collected from Meaford (Me2, Me3) and Lincoln (Li6), Ontario. Sixty-two bacterial strains suspected to be Pss, based on plating morphologies, were isolated from adzuki seed lots from 17 farm locations (Meaford, Elmvale, Lake Simcoe, Oakwood, Kawartha Lakes, Port Perry, Rice Lake, Cobourg, Ayton, Milton, Exeter, Stratford, Alisa Craig, Tavistock, Ayra, Thamesford, and Lincoln) across Ontario (courtesy Hensall Coop), and one leaf sample from Woodstock ON. 16S rRNA partial gene sequencing was used to identify Pss strains up to the species level and whole genome Illumina sequencing was used to identify Pss strains to the pathovar level. The software RagTag was used to assemble contigs of strain sequences into whole scaffolds and annotation was performed by the Rapid Annotations using Subsystems Technology (RAST) server. Virulence factors in the annotated whole genome assemblies of the Pss strains were identified with the VFanalyer tool of the Virulence factor database (VFDB) (Liu *et al*., 2022). Pathogenicity screening was done by inoculating two adzuki bean varieties (Erimo and a Chinese variety) with the Pss strains under controlled conditions. The experiment was a completely randomized design with 8 treatments, 2 controls, and three replicates. Six week-old-plants (onset of flowering) were transferred to a misting tent for 48 hours at a relative humidity of 100 %. After 48 hours, trifoliate leaves were inoculated with liquid cultures of the Pss strains via wounds created with a multiple-pin device (floral frog). The plants were left in the tent without misting for 48 hours and then transferred back to the growth room. Visual disease estimates were conducted weekly for up to four weeks post-inoculation, using a 1 – 9 (no symptoms to severe symptoms) scale, and the area under the disease progress curve (AUDPC) was calculated.

RESULTS AND DISCUSSION: Of the 62 Pss suspect isolates, 16S rRNA sequencing and NCBI BLAST search identified 57 as *Pseudomonas* sp. and 5 as *P. syringae*. Whole genome sequencing confirmed that 3 of the strains (Me2, Me3, and Li6) were Pss. Pss27 was also confirmed to be Pss, following the same methods. A comparison of the complete virulence factor repertoires among the strains showed 170 conserved genes, 10 unique genes in Pss27, 4 unique genes in Me2, 1 unique gene in Me3, and no unique genes in Li6. Results from inoculation with the Pss strains showed that Pss27 was the most aggressive ($p<0.001$). Erimo showed more resistance than the Chinese variety ($p<0.001$). A quantitative trait locus (QTL) for resistance to BBS was identified with the Pss strains used in this study (Unpublished). The next phase of this study will match unique virulence factors in Pss27 to cognate host gene targets within the QTL region to identify molecular markers associated with resistance/susceptibility to BBS in adzuki bean. Identifying the virulence factors most implicated in host-pathogen interactions can be a strategy to pinpoint candidate genes in QTL regions.

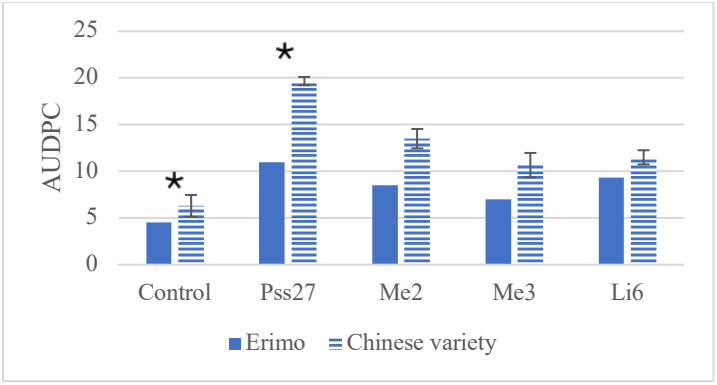

Figure 1: Inoculation of adzuki varieties with Pss strains; n=3 ± SE. (asterisk indicates significantly different means)

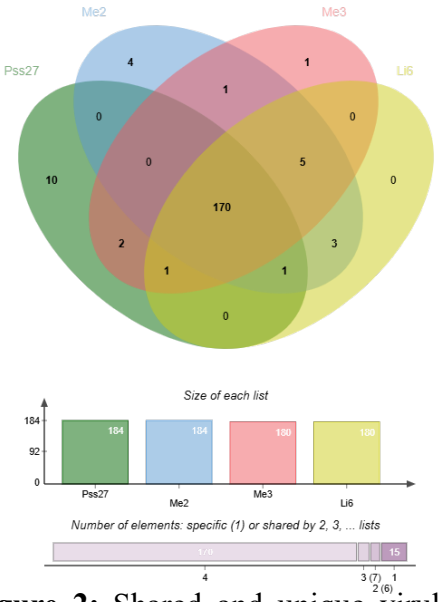

Figure 2: Shared and unique virulence factors in Pss strains. Pss27, Me2, Me3, and Li6. The unique factors in different isolates include: Pss27 (ahlI, ahlR, ppkA, avrRpm1, hopAB1, hopAF1, hopAP1, hopH1, hopL1, sypA); Me2 (hcp1, acrB, rmlA, rmlD); and Me3(fha1).

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k-MER GENOME-WIDE ASSOCIATION STUDY FOR COMMON BACTERIAL BLIGHT RESISTANCE IN A TEPARY BEAN (*PHASEOLUS ACUTIFOLIUS*) DIVERSITY PANEL

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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. For example, resistance to common bacterial blight (CBB) exists in tepary bean. To better understand the genetic basis of CBB resistance, this research aims to augment knowledge of loci encoding bacterial blight-resistant traits in tepary bean via *k*-mer-based genome-wide association studies (GWAS). Unlike traditional SNP-based approaches, *k*-mer-based GWAS utilizes short, fixed-length DNA sequences (*k*-mers) as genetic markers, allowing for a more comprehensive and unbiased analysis of genetic variation associated with traits of interest.

MATERIALS AND METHODS: Whole genome sequencing was conducted on 290 tepary accessions using the Illumina NovaSeq 6000 platform. CBB phenotypic data were obtained from a previous study that evaluated disease severity on a 1–9 scale, where 1 = no symptoms and 9 = complete infection based on visual symptoms (Bornowski et al., 2023). For genetic association analysis, a *k*-mer-based GWAS was performed on 146 accessions by extracting *k*-mers from sequencing reads using the kmersGWAS pipeline (Voicheck and Weigel, 2020). Identified CBB-associated *k*-mers were then mapped to the tepary bean reference genome G40001 and six other *de novo* tepary bean genome assemblies (Table 1) to determine their genomic distribution. Visualization and downstream analyses were performed using R and Python tools to identify candidate regions and potential resistance loci.

RESULTS AND DISCUSSION: A total of 1.08 billion *k*-mers were identified, with 5,241 *k*-mers significantly associated with CBB resistance. These *k*-mers were mapped across tepary bean genomes differential in CBB resistance. Notably, 88.5% of the *k*-mers mapped to TDP-154, highlighting its strong association with resistance (Table 1). TDP-136 also showed a high mapping rate (88%), aligning with its low CBB score (1.50). In contrast, TDP-159 and TDP-176, with higher CBB scores (9.00 and 7.25), had much lower mapping rates (8.17% and 7.14%, respectively). Despite similar low CBB scores (~1.50), TDP-001, TDP-013, and TDP-312 exhibited lower mapping percentages (6.75–6.81%), suggesting their resistance may involve different genetic factors. Manhattan plots of significant *k*-mers revealed a prominent peak located in contig 750 of TDP-154 (Fig. 1A), suggesting a genomic region associated with resistance. Synteny analyses across multiple tepary bean genomes revealed a syntenic block in which *k*-mers

associated with CBB resistance were aligned (Fig. 1B); in TDP-136 and TDP-154, a putative NBS-ARC resistance gene was present, which was located on chromosome 7.

Table 1. *k*-mers associated with common bacterial blight resistance (strain 484A) that mapped to the respective genome assemblies.

Genomes	Accession No.	CBB score	# k-mers mapped	% k-mers mapped
TDP-001	G40001_Frijol_Bayo	1.50	357	6.81
TDP-013	G40022	1.58	354	6.75
TDP-136	G40168	1.50	4,612	88.00
TDP-154	G40177E1	1.67	4,637	88.48
TDP-159	G40178	9.00	428	8.17
TDP-176	G40199	7.25	374	7.14
TDP-312	TARS-Tep 23	1.00	355	6.77
TDP-302	PI_638833__18	NA	387	7.38

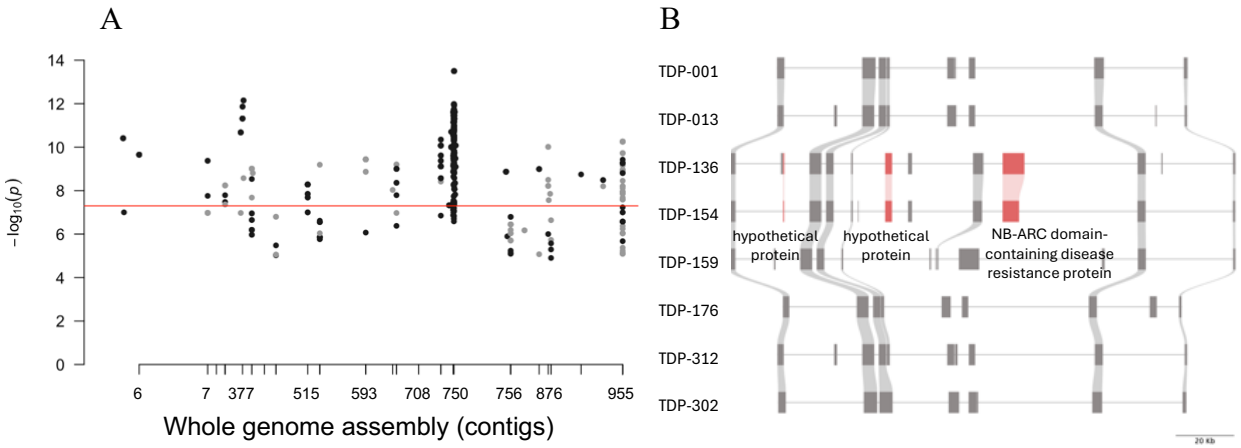

Figure 1. A. Manhattan plot of *k*-mer-based GWAS for CBB resistance, with *k*-mers mapped to the TDP-154 genome. B. Riparian plot showing syntenic regions in tepary bean genomes where *k*-mers associated with CBB resistance aligned, with red-highlighted genes in TDP-136 and TDP-154.

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EFFECT OF RHIZOBIUM TROPICI, AZOSPIRILLUM BRASILENSE, AND BACILLUS SPP. INOCULATION SNAP BEAN

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INTRODUCTION: Snap bean (*Phaseolus vulgaris L.*) is an economically and nutritionally important legume. Inoculation with plant growth-promoting bacteria, such as Rhizobium tropici, Azospirillum brasilense, and Bacillus spp., has been studied as a sustainable alternative to increase productivity and reduce the need for chemical fertilizers. This study aimed to evaluate the effect of isolated and combined inoculation of these bacteria on the growth and yield of snap bean with a determinate growth habit, cultivated in a greenhouse.

MATERIAL AND METHODS: The experiment was conducted from April to June 2024, in the greenhouse of the State University of Londrina (UEL). Nine-liter pots containing nitossolo were used, with the following chemical characteristics: pH ($CaCl_2$) of 5.5, organic matter of 3.2%, P of 15 mg/dm³, K of 3.5 mmolc/dm³, Ca of 40 mmolc/dm³, Mg of 10 mmolc/dm³, Al of 0.5 mmolc/dm³, H+Al of 30 mmolc/dm³, CEC of 84 mmolc/dm³, and base saturation of 64%. During the experimental period, the average temperature in the greenhouse ranged from 18°C to 30°C, with relative humidity maintained around 70%. The snap bean variety used was 'Macarrão', known for its determinate growth habit and 65-day pod harvest cycle. The experimental design was completely randomized, with five treatments and four replications: control (no inoculation), inoculation with Rhizobium tropici, inoculation with Azospirillum brasilense, inoculation with Bacillus spp., and inoculation with a combination of the three bacteria. The seeds were treated with bacterial suspensions (1 x 10^9 cells/mL) for 30 minutes before sowing. Three seeds were sown per pot, and after 10 days, thinning was performed to leave only one plant per pot. Sowing was carried out in April 2024. Initial fertilization in the sowing furrows was calculated based on soil analysis, following the recommendations of Pauletti and Motta (2019), with a specific formulation of 180 kg/ha of the 8-28-16 formula applied. Weed control, 25 days after emergence, involved the application of the herbicide fluazifop-p-butyl + fomesafen (200 + 250 g/ha 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. At 65 days, the plants and pods were harvested at the beginning of the R8 stage. Evaluations included height, stem diameter at the first node, pod diameter and average length, number of pods per plant, and yield. Data were subjected to analysis of variance, and means were compared by Tukey's test at 5% significance.

RESULTS AND DISCUSSION: The results showed that inoculation with plant growth-promoting bacteria had a significant effect on the analyzed variables (Tables 1 and 2). Plants inoculated with the combination of the three bacteria showed the greatest improvements in all measured variables. Specifically, these plants exhibited significantly higher height (55.3 cm), stem diameter (7.2 mm), and pod diameter (9.5 mm) compared to the control, which had values of 45.2 cm, 5.8 mm, and 8.2 mm, respectively. These results are consistent with previous studies that

demonstrated the positive effect of inoculation with Rhizobium tropici and Azospirillum brasilense on the growth and yield of snap bean.

Table 1. Effect of inoculation on height, stem diameter, and pod diameter of snap bean

Treatment	Height (cm)	Stem Diameter(mm)	Pod Diameter (mm)
Control	45.2 c	5.8 c	8.2 c
Rhizobium tropici	50.1 b	6.5 b	9.0 b
Azospirillum brasilense	48.7 b	6.3 b	8.8 b
Bacillus spp.	49.5 b	6.4 b	8.9 b
Combination of three bacteria	55.3 a	7.2 a	9.5 a
CV (%)	**15.2**	**14.8**	**13.9**

Table 2. Effect of inoculation on pod length, number of pods per plant, and yield of snap bean

Treatment	Pod Length	Pods per Plant	Yield (g/plant)
Control	12.5 c	15.3 c	150.3 c
Rhizobium tropici	13.8 b	18.5 b	170.5 b
Azospirillum brasilense	13.5 b	17.8 b	165.2 b
Bacillus spp.	13.6 b	18.0 b	168.0 b
Combination of three bacteria	14.5 a	20.2 a	185.7 a
CV (%)	**14.5**	**6.3**	**8.1**

Furlan et al. (2016) found that Rhizobium tropici significantly increased plant height and pod yield in snap bean cultivars with a determinate growth pattern. In terms of pod length, number of pods per plant, and yield, the combination of the three bacteria also outperformed the other treatments. The combination treatment resulted in a pod length of 14.5 cm, 20.2 pods per plant, and a yield of 185.7 g/plant, significantly higher than the control, which had values of 12.5 cm, 15.3 pods per plant, and 150.3 g/plant, respectively. This enhanced performance is likely due to the synergistic effects of the three bacteria, which may have improved biological nitrogen fixation and overall plant growth.

CONCLUSION: Inoculation with Rhizobium tropici, Azospirillum brasilense, and Bacillus spp., either isolated or combined, significantly improved the growth and yield of snap bean. The combination of the three bacteria was the most efficient, highlighting its potential as a promising practice for sustainable agriculture.

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MAPPING OF FLUMIOXAZIN TOLERANCE IN SNAP BEAN DIVERSITY PANEL

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Introduction

Effective weed management is crucial for maintaining profitable snap bean (Phaseolus vulgaris L.) production. Waterhemp (Amaranthus tuberculatus) is a major pest in snap bean farming, and flumioxazin, a PPO-inhibiting herbicide, has shown potential in controlling it. However, limited knowledge exists on snap bean tolerance to flumioxazin. This study aimed to quantify snap bean tolerance levels and identify genetic factors influencing this trait.

Materials and Methods

A genome-wide association study (GWAS) was conducted using 377 genotypes from the Snap Bean Association Panel (SnAP) to assess flumioxazin tolerance. Field trials were conducted at the University of Illinois Vegetable Crop Farm in 2021 and 2022. Two treatment levels were applied: flumioxazin at 378 g a.i. ha-1 and a non-treated control. Plant density (PD) and shoot biomass (BP) were measured as indicators of herbicide tolerance. GWAS was performed using 20,619 SNPs to identify loci associated with flumioxazin tolerance.

Results and Discussion

GWAS identified a key genomic region on chromosome 02 linked to flumioxazin tolerance. The tolerance was influenced by factors such as seed size, herbicide metabolism, and detoxification pathways. Transcriptomic analysis revealed the involvement of oxidoreductase processes and programmed cell death pathways. A transcription factor within the identified genomic region may regulate the expression of tolerance-related genes. Romano-class cultivars (e.g., Bush Romano 350, Roma II) exhibited high flumioxazin tolerance, making them valuable candidates for breeding programs.

Conclusion

This study identified a major genomic region influencing flumioxazin tolerance in snap bean. Insights into the underlying genetic and physiological mechanisms provide a foundation for breeding herbicide-tolerant snap bean varieties.

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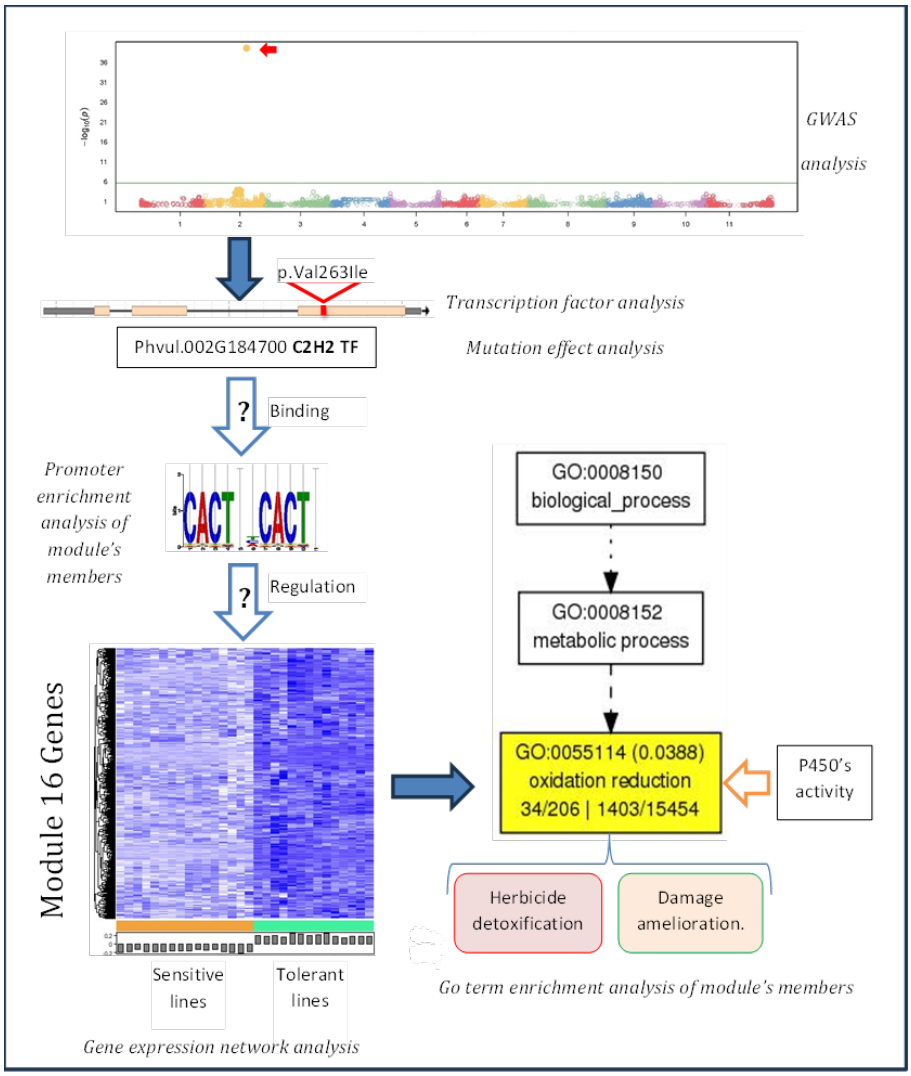

Graphical Summary: Genome-wide association study (GWAS) analysis of the SnAP 377 genotypes identified a single genomic region on chromosome 2 associated with tolerance to the soil-applied herbicide flumioxazin. The associated SNP is highlighted by a red arrow in the Manhattan plot. Within the linked region, a putative C2H2 transcription factor harbors a missense mutation correlated with the tolerance phenotype. Co-expression network analysis of gene expression in tolerant and sensitive lines revealed clusters of genes with similar expression patterns. One of these clusters, **module 16**, consists of genes upregulated in tolerant lines, with expression levels represented by the intensity of blue in the heatmap. Promoter motif analysis of sequences upstream of module 16 genes identified an enrichment of predicted binding sites for the C2H2 transcription factor. Gene ontology (GO) term enrichment analysis of module 16 revealed a significant presence of genes involved in oxidation-reduction processes, a common feature in genes associated with herbicide tolerance.

Acknowledgments: This research was supported by USDA-ARS.

INTEGRATION OF ANGULAR LEAF SPOT RESISTANCE LOCI AND RLK AND NB-LRR-ENCODING GENES IN COMMON BEAN CHROMOSOMES Pv01, Pv04, Pv08 AND Pv10

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Introduction

Common beans are among the most important crops, being one of the most widely consumed legumes in Brazil and worldwide. However, diseases caused by fungi, bacteria, and viruses are major factors limiting productivity, with angular leaf spot (ALS), caused by the pathogen *Pseudocercospora griseola*, being particularly damaging to the crop. Resistance to angular leaf spot is primarily conferred by single dominant resistance genes and quantitative trait loci (QTLs), most of them mapped on Pv01, Pv04, Pv08 and Pv10. The plant immune response is largely mediated by proteins containing nucleotide-binding domains and leucine-rich repeat domains (NB-LRR), as well as proteins with receptor-like kinase (RLK) domains. This study aimed to identify typical resistance proteins that are located near genes conferring resistance to angular leaf spot, previously mapped in common bean chromosomes Pv01, Pv04, Pv08 and Pv10.

Material and Methods

Data on molecular markers linked to resistance genes and QTLs for angular leaf spot on chromosomes Pv01, Pv04, Pv08, and Pv10 were collected from the literature. To identify the physical position of ALS resistance loci in the reference genome, we performed a BLASTn search using the sequence of the molecular marker linked to the ALS resistance gene/ QTL. Based on the physical positions of these molecular markers, candidate genes encoding NB-LRR proteins and kinases located within a 500 Kb region of the resistance loci were investigated in the reference genome (version 2.1) available in the Phytozome molecular database. Thus, an integrated map of ALS resistance loci and candidate genes (encoding defense response-related proteins) was constructed using MapChart software. This map contains candidate genes for all ALS resistance genes and QTLs previously described in the literature.

Results

At total 33-LRR NB proteins and 42 protein kinases were detected close to ALS resistance loci on chromosomes Pv01, Pv04, Pv08, and Pv10. Candidate genes for all ALS resistance genes and QRLs, previously described in the literature were detected. These candidate genes may be useful for further studies to validate their function in ANT response, and to understand how they interact with metabolic pathways. On chromosome Pv01, two angular spot resistance genes were mapped. *Phg-1*, present in the cultivar AND 277 (Gonçalves-Vidigal et al. 2011) and $PhgPv01^{CDRK}$ identified in the cultivar California Dark Red Kidney (CDRK) by Gonçalves-Vidigal et al. (2020). In Pv04, the ALS genes *Phg-3* in the cultivar Ouro Negro and *Phg-4* in the cultivars G5686 and CAL 143 are described by Gonçalves-Vidigal et al. (2013) and Keller et al. (2015), respectively. In this chromosome, a large number of candidate genes were found close to *Phg-4*, mainly candidate genes that encode kinases. At chromosome Pv08, the *Phg-2* resistance locus was found in the cultivar Mexico 54 linked to the molecular marker SCAROPN02 (Sartorato et al., 1999), as well as the second allele of this locus, $Phg\text{-}2^2$ found in the cultivar BAT 332. Unlike Pv04, in Pv08 a greater number of candidate genes encoding NB-LRR were found. On the Pv10 chromosome, the *Phg-5* gene, later classified as QTL ALS10.1, was mapped under natural field conditions and

for races 0-39 and 31-0 in the cultivars CAL 143 and G5686 (Oblessuc et al. 2012, 2013; Keller et al. 2015).

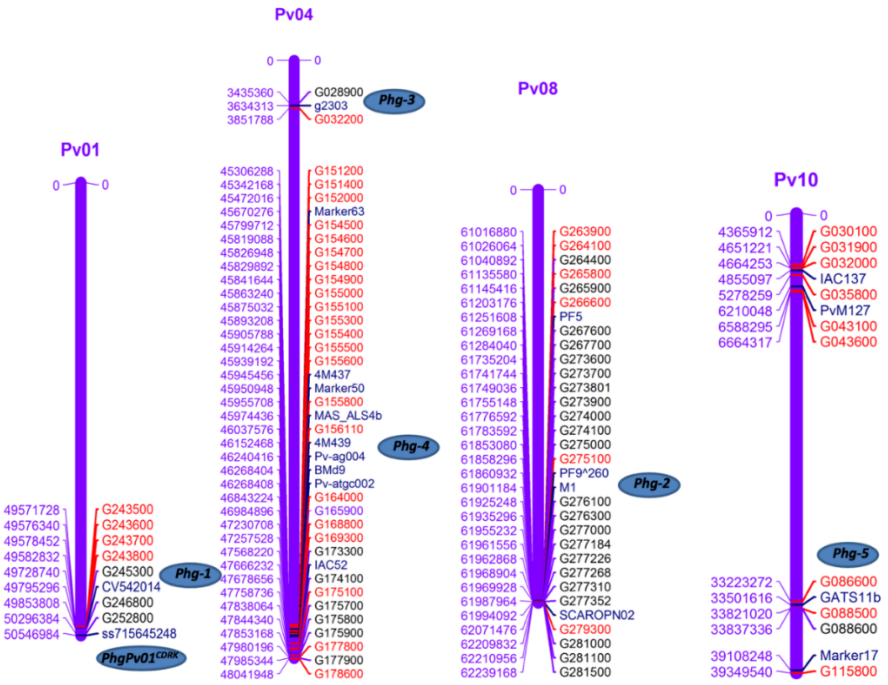

Fig. 1 Integrated map of chromosomes Pv01, Pv04, Pv08, and Pv10 with candidate genes encoding NB-LRR and kinases proteins. Resistance genes to angular leaf spot are marked in circles. Candidate genes are represented by the last seven digits of their annotation. For example, G243500 on Pv01 corresponds to Phvul.001G243500. Genes encoding NB-LRR proteins and kinases are represented in black and red, respectively. Molecular markers are marked in blue.

Conclusion

The literature review of studies mapping resistance genes and QTLs for angular leaf spot in common bean led to the identification of molecular markers linked to these genes and QTLs. Using these markers, the physical locations of resistance genes and QTLs for angular leaf spot resistance were found in the common bean reference genome, providing valuable insights for bean breeding programs. Furthermore, maps were created that highlight candidate genes encoding resistance-related proteins (NBS-LRR and kinases) linked to ALS resistance genes and QTLs on chromosomes Pv01, Pv04, Pv08, and Pv10. A total of 33 candidate genes encoding NBS-LRR proteins and 42 encoding kinases were identified near the genomic regions conferring resistance to angular leaf spot. Future research should focus on validating the functional role of these genes in the response to angular leaf spot.

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COMBINING DATASETS ACROSS BREEDING PROGRAMS TO DEVELOP A ROBUST GENOMIC PREDICTION MODEL: THE COMMON BEAN PAN-GS CONSORTIUM

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INTRODUCTION:

Breeding for yield is challenging because it is a complex quantitative trait controlled by tens to hundreds of genes each with minor allelic effects. Genomic selection (GS) aims to overcome this challenge by using genomic data from high-density genome-wide markers paired with phenotype data from a training population, to train a prediction model that can estimate these minor allelic effects for each line within a breeding population. The sum of these estimated allelic effects, known as the genomic-estimated breeding value (GEBV), are used to select for lines that are predicted to result in progeny with improved yield. Undesirable low GS predictive accuracy commonly occurs with small training populations, with low genetic diversity, and low genetic relatedness to the breeding population. To overcome these requirements this project seeks to combine the genomic and phenotypic data from multiple common bean breeding programs to create a 'Pan-GS' predictive model that should be more robust, accurate, longer-lasting, and enable greater genetic gain than a single breeding program trained-GS model (Plavšin et al., 2021, Merrick et al. 2022). This universal Pan-GS model will become freely available thereby removing the barrier of entry for implementing GS in common bean breeding programs. This has led us to the formation of the Pan-GS Phaseolus research consortium. This is a report of the activities carried out thus far and next steps.

MATERIALS AND METHODS:

A combined phenotypic dataset from 14 common bean breeding programs was assembled that consisted of one to two years of the most recent raw yield data from pre-registration field trials, totalling 23,957 plots growing 3,763 breeding lines and 280 check lines across 33 locations in Canada, the United States of America, Puerto Rico, Colombia, France, and Uganda. This dataset also included sowing and harvest date, plot, days to maturity, plot area and plot weight, 100 seed weight, seed moisture, market class, and pedigree. Pedigree information was used to identify sibling lines within the same family. To eliminate genetic redundancy with the training population, the highest yielding sibling from each family was identified based on the highest BLUP score calculated using the ASReml-R package. Using this approach, 2,479 sibling lines that were not the highest yielding were eliminated from the training population, resulting in a final training

population of 1,284 breeding lines each of which had a unique pedigree, as well as 245 checks lines, of which 42 of these checks were shared across at least two breeding programs.

PRELIMINARY RESULTS:

Whilst the Pan-GS training population consisted of slightly more entries originating from the Meso-American gene pool (895 entries) than the Andean gene pool (507 entries), the major market classes are well represented with similar number of entries for each class (**Figure 1**). The even representative of market classes should reduce any potential market-class specific bias when training the predictive models that should ensure these models can be used by any common breeder regardless of their market class of choice.

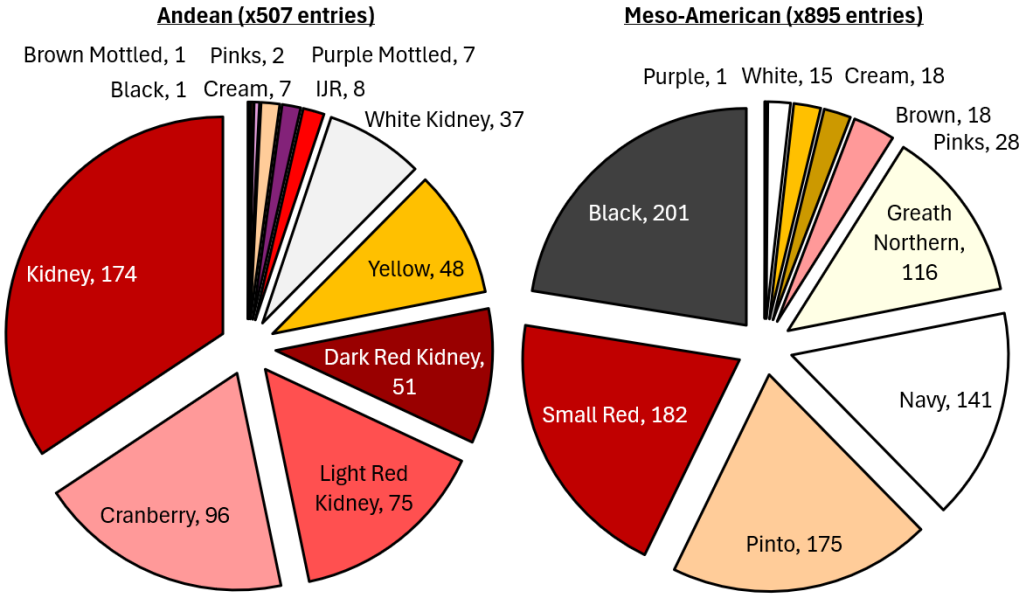

Figure 1. Distribution of market classes within the Pan-GS training population split by Andean and Meso-American gene pools. Does not include 127 snap bean entries.

NEXT STEPS:

Genomic DNA from the training population will be extracted and sent for genotyping using BARCBean12K Beadchips. The resulting sequencing data will be combined with the phenotype and pedigree data to train and compare the predictive accuracy of different predictive models. This will include models trained on the complete training population, models trained by gene pool, by closely related market-class, by a single market-class, and to determine if the performance of rarer market classes can be predicted using data from the same gene pool. Using this dataset, we will also be able to identify shared and rare alleles between breeding programs either within the same or different geographic regions. The rate of genetic gain for each breeding program could also potentially be compared using the shared checks as controls. The end goal is to develop the highest performing Pan-GS predictive model or models that by being publicly available through a web-based user interface will remove the major barriers of entry for performing GS by common bean breeders worldwide.

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DIAGNOSTIC MOLECULAR MARKERS ASSOCIATED WITH THE DOMINANT CRANBERRY (*S*) AND PINTO (*Pi*) SEEDCOAT PATTERNING PHENOTYPES

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INTRODUCTION: Genetic analysis of seedcoat patterning in common bean (*Phaseolus vulgaris* L.) began with the rediscovery of Mendel's laws. In 1901, Tschermak (1) initially discovered that mottling could be controlled by dominant or heterozygous gene action, and this was later confirmed by Shull (2) and Emerson (3). Shull (2) assigned the symbol *M* to the dominant acting mottling gene. Utilizing F_2 populations developed by crossing two solid-colored parents, Shull (2) and Emerson (3) observed the following ratio: ¼ dark pattern seedcoats: ½ mottled seedcoats: ¼ light patterned seedcoats. This deviated from the expected 3:1 ratio for dominance inheritance. Since heterozygous mottled progeny could not be selfed to homozygosity, as with the dominant *M* gene, the mottling phenotype was considered to be "ever-segregating". Kooiman (4) initially gave the genetic symbol *B* for the "ever-segregating" phenotype which was changed to *C* by Lambrecht (5) since *B* was also used for the brown factor.

Tjebbes and Kooiman (6) performed the first genetic analysis of the striped seedcoat pattern using the Kievitsboon cranberry bean. They observed the striping pattern was dominant over solid color and assigned the symbol *S* for the dominant striping phenotype. As far as we know, Prakken (7) was the first to consider the genetics of the pinto pattern and used the gene symbol *Pi* for this phenotype. He did not study the inheritance of the pinto pattern, but the experience of plant breeders suggested the pinto pattern is dominant to solid color pattern. Other dominant patterning genes that gave marbled, speckled and other seed coat patterns were described by Lambrecht (8). Tjebbes and Kooiman (6) were the first to observe close linkage between the "ever-segregating" *C* patterning and the dominant mottling gene when recombination was not observed between the phenotypes. Prakken (8) later demonstrated *S* and *M* genes tightly linked. He then described the "complex *C* locus" as a group of linked genes regulating seedcoat patterning and color. Here we mapped the dominant acting *S* and *Pi* genes and developed diagnostic PACE markers linked to each pattern.

MATERIALS AND METHODS: To study the gene action of the *Pi* gene, an F_2 population from the cross of the pinto UI114 (*Pi*) and the solid brown colored Golden Gate Wax (GGW; *pi*) was analyzed. A logistic regression genome-wide association study (GWAS) that involved the cranberry (*S*; n=31) vs. the solid red colored (*s*; n=147) members of the Andean Diversity Panel (ADP) utilizing 219,055 SNPs was performed to physically place *S* on the G198333 ver.2 reference genome. A logistic regression GWAS that involved the pinto (*Pi*; n=92) vs. pink and Durango Red (*pi*; n=43) members of the Middle American Diversity Panel (MDP) utilizing 1,107364 SNPs was performed to physically place *Pi* on the UI111 ver.1 reference genome.

RESULTS AND DISCUSSION: Seed coat pattern was scored for 241 F_2 plants derived from the UI114 x GGW population. Of these F_2s, 174 expressed pinto seedcoat (*P*i_), while 67 showed

solid brown seedcoat (*pipi*). This result fits a 3:1 ratio (X^2 $P_{3:1}$=0.54) indicating the seedcoat pinto pattern is controlled by a dominant acting (*Pi*) gene.

The GWAS for the *S* gene identified a peak SNP (S08_3141933; -log10(*P*) =19.44; Fig. 1A), and two neighboring SNPs (S08_3142008, S08_3142010) with nearly equivalent *P* values, that were located in G19833 gene model Phvul.008G.038000. The model is located within a cluster of MYB genes (Phvul.008G038000 to Phvul.008G038600) that are orthologous to other plant MYB genes associated with flavonoid production. GWAS for the *Pi* gene identified a 211kb LD block of SNPs with identical *P* values (-log10(*P*) =19.44; Fig. 1B) that spanned the UI111 MYB cluster homologous to the G19833 cluster discovered with the *S* GWAS. Physical mapping relative to the UI111 reference genome identified a ~57 kb deletion in pinks and Durango reds that spanned gene models PvUI111.08G0405 to PvUI111.08G0409 which included four MYB genes.

These results are consistent with the previous mapping of the complex C locus to Pv08 (10), and the observations that the locus is located in the vicinity of a cluster of MYB genes (11-15). Further, the observation that *S* and *Pi* map in different physical regions of the C locus is consistent with Prakken's (8) concept that the *C* locus is a cluster of genes controlling seedcoat patterning.

For mapping purposes, we developed a *Pi* PACE marker in gene model PvUI111.08g040900 fully diagnostic for the MDP and all but three red mottled genotypes in the ADP. Similarly, an *S* PACE marker in gene model Phvul.008G038000 was fully diagnostic in the MDP, while for the ADP it was diagnostic for all members except for two white kidney genotypes and one Carioca genotype. We only consider these to be linked markers, and the fact they are located in genes does not indicate at this time that they are causative genes. The primers for the *Pi* and *S* markers are listed in Table 1.

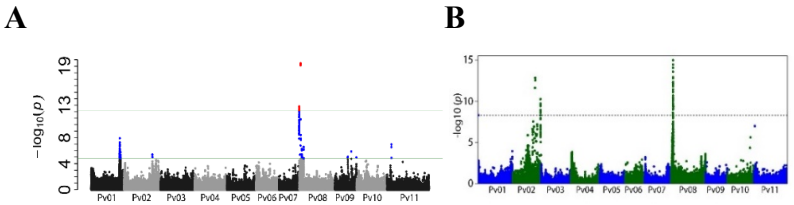

Figure 1. Manhattan plots depicting significant peaks on Pv08 associated with the cranberry (**A**) and pinto (**B**) dominant patterning phenotypes.

Table 1. Diagnostic PACE markers for the *Pi* and *S* seedcoat patterning genes.

***Pi* PACE primers**	
UI111-8-409-3360423 R1	GAAGGTGACCAAGTTCATGCTTATCACTTGGTGGGGTTTCCAACT
UI111-8-409-3360423 R2	GAAGGTCGGAGTCAACGGATTATCACTTGGTGGGGTTTCCAACA
UI111-8-409-3360423 CF	CACTCTCACAACAAAGATGACAAGAACAA
***S* PACE primers**	
G19833-8-380-3141933-F1	GAAGGTGACCAAGTTCATGCTCCATTCTCTAATTGGCACTTGTGCT
G19833-8-380-3141933-F2	GAAGGTCGGAGTCAACGGATTCCATTCTCTAATTGGCACTTGTGCA
G19833-8-380-3141933-CR	GTAATACTGGAGCTGAAGAAGATGATGAT

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DEPTH CAMERA-BASED POD COUNT ESTIMATION: A POTENTIAL TOOL FOR SEED YIELD ESTIMATION IN DRY BEAN BREEDING

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INTRODUCTION

Conventional breeding involves crossing two genetically diverse parents and selecting superior lines over multiple generations to develop high-yielding crop varieties. While this approach has been successful in improving crop performance (Kaiser et al., 2020), it has certain limitations, as developing a new variety typically takes 7–15 years depending on the crop, and requires significant financial investment (Tuberosa, 2012). In plant breeding programs, yield potential is generally assessed in later generations through yield trials, where lines are tested at multiple locations and replicated within sites based on seed availability. The drawback of this approach is that yield data is only available after harvest, meaning some lines may be advanced through early generations without a clear understanding of their yield potential. Identifying high-yielding lines earlier in the breeding pipeline could help reduce costs and improve the rate of genetic gain. To overcome this challenge, we are testing a rover-based phenotyping platform that captures side-view videos of bean plants in the field coupled with a machine learning model (YOLOv11) for pod detection (Figure 1C) and counting. Moving forward, we aim to refine this system to use pod and seed counts for predicting seed yield and improving selection efficiency in dry bean breeding.

MATERIALS AND METHODS

The study was conducted in field trials of the dry bean breeding program at North Dakota State University, during the 2024 growing season at Hatton, ND. A rover platform was developed by the Agricultural and Biosystems Engineering Department at NDSU to capture side-view videos of dry bean rows across different plots. This study aimed to assess the accuracy and precision of pod identification using a deep learning model. For field data collection, a camera was mounted 20 cm above the ground on the rover platform to ensure the full length of the plants was captured in each frame. Videos were recorded using an Intel® RealSense™ Depth Camera D405 (©Intel Corporation, Santa Clara, CA, USA). Detailed camera specifications can the found in Mathew et al. (2023). The idea behind using the depth camera was to remove background elements using a depth threshold, keeping only the objects of interest (plants in the dry bean row closest to the camera) in each image. The platform was manually moved across the field during data collection, and an integrated camera network architecture was used to control the video capture process. Data was collected across different advanced yield trials (AYT) from six market classes, including pinto (PAYT), slow-darkening pinto (SDPAYT), great northern (GNAYT), navy (NAYT), black (BAYT), and red and pink (RPAYT) beans. For this preliminary analysis, only BAYT was assessed. Videos were recorded at the full maturity (R8) stage of crop growth, and 906 images were extracted from the videos. Make Sense AI (https://www.makesense.ai/), an open-source image labeling tool, was used to annotate the pods, into YOLO format. Finally, the dataset was split into training and test sets in a 90:10 ratio. The performance of the model was evaluated using MS COCO evaluation metrics, such as Precision (P), Recall (R), mean Average

Precision (mAP) at 50% and mAP at 50:95% with a 65% Intersection over Union (IoU) threshold.

RESULTS AND DISCUSSIONS

Using a training set of 815 images, the YOLOv11 model achieved 71% true positive rate, correctly identifying most pods; however, it missed 29% of them. In contrast, a soybean study by Mathew et al. (2023) using 35,082 images resulted in a 97% true positive rate and only 2% false negatives, highlighting the impact of a larger dataset on detection accuracy. In terms of mean average precision (mAP), the model achieved a mAP@50 of 82% (Figure 1A), indicating that the detections were generally reliable when the overlap with the ground truth was at least 50%. However, the mAP@50:95 was 56% (Figure 1B), indicating reduced accuracy at higher overlap thresholds, suggesting some localization errors. The recall was 73%, meaning the model correctly identified 73% of all objects present in the image. High precision and recall values indicate the model's strong ability to accurately detect pods. These results highlight the need for a larger training dataset to improve model performance. To address this, additional images from all market classes will be incorporated into future training. Furthermore, new data will be collected during the 2025 season for testing, allowing for a comprehensive evaluation of the model's predictive ability. Once improved, the model will be used to estimate pods per plot and potentially, seeds per pod, contributing to dry bean seed yield predictions. This research has been suppported by USDA-ARS and Nrtharvest Bean Growers Assoc.

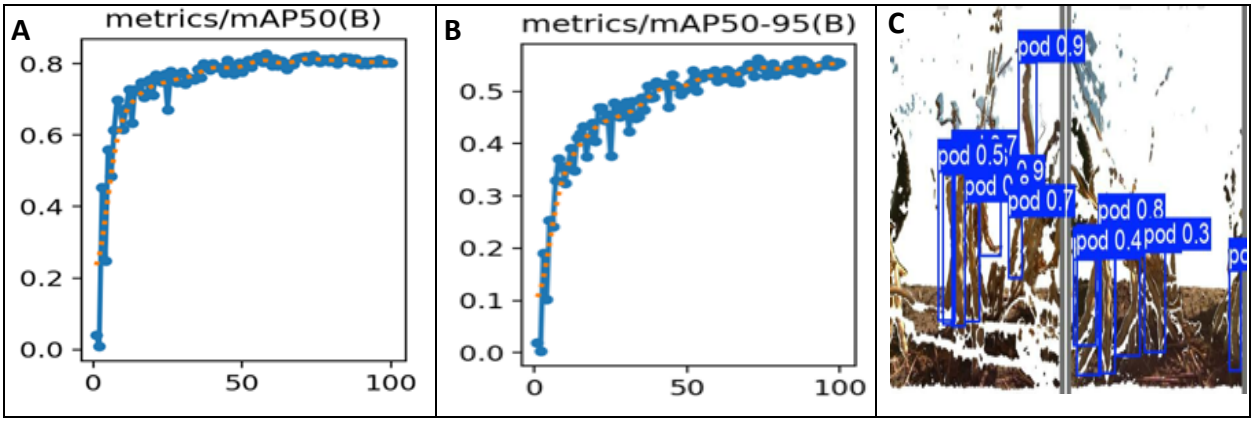

Figure 1. YOLOv11 performance metrics and its ability to detect dry bean pods in video frames captured with a depth camera. A) Mean average precision at an intersection over union (IoU) threshold of 0.5 (mAP@50). B) Mean average precision at IoU thresholds ranging from 0.5 to 0.95 (mAP@50:95). C) Pod detection in the validation set with respective confidence scores.

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DEVELOPING HIGH-THROUGHPUT PHENOTYPING TECHNIQUES FOR COMMON BEAN EMERGENCE AND YIELD CORRECTION

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Introduction

While plant genomic techniques have rapidly increased the capacity for breeders to identify and select for specific genes, breeding remains limited by the data quality of trait evaluation under field conditions. One area where phenotyping is deficient is the evaluation of emergent seedlings in breeding plots. Traditionally, emergence is evaluated through manual counts early in the season on a subset of plots, or later as a score of total plot stand. However, this evaluation is time and labour intensive, reducing the number of lines a breeding program can evaluate (Araus *et al.,* 2018) with these methods lacking precision and prone to bias (Yates, 2019). Using an Unmanned Aerial System (UAS) imaging phenotyping platform integrating an RGB + multispectral camera, plant seedling distribution can be detected with high sensitivity and provide previously unavailable data. Using this spatial data, two important attributes of breeding plots can be evaluated: the uniformity of seedling distribution, and the plot's predicted harvestable area. This report demonstrates the capacity for a UAS imaging platform to detect emergent seedlings with high accuracy, and how spatial data can be used to characterize seeding and emergence quality, the spatial characteristics of plots, and provide methods for yield correction to allow breeders to assess breeding lines more accurately in field trials.

Materials and Methods

Using a Mavic 3 UAS system carrying an integrated multispectral camera, imagery from the McGill University Pulse Breeding and Genetics Laboratory registration field trials (designed as a set of 3 randomized complete block designs) was collected weekly from seeding to harvest, encompassing 18 flights from June to August 2024. During this same time, ground truths of crop emergence and stand evaluation was captured. From each flight, imagery was processed into orthomosaics using Agisoft Metashape (agisoft.com), then imported into qGIS (qgis.org) for analysis. Using qGIS, NDVI (normalized differentiated vegetative index) was calculated to create a plant-sensitive raster of the studied breeding plots, which was then evaluated using a threshold value of 0.5 to separate plant from non-plant material.

To isolate seedlings from soil background around emergence, thresholder imagery was polygonised and individual seedlings were converted into countable centroids. Using tools integrated into qGIS, the number of emergent seedlings in each plot was counted, and a quadrat method was used to evaluate the aggregation of measured seedlings. To identify the harvestable area (HA), a buffer corresponding to the seeding width was generated around detected seedlings. The seedling-derived harvestable area was compared to the area predicted by the seeding design (row length * height) to generate a percentage used for evaluation. Pearson correlations for this predicted harvestable area were compared to ground truths of emergence and crop stand, as well as drone derived versions of those traits. The correlations between harvestable yield, seedling dispersion index and yield were also evaluated. Finally, for each registration trial, plot yields were adjusted by the estimated harvestable area, and coefficients of variation between corrected and uncorrected yields for those trials were compared. To minimize overadjustment due to minor fluctuations in peak harvestable area (HA) or severe reductions in harvestable area, a third model only adjusting plots with 50 – 90% of target harvestable areas was also calculated.

Results and Discussion

Using the correlation to yield as the heuristic for the detection of emergent seedlings, the high-throughput phenotyping method (r = 0.68) outperformed the ground truth (r = 0.47). Seedling-derived harvestable area outperformed both ground truth scores at predicting a plot's peak stand (r = 0.76). Both methods of emergence count ranked highest for correlation to crop yield, with predicted harvestable area and ground truth stand scores having comparable correlations. The seedling dispersion index, measured as the variance-to-mean ratio of quadrat counts of seedlings, was strongly correlated to peak crop stand and had a significant but weak negative correlation to crop yield. This correlation indicates that irrespective of variety or emergence, plots inclined to 'clumped' seeding resulted in reduced yield compared to plots with uniform seeding patterns. A method for yield correction was demonstrated using two methods: a direct correction generated by yield divided by harvestable area, and a conditional correction only correcting plots with harvestable area between 60 and 90% of target. Techniques showed improved coefficients of variation, with corrections improving as variability in harvestable area increased.

*Table 1. Pearson correlations between plot peak crop stand (identified as July 10, 2024) and final crop yield, compared to drone-derived and ground measured emergence traits. * indicates P<0.05, ** indicates P<0.01, *** indicates P<0.0001.*

	Peak Stand	Crop Yield
Ground Truth Emergence	0.44683**	0.47318**
Drone Emergence	0.37712**	0.68487***
Predicted Harvestable Area	0.76433***	0.36958**
Ground Truth Stand Scores	0.47194**	0.37357**
Seedling Dispersion Index	-0.73531***	-0.2494*

Table 2. Coefficients of variation for yield for three registration trials in the McGill Pulse Breeding and Genetics program. Brackets indicate change from unadjusted HA to corrected HA coefficients of variation for yield.

Registration Trial	Trts	Blocks	Mean HA %	Variance HA %	Unadjusted CV %	HA Corrected CV %	HA Corrected CV % (60 - 90%)
Black Bean	5	4	93.47	0.09	9.32	9.80 (0.48)	9.32 (0)
Pinto Bean	5	4	89.81	0.46	21.17	18.92 (-2.26)	18.64 (-2.54)
Cranberry Bean	4	4	85.65	2.18	43.99	37.59 (-6.40)	39.61 (-4.38)
Total	14	4	93.56	0.79	54.94	51.89 (-3.05)	53.56 (-1.38)

These results show great promise for utilizing UAS systems to evaluate field trial emergent traits with high precision and reduced variance. UAS evaluation of harvestable area shows promise as a tool for breeders to evaluate and reduce field trial variation, allowing breeders greater capacity to discern superior genetics in the field, especially when the evaluated spatial variance from seeding is high.

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EXPANDING OUR KNOWLEDGE AND THE AVAILABILITY OF ARID-ADAPTED WILD TEPARY BEAN GERMPLASM FROM NEW MEXICO

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INTRODUCTION

Food systems dependent on mesic crops such as common bean *P. vulgaris* L. are highly vulnerable to irregular weather patterns and the drying of western North America down to the Central American Dry Corridor presents climatic and historic features with profound implications for the future of agricultural systems (Williams et al. 2020, McKinnon et al. 2021).

Tepary bean (*Phaseolus acutifolius* Asa Gray) has long been known as a drought and heat tolerant crop (Freeman 1912). It was grown by the peoples of the Southwest in pre-Columbian times (Carter 1945). New irrigation techniques in the colonial period and new technologies from the 1930s onwards resulted in the loss of many landraces as more water-use-intensive cropping systems supplanted traditional ones (Nabhan & Felger 1978, Pratt et al. 2022). From the historic range of tepary cultivation (from southern Arizona down to Guanacaste in Costa Rica), some cultivated germplasm has been collected and conserved in genebanks. Once internal duplicates are eliminated, the number of different landraces in the major germplasm collections for tepary (USDA, CIAT, INIFAP) would total only about 100. Thus, the major reservoir of genetic diversity is found in its wild forms and the sister species, *P. montanus* Brandegee.

Wild teparies are distributed from Arizona south to Michoacán, while *P. montanus* thrives from Arizona south to Jalapa (Debouck 2021) in mid-elevation mountainous regions. The number of accessions of wild teparies from New Mexico in genebanks was less than five at the beginning of this work in 2023.

We undertook an exploration and collection of wild tepary germplasm in southern New Mexico during 2023 and 2024. We sought to locate previously reported populations and find new ones. Herbarium specimens and seed samples were obtained whenever possible.

RESULTS AND DISCUSSION

While the exploration of 2023 yielded little seed germplasm because of low and late rainfalls, the exploration of 2024 resulted in seed germplasm for populations at all locations. A total of eighteen populations of wild tepary were found (Table 1), with already some extremes in elevation (1288 m to 1918 m) and latitude from 31° 30’ 56.8” in the Peloncillo Mountains to 33° 13’ 35.9” in the Gila Cliff Dwellings. Three populations of *P. montanus* were found in the Chiricahua Mountains area, AZ, not far from the border with New Mexico. All these materials are presently under seed increase at USDA-Pullman to make them available to the bean community. Soil samples collected

in the rhizosphere of all populations listed in Table 1 (except six) are available for study (currently held at the National Rhizobium Germplasm Resource Collection USDA-Beltsville, MD).

Table 1 – Populations found in chronological order while those highlighted in grey refer to populations found in 2023 for which seeds were recovered in 2024.

Collection No.	Species	Latitude N	Longitude W	Elevation (masl)	Date
3406	*acutifolius*	32° 17' 48.4"	106° 36' 38.4"	1565	3-X-2024
3407	*acutifolius*	32° 18' 19.0"	106° 35' 32.0"	1740	3-X-2024
3408	*acutifolius*	32° 18' 44.4"	106° 34' 43.2"	1859	3-X-2024
3387	*acutifolius*	32° 22' 08.6"	106º 33' 34.7"	1750	4-X-2024
3409	*acutifolius*	32° 21' 50.8"	106° 33' 55.5"	1893	4-X-2024
3390	*acutifolius*	32° 20' 16.6"	106° 35' 10.7"	1757	5-X-2024
3410	*acutifolius*	32° 17' 33.5"	106° 35' 40.2"	1639	5-X-2024
3412	*acutifolius*	32° 02' 18.8"	106° 57' 23.0"	1288	6-X-2024
3413	*acutifolius*	31° 53' 13.6"	109° 12' 30.6"	1663	7-X-2024
3414	*montanus*	31° 53' 13.6"	109° 12' 30.6"	1663	7-X-2024
3416	*montanus*	31° 54' 33.1"	109° 15' 09.7"	1964	7-X-2024
3417	*montanus*	31° 55' 44.6"	109° 13' 10.4"	1693	7-X-2024
3418	*acutifolius*	31° 55' 44.6"	109° 13' 10.4"	1693	7-X-2024
3420	*acutifolius*	32° 39' 01.3"	108° 31' 56.8"	1790	8-X-2024
3421	*acutifolius*	32° 47.1' 17.8"	108° 29' 38.2"	1518	8-X-2024
3422	*acutifolius*	32° 51' 04.8"	108° 35' 26.0"	1327	8-X-2024
3423	*acutifolius*	33° 02' 59.0"	108° 30' 03.4"	1535	9-X-2024
3428	*acutifolius*	33º 13' 35.9"	108º 16' 11.5"	1795	10-X-2024
3429	*acutifolius*	33º 10' 45.0"	108º 12' 19.2"	1698	10-X-2024
3430	*acutifolius*	33º 05' 16.6"	109º 05' 21.8"	1827	11-X-2024
3431	*acutifolius*	32º 57' 04.6"	108º 57' 35.8"	1918	11-X-2024

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