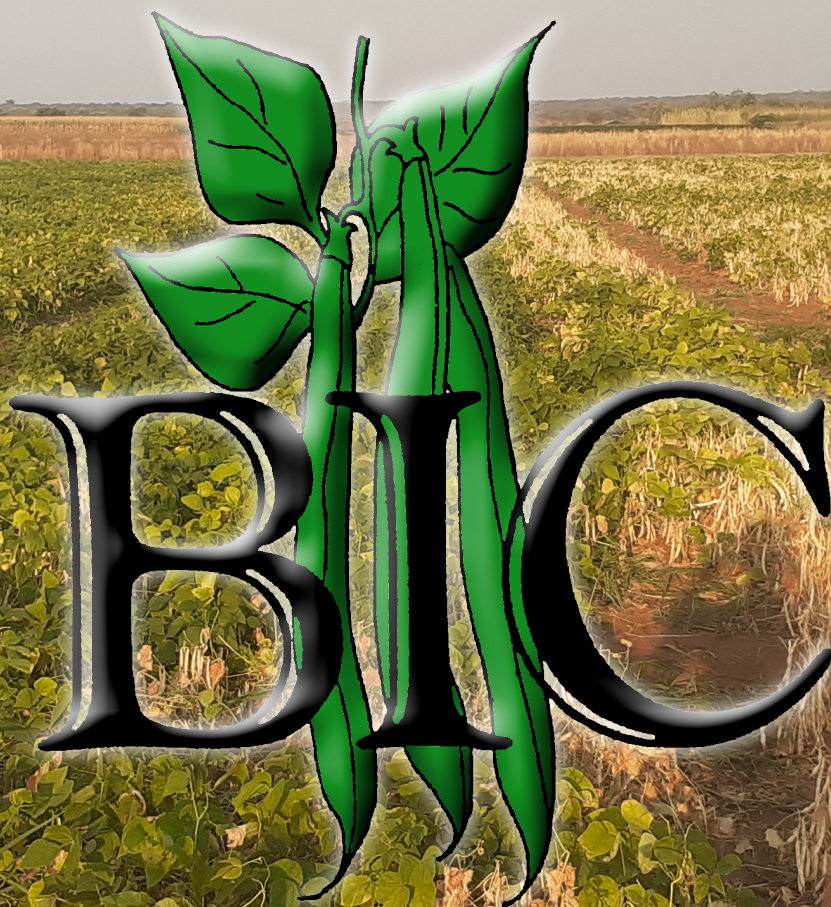


**ANNUAL REPORT OF THE**  
**BEAN IMPROVEMENT**  
**COOPERATIVE**

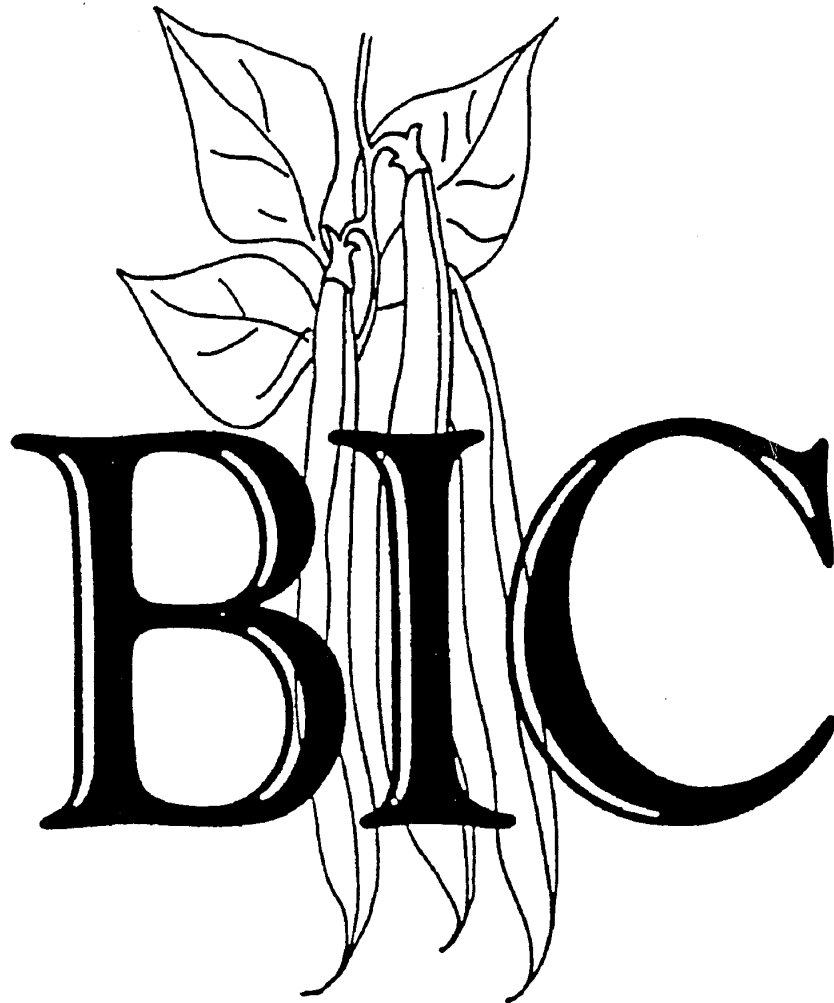


**A VOLUNTARY AND INFORMAL ORGANIZATION  
TO EFFECT THE EXCHANGE OF INFORMATION AND MATERIALS**

**VOLUME 65**  
**2022**



**ANNUAL REPORT OF THE  
BEAN IMPROVEMENT  
COOPERATIVE**



**AVOLUNTARY AND INFORMAL ORGANIZATION  
TO EFFECT THE EXCHANGE OF INFORMATION AND MATERIALS**

**VOLUME 65  
2022**





**THE LXV**

Report of The

**BEAN IMPROVEMENT COOPERATIVE**

No. 65

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**Coordinating Committee**

Kirstin Bett  
Karen Cichy  
Kelvin Kamfwa  
Ken Kmiecik  
Phil Miklas (Ex officio)  
Jim Myers

Juan Osorno  
Peter Pauls  
Timothy Porch (President)  
Thiago Souza  
Dan Wahlquist

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

Dr. Timothy Porch  
USDA-ARS-TARS  
2200 P.A. Campos Ave., Suite 201  
Mayaguez, P.R. 00680  
Phone: 787-831-3435 x254  
FAX: 787-831-3386  
[timothy.porch@usda.gov](mailto:timothy.porch@usda.gov)

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

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<b>COVER:</b> Off-season 2021 drought trial of the University of Zambia Bean Breeding Program at the Golden Valley Agricultural Research Trust Farm, Chisamba, Zambia. Photo credit: Kelvin Kamfwa.	

## THE 65th ANNUAL REPORT OF THE BEAN IMPROVEMENT COOPERATIVE

**The Bean Improvement Cooperative (BIC)** celebrated its 31<sup>th</sup> Biennial Meeting in November, 2021. For the first time, due to the covid pandemic, the meeting took place on a virtual platform with both the BIC and NAPIA meetings held concurrently. We would like to thank the local BIC meeting organizing committee, Dr. Kirstin Bett and Laura Jardine of the University of Saskatchewan, for their excellent meeting organization despite the challenges with this new format. There were a total of 184 registrants that included participants from Africa, Asia, Europe, and North, Central and South America. The online format made it easier and more cost effective for international participation and for collaboration and cross-pollination between the BIC and NAPIA communities. The plenary BIC session was presented by Dr. Paul Gepts, Professor at UC Davis, and is included in this volume as a longer research note titled “Intriguing observations in *Phaseolus* and potential future research tracks.” There were a total 24 oral presentations and 16 poster presentations.

The meeting received generous support from a number of donors including: at the Platinum level, Manitoba Pulse and Soybean Growers and Saskatchewan Pulse Growers; at the Gold level, Vilmorin-Mikado, HM Clause, Limagrain and Hazera Seeds of Growth; at the Silver level, Northern Pulse Growers Association, USA Dry Pea and Lentil Council, Syngenta, and North Dakota Crop Improvement and Seed Association; and at the Bronze level, American Pulse Association, Ontario Bean Growers, and Treasure Valley Seed Company.

Given the online format and absence of an Awards Banquet, awards were not presented during the 2021 BIC Meeting. The BIC Coordinating Committee decided to postpone the awards, including the Frazier-Zaumeyer Distinguished Lectureship, BIC Meritorious Service Award, BIC Achievement Award, and BIC Technical Merit Award until the 2023 BIC meeting. However, we would like to congratulate the three students that tied for the best oral presentation award during the 2021 meeting: Henry Cordoba, Kimberly Gibson, and Hannah Jeffrey, and María Jurado Cañas for the best student poster.

We look forward to the 2023 BIC meeting to be hosted at Clemson University. Additional information regarding this meeting will be shared as it is organized. Please share information about the BIC with interested colleagues who might like to attend the 2023 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 Coordinating Committee welcomes Dr. Kelvin Kamfwa as a new member and thanks Dr. Jennifer Trapp for her 5 years of service on the Coordinating Committee. 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 safe and successful year. Warm regards,  
**Tim Porch, BIC President**

## BIC COMMITTEE MEMBERSHIP - 1957 to 2022

### 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 (ex officio), Myers, Osorno, Pauls, **Porch**, Souza, Wahlquist

### Awards Committee:

1971	Baggett, Briggs, Burke, Dean, Wallace		
1973	Burke, Dean, Mauth, Zaumeyer	1995	<b>Coyne</b> , Dickson, Stavely
1975	Ballantyne, Frazier, Mauth	1997	<b>Coyne</b> , Schwartz, Stavely
1977	Ballantyne, Curme, Frazier, Schuster	2001	<b>Hosfield</b> , Magnuson, Schwartz
1979	Ballantyne, Schuster, Silbernagel, Temple	2004	Hosfield, <b>Schwartz</b> , Singh
1981	Abawi, Bliss, Monis, Silbernagel	2012	Noffsinger, <b>Schwartz</b> , Singh
1983	Adams, Bliss, Burke, Dean, Morris	2014	<b>Beaver</b> , Noffsinger, Urrea
1985	Emery, Hagedorn, Sandsted, Schwartz	2015	<b>Beaver</b> , Myers, Urrea
1987	Emery, Hagedorn, Sandsted		
1989	<b>Coyne</b> , Silbernagel, Wallace		

## Genetics Committee

- 2004 **Bassett** (Chair), Beaver, Blair, Gepts, McClean, Miklas, Welsh (ex officio)
- 2005 **Beaver** (Acting Chair), Blair, Gepts, McClean, Miklas, Porph, 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, Porph, Urrea
- 2018 Bett, Ferreira, Gepts, Goncalves-Vidigal, Kalavacharla, **Kelly** (Chair), Kisha (ex officio), McClean, Osorno, Porph, Urrea
- 2020 Bett, Ferreira, Gepts, Goncalves-Vidigal, Kalavacharla, Kelly, McClean, **Miklas** (Chair), Osorno, Porph, Urrea
- 2021 Bett, Ferreira, Gepts, Goncalves-Vidigal, Kalavacharla, Kelly, McClean, **Miklas** (Chair), Osorno, Parker, Porph, Urrea

## BIC GENETICS COMMITTEE MEETING MINUTES

**Location:** Virtual zoom meeting hosted by Carlos Urrea (U. of Nebraska, Scottsbluff)

**Date:** Friday, August 20, 2021, 3:00 - 4:00 pm MST

**Committee Members:** Bett, Ferreira, Gepts, Goncalves-Vidigal, Hoyos-Villegas, Kalavacharla, McClean, Miklas (Chair), Osorno, Porch, and Urrea.

**Present:** Ferreira, Gepts, Goncalves-Vidigal, Vazbisneta, Hoyos-Villegas, McClean, Miklas (Chair), Osorno, Porch (Acting Secretary), Heitholt, Urrea, Gomez, Raatz, Pastor-Corrales, Parker

### 1. Old Business:

The Genetics Committee meeting minutes from 2020 and published in the 2021 BIC v64 were approved.

### **Bean Gene List: Propose the addition of a modified Introduction section highlighting newly developed genomic tools**

**Decision:** The genomic tools section will be organized in bullet form in the Bean Gene List. Further consultation is needed regarding curation of data on the LIS (Legume Information System; <https://legumeinfo.org/>) database for specific traits of interest. One idea is for each lab group to curate their own data as they publish it and then share it with LIS.

### **New SNP markers – to replace/complement the SCAR marker Table**

This is an effort to replace or complement the **SCAR Table**

[http://www.bic.uprm.edu/?page\\_id=91](http://www.bic.uprm.edu/?page_id=91) with the SNP-based markers for bean community use using the Intertek Company platform ([agritech.sweden@intertek.com](mailto:agritech.sweden@intertek.com)) for economical and efficient DNA extraction and genotyping. Coordinated by the GIAR Excellence in Breeding Platform (EiB) CIAT, led by Bodo Raatz, and the USDA-ARS-WA, SNP/InDel markers were developed and converted to KASP markers (Intertek-Lab [agritech.sweden@intertek.com](mailto:agritech.sweden@intertek.com)).

**Decision:** An excel spreadsheet has been developed with tested and verified markers. Before posting on the BIC webpage, the markers will be confirmed as being based on the G19833 v2.1 genome sequence. In addition, the sequence information and genetic background used for testing will be added to the excel spreadsheet. These same markers can also be used with temperature shift assays. This information will be posted on the BIC website so that users can share their results in different genetic backgrounds, provide suggestions, and decide on which platform to use.

***bc-u* symbol was changed to *bc-u<sup>d</sup>* to reflect its origin from race Durango and different interactions.** Namely, *bc-u* was absent from host groups 2, 4, 5, and 7 and present in HG-10 which differs from the Drijfhout (1978) description.

**Decision:** The committee reviewed Soler-Garzon et al. 2021b and approved that the *bc-u* symbol be changed to *bc-u<sup>d</sup>* to reflect its origin from race Durango.

## New business

### Membership

**Decision:** Travis Parker was nominated by Paul Gepts and seconded by Phil McClean to be a new Genetics Committee member. All were in favor.

### Gene description amendments:

**Add the candidate gene PvNAC1** to the description for *bgm* (syn *bgm-1*) based on Soler-Garzon (2021a).

**Decision:** Add the candidate gene PvNAC1 reference to the description for *bgm* (syn *bgm-1*) in the Bean Gene List based on the evidence in Soler-Garzon (2021a).

**Additional updates of candidate genes** can be made to those genes below. The candidate, the gene model, and the reference genome used should be included. Mutation analysis is needed within the genes themselves to show a high confidence candidate gene in order to include the gene in the Bean Gene List.

Researchers can send their updates of candidates to Phil Miklas to indicate which candidates or genes have been identified for addition to the Bean Genes List. An initial list of candidates/genes include:

- *fin* gene (Paul Gepts)
- *Ppd* (Paul Gepts)
- Dormancy (Phil McClean)
- Pod Shattering candidate genes (Paul Gepts)

**Add the KTR2/3 (truncated CRINKLY4 kinase) candidate gene** to the description for *Co-1* cluster alleles (MDRK, *Co-1*; Kaboon, *Co-1*<sup>2</sup>; Perry Marrow, *Co-1*<sup>3</sup>; AND277, *Co-1*<sup>4</sup>). It is also found in CDRK but not in *Widusa* (Richard et al., 2021).

**Decision:** Phil Miklas will contact Valerie Geffroy to see if she would like to add KTR2/3 (truncated CRINKLY4 kinase) as a candidate gene to the description of the *Co-1* cluster of alleles according to the results of Richard et al. (2021).

## New Gene Symbols

### *bc-4*

*bc-4* is a new recessive gene locus that interacts with *bc-2* to condition resistance to BCMV. *bc-4* was found in host groups 4, 5, and 7. Genes encoding Vps4 AAA<sup>+</sup> ATPase ESCRT proteins on Pv11 and Pv05 are candidates for *bc-2* and *bc-4*. A draft of the manuscript was sent to the committee on 7-29-21. Soler-Garzon et al. are planning on submitting this paper in the next several weeks for publication.

**Decision:** Members were asked to respond by Sept. 10, 2021 regarding their approval of the *bc-4* gene symbol. New symbol was subsequently approved by the committee.

## Same gene with different mutations

The use of superscripts in brackets is proposed to denote different mutations for the same gene (ie. the different mutations are not different alleles in the genetic sense). *bc-2*<sup>[d]</sup> denotes a 10 kb deletion of Durango origin and *bc-2*<sup>[n]</sup> a single SNP deletion found in a navy bean landrace



selection (Robust) for the gene encoding Vps4 AAA<sup>+</sup> ATPase ESCRT on Pv11. Both mutations (frameshift) result in truncated proteins.

**Decision:** Support the use of superscripts in brackets to denote different mutations for the same gene. These mutations are not different alleles in the genetic sense since different mutations may result in the same phenotype. E.g. *bc-2*<sup>[common red]</sup> denotes a 10 kb deletion in the common red line of Durango origin and *bc-2*<sup>[robust]</sup> a single SNP deletion in the line Robust.

**Move *bc-1*<sup>2</sup> and *bc-2*<sup>2</sup> to the obsolete symbol list.**

There is only one resistance allele for the *Bc-1* and *Bc-2* loci. The differential interaction for *bc-1* is due to presence versus the absence of *bc-u*<sup>d</sup>. The differential interaction for *bc-2* is due to the presence versus the absence of *bc-u*<sup>d</sup> and *bc-4*.

**Decision:** Move *bc-1*<sup>2</sup> and *bc-2*<sup>2</sup> to the obsolete symbol list. Consider moving the *bc-u* symbol to the obsolete symbol list in the next meeting.

## LIST OF GENES - *Phaseolus vulgaris* L.

The original comprehensive gene list was prepared by S.H. Yarnell (Bot. Rev. 31:247-330, 1965) and published in the BIC 8:4-20, 1965. An updated list was prepared by M.H. Dickson and associates and published in the BIC 25:109-127, 1982. The next update (BIC 32:1-15, 1989) was prepared by M.J. Bassett, involving extensive additions, corrections, revisions, and style changes. Subsequent updates (BIC 36:vi-xxiii, 1993; BIC 39:1-19; and BIC 47:1-24) were prepared by M. J. Bassett. Updates were completed by T.G. Porch in 01/2008, 12/2009, 02/2011, and 10/2013; by K.E. Bett in 02/16; by P. Miklas in 11/17, 03/21, and in BIC 65 (2022).

### **Coordination of Genes and Gene Symbol Nomenclature - BIC Genetics Committee.**

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

We strongly recommend that any researcher conducting studies of potentially new, qualitatively inherited traits of common bean submit the manuscript to the committee prior to publication (concurrent submission can be made to the genetics committee and the journal). The committee will evaluate the data to determine 1) if sufficient evidence exists to establish the inheritance hypothesis, 2) whether any issue of potential allelism of the trait has been met, and 3) whether the proposed gene symbol has been previously assigned to another gene. The evidence must include 1) data from one generation to formulate a hypothesis, 2) data from subsequent generations to test that hypothesis, and 3) molecular data including linkage and physical mapping to support naming new genes within or near known gene clusters (e.g. the *Co-1* cluster on Pv01). The population sizes used must be sufficiently large to distinguish (with statistical significance) among potential segregation hypotheses.

For example, during 2020, the symbol  $p^{sd}$  with supporting data was submitted to the committee for approval, which was granted.

### **The following is the review process for new traits and gene symbols:**

- a. The committee will evaluate the data to determine:
  - i. if sufficient evidence exists to establish the inheritance hypothesis
  - ii. whether any issue of potential allelism of the trait has been met
  - iii. whether the proposed gene symbol has been previously assigned to another gene
- b. The evidence must include:
  - i. data from one generation to formulate a hypothesis
  - ii. data from subsequent generations to test that hypothesis
  - iii. for hyper-variable pathogens: family mean testing (F2:3 progenies, or recombinant inbred lines – RILs), and use of multiple, specific races of the pathogen to separate effects of individual genes in gene clusters

- iv. molecular marker data and genetic linkage map and physical map (preferred) positions when available with available resources for such determinations detailed in “genomic resources” section below. When associating an annotated gene with a gene symbol include the gene model and reference genome.
- c. Lastly – parent, germplasm line, or cultivar source of new genes accepted by the committee must be made publically available via seed deposit with the U.S. National Plant Germplasm System - Plant Introduction Station in Pullman, WA, as a Genetic Stock (this enables others to access the gene source for subsequent allelism tests, genetic studies, etc.). This requirement is unnecessary for well known and easily accessible materials.

**Genomic resources** – as genomic resources have improved for *Phaseolus*, they have correspondingly been useful to characterize (physical position, candidate genes, sequenced-based mutant alleles) and discern new and existing genes. There are now multiple reference genomes available for alignment of marker and sequence data to assist in locating genes to specific regions of the genome.

- Andean Landrace Chaucha Chuga (G19833; Schmutz et al. 2014) version 2.1, is available on Phytozome 13 ([https://phytozome-next.jgi.doe.gov/info/Pvulgaris\\_v2\\_1](https://phytozome-next.jgi.doe.gov/info/Pvulgaris_v2_1)).
- The reference assembly and annotation for the race Durango pinto genotype UI 111 is available at Phytozome 13 ([https://phytozome-next.jgi.doe.gov/info/PvulgarisUI111\\_v1\\_1](https://phytozome-next.jgi.doe.gov/info/PvulgarisUI111_v1_1)).
- The Guatemalan race genotype Labor Ovalle is available at Phytozome 13 [https://phytozome-next.jgi.doe.gov/info/PvulgarisLaborOvalle\\_v1\\_1](https://phytozome-next.jgi.doe.gov/info/PvulgarisLaborOvalle_v1_1)).
- The Mesoamerican race genotype 5-593 is now available as well [https://phytozome-next.jgi.doe.gov/info/PvulgarisLaborOvalle\\_v1\\_1](https://phytozome-next.jgi.doe.gov/info/PvulgarisLaborOvalle_v1_1)
- The BAT 93 (race Mesoamerican) was released (Vlasova et al., 2016).
- The interspecific genotype OAC Rex (race Mesoamerican + tepary) is also available on a different site (<http://www.ncbi.nlm.nih.gov/sra/?term=OAC+Rex>).
- Frijol Bayo (dom. Tepary) is available at [https://phytozome-next.jgi.doe.gov/info/Pacutifolius\\_v1\\_0](https://phytozome-next.jgi.doe.gov/info/Pacutifolius_v1_0), and W6 15578 (wild tepary) at [https://phytozome-next.jgi.doe.gov/info/PacutifoliusWLD\\_v2\\_0](https://phytozome-next.jgi.doe.gov/info/PacutifoliusWLD_v2_0)).
- The assembly and annotation of Lima bean G27455 (Garcia et al., 2021) is also available at Phytozome 13 ([https://phytozome-next.jgi.doe.gov/info/Plunatus\\_V1](https://phytozome-next.jgi.doe.gov/info/Plunatus_V1)).

In addition to reference genomes WGS data for hundreds of accessions (eg. Lobaton et al., 2018; Wu et al., 2020) also exists and can be useful for gene identification and characterization as well; however, to date, centralized access to resequencing data sets is unavailable. But that is changing with the development of the Legume Information System (LIS) platform– excerpt from Parker et al. [(2021) BIC 64:65-66.] “LIS offers a continuously updated, highly integrated platform for comparing genetic and phenotypic data among distinct genomes assemblies and species. Recent updates in the data deposition system (available at [https://legumeinfo.org/submit\\_data](https://legumeinfo.org/submit_data)) now facilitate the process of adding QTL mapping or GWAS data to the repository. Resources include genome browsers, tools for accessing gene annotation and expression data, trait and map databases, and numerous comparison tools. The portal now includes example data sets that can be used as templates for future submissions. Under this proposal, submission by the authors of QTL mapping and GWAS results to LIS as a

routine part of the publication process in *Phaseolus* is strongly encouraged. These data can be reviewed by LIS developers and rapidly incorporated into the database.”

The BIC website ‘Genetics’ section [http://www.bic.uprm.edu/?page\\_id=91](http://www.bic.uprm.edu/?page_id=91) has a new Beyond SCARs Table for trait-linked snps and indels converted to tm-shift assays (see Soler-Garzón et al., BIC v65: this issue). Please submit gene-linked markers with utility for MAS to add to this list.

Questions or comments should be addressed to the chairperson of the committee:  
Phillip Miklas, USDA-ARS: [phil.miklas@usda.gov](mailto:phil.miklas@usda.gov)

## GENE LIST

- Acc* *Accompanying* colors, i.e., the formerly "pleiotropic effects of *R*<sup>st</sup> on the color of pods, the top edge of the standard, and the hypocotyl" (Prakken 1974).
- ace* *acera* (Latin): produces shiny pod (Yen 1957). *Ace* is linked to *V* (Bassett 1997a), which is located on Pv06 (McClellan et al. 2002).
- Adk* structural gene for *adenylate kinase* enzyme (Weeden 1984).
- Am* *amaranth*: with *No* and *Sal* geranium flower color, and scarlet flower with *Beg No Sal* (Lamprecht 1948b, 1961a). Scarlet flower (Fan 1, 43C; Royal Hort. Soc. fans) is expressed by *Sal Am V*<sup>wf</sup> (or *v*), and *Sal Am v* expressed oxblood red seed coats (vs. mineral brown) due either to a pleiotropic effect of *Am* or a very closely linked dominant gene (Bassett 2003b). *Am* has no expression with *sal*, and *Am* is located 9 cM from *V* (Bassett 2003b) on Pv07 (McClellan et al. 2002).
- Amv-1* high level resistance to a strain of *alfalfa mosaic virus* (Wade and Zaumeyer 1940).
- Amv-2* resistance to the same strain of *alfalfa mosaic virus* as for *Amv* (Wade and Zaumeyer 1940).
- Ane* *Anebulosus* (Latin): produces nebulosus-mottling on testa (Prakken 1977a); observable only in *c*<sup>u</sup> *J* and *C/c*<sup>u</sup> *J* backgrounds. Not allelic with *V* or *R*, but linked to *B* (Lamprecht 1964). This trait is more commonly known as strong (grayish brown) vein pattern of seed coats (Bassett, editor).
- aph* *aphyllus* (Latin): plants are sterile and have only two (unifoliate) leaves and 4 to 6 nodes. (Lamprecht 1958).
- Arc* *arcus* (Latin): with *Bip* gives virgatus seed coat pattern, with *bip* gives virgata; *arc* with *Bip* gives *arcus*, with *bip* gives bipunctata; extends seed coat color in partly colored seeds (Lamprecht 1940b). The arcus pattern is also expressed by *t z Bip J Fib*; possible allelism between *Arc* and *Fib* has not been tested (Bassett and McClellan 2000; Lamprecht 1940b), whereas *J* and *Fib* are not allelic (Bassett 2001).
- arg* *argentum* (Latin): with *Y* produces a "silver" or greenish gray pod (Lamprecht 1947b), formerly *s* (Currence 1930, 1931); *arg* with *y* gives a white pod (Currence 1931; Lamprecht 1947b).
- Arl (Arc)* structural gene for the seed protein *arcelin* (Osborn et al. 1986).
- asp* *asper* (Latin): very dull (non-shiny) seed coat that is slightly rough textured due to the pyramidal shape of the outer epidermal palisade cells (Lamprecht, 1940c). With *P C J G B V*, *asp* seed coats had only 19% of the total anthocyanin content (delphinidin 3-*O*-glucoside, petunidin 3-*O*-glucoside, and malvidin 3-*O*-glucoside) compared with *Asp*; this was achieved by *asp* changing the size and shape of the palisade cells of the seed coat epidermis, making the cells significantly smaller than with *Asp* (Beninger et al. 2000). *Asp* is located Pv07 (Miklas et al. 2000).
- B (Br, Vir)* as used by Lamprecht (1932a, 1939, 1951a); the greenish brown factor of Prakken (1970). Similar or equivalent genes, according to Feenstra (1960), are the *C* of Tschermak (1912), the *D* of Shull (1908), the *E* of Kooiman (1920), the *H* of Shaw and Norton (1918), and the *L* of Sirks (1922). Smith (1961) used the gene symbol *Br* for *B*, according to Prakken (1972b).

- Lamprecht (1932b) used the gene symbol *Vir* for the effects of segregation at *B* in the genotype *P C j g B/b v*, according to Prakken (1970). The interactions of *B* with nearly all combinations of genes for seed coat color were summarized by Prakken (1972b). With *P C J G V Asp*, the *B* gene acts to regulate the production of precursors of anthocyanins in the seed coat color pathway above the level of dihydrokaempferol formation (Beninger et al. 2000). With *P C J G v Asp*, the *B* gene acts to regulate the production of astragalin and kaempferol 3-*O*-glucoside (Beninger et al. 1999). *B* is very tightly linked (Kyle and Dickson 1988) to the virus resistance gene *I* on Pv02 (Freyre et al. 1998; Vallejos et al. 2000).
- bc-u*<sup>d</sup> strain-*unspecific* complementary gene, giving resistance to certain strains of *bean common mosaic virus* (BCMV/BCMNV) only when together with one or more of the strain-specific resistance genes. Originally named *bc-u* by Drijfhout (1978b) but renamed by Soler-Garzón et al. (2021b) to reflect Durango race origin and absence from host groups (HG) 2, 4, 5, 7 and presence in HG-10. A basic Leucine Zipper (bZIP) transcription factor protein is the candidate gene for *bc-u*. bZIP protein gene Phvul.005G124100 (G19833 v2.1) carries a unique non-synonymous mutation at codon 14 in the first exon (Pv05: 36,114,516 bases), resulting in a premature termination codon that causes a nonfunctional protein. A marker for this mutation is listed in the Beyond SCARs table ([http://www.bic.uprm.edu/?page\\_id=91](http://www.bic.uprm.edu/?page_id=91)).
- bc-1* alone provides resistance to pathogroup PG-1 strains and combined with *I* gene provides protection (localized vein necrosis - VN) to NL-8 strain (PG-3). Combined with *bc-u*<sup>d</sup> it provides resistance to PG-2, -3, and -5, and combined with *I* gene and *bc-u*<sup>d</sup> it provides VN resistance to PG-6 (NL-3 strain) (Soler-Garzón et al. 2021a). A marker linked with this gene on Pv03 is listed in the Beyond SCARs table ([http://www.bic.uprm.edu/?page\\_id=91](http://www.bic.uprm.edu/?page_id=91)). Gene was first described by Drijfhout (1978b).
- bc-2* combined with *bc-u*<sup>d</sup> gives resistance to BCMV and BCMNV except PG-7, and combined with *bc-4* provides resistance to all BCMV (except PG-5) but not BCMNV (Soler-Garzón et al. 2021b). Phvul.011G092700 (G19833 v2.1), a vacuolar protein-sorting 4 (Vps4) AAAC ATPase endosomal sorting complexes required for transport (ESCRT) protein, is the *bc-2* candidate gene. Two different variants within the candidate gene are noted *bc-2*<sup>[UI 111]</sup>, contains a 10-kb deletion, and *bc-2*<sup>[Robust]</sup> consists of a single nucleotide polymorphism (SNP) deletion. Markers for these different variants are listed in the Beyond SCARs table ([http://www.bic.uprm.edu/?page\\_id=91](http://www.bic.uprm.edu/?page_id=91)). Gene was first described by Drijfhout (1978b).
- bc-3* with *bc-u* gives resistance to all strains of BCMV (Drijfhout 1978b). This gene is located on Pv06 (Johnson et al., 1997).
- bc-3*<sup>2</sup> Previously *cyv*, conditions resistance to *clover yellow vein virus*. Allelic to *desc*, *cyv*, and *bc-3*, located on Pv06, and linked to PveIF4E (Hart and Griffiths, 2013).
- bc-4* when combined with *bc-2* provides resistance to all BCMV (except PG-5) but not BCMNV (Soler-Garzón et al. 2021b). Gene model Phvul.005G125100, a Vps4 AAAC ATPase ESCRT protein, was identified as the candidate gene for *bc-4* gene, and a marker for the putative causal mutation is listed in the Beyond SCARs table ([http://www.bic.uprm.edu/?page\\_id=91](http://www.bic.uprm.edu/?page_id=91)).
- Bcm* confers temperature-sensitive resistance to *blackeye cowpea mosaic virus*. Tightly linked, if not identical, to the *I* gene for resistance to bean common mosaic virus (Kyle and Provvidenti 1987; Provvidenti et al. 1983).
- Bct* (*Ctv-1*) a gene conditioning resistance to *beet curly top virus* discovered by Schultz and Dean (1947). The *Ctv-1* symbol was proposed by Provvidenti (1987) and updated to *Bct* by Larsen and Miklas (2004). *Bct* is located between the *Phs* and *Asp* loci on Pv07 (Miklas et al. 2000).
- Bdm* confers resistance to *Bean dwarf mosaic virus* (BDMV) through the blockage of long-distance movement in the phloem (may or may not be associated with a hypersensitive response) (Seo et al. 2004).
- Beg* with *P v* (Line 214), gives *begonia* red flower color by fully dominant action, but with *P v*<sup>lae</sup>, expresses partial dominance for *begonia* red flower (Lamprecht 1948b). Allelism of *Beg* with *Sal* was not tested (Bassett 2003b).

<i>bgm</i>	( <i>syn with bgm-1</i> ) confers resistance (prevents a chlorotic response) to bean golden yellow mosaic virus (BGYMV) (Velez et al. 1998), found in A429 (Urrea et al., 1994), and located on Pv03 (Blair et al., 2007). A mutation (5 bp deletion) in the NAC (No Apical Meristem) domain transcriptional regulator superfamily protein gene model Phvul.003G027100 (G19833 v2.1) corresponds with the recessive <i>bgm</i> resistance allele (Soler-Garzón et al. 2021c). A marker for the causal mutation is listed in the Beyond SCARs table ( <a href="http://www.bic.uprm.edu/?page_id=91">http://www.bic.uprm.edu/?page_id=91</a> ).
<i>bgm-2</i>	from DOR 303 confers resistance (prevents a chlorotic response) to BGYMV (Velez et al. 1998).
<i>bgm-3</i>	from <i>P. coccineus</i> confers resistance to leaf chlorosis in the presence of BGYMV (Osorno et al. 2007).
<i>Bgp</i>	prevents pod deformation in the presence of BGYMV (may require <i>bgm</i> for expression) and found in DOR 482 ('Don Silvio') (Acevedo-Román et al., 2004).
<i>Bgp-2</i>	from <i>P. coccineus</i> prevents pod deformation in the presence of BGYMV (Osorno et al. 2007).
<i>bic</i>	<i>bic</i> confers bicolor flowers (colored banner and white wings) and dark olive brown seed coat (Bassett and Miklas 2007).
<i>Bip</i>	<i>bipunctata</i> (Latin): <i>Bip</i> and <i>bip</i> combine with <i>Arc</i> and <i>arc</i> to form seed coat patterns based on the hilum; extends seed coat color in partly colored seeds (Lamprecht 1932d, 1940b). Genotype <i>t z bip</i> expresses the bipunctata pattern of partly colored seed coats; whereas <i>t z Bip</i> expresses virgarcus pattern (Bassett 1996c; Schreiber 1940). <i>Bip</i> is linked to <i>J</i> and is located on Pv10 (McClellan et al. 2002).
<i>bip<sup>ana</sup></i>	Anasazi pattern of partly colored seed coats is expressed by genotype <i>t Z bip<sup>ana</sup></i> ; whereas <i>t z bip<sup>ana</sup></i> expresses the Anabip pattern (Bassett et al. 2000).
<i>blu</i>	<i>blue</i> flower color mutant (Bassett 1992a).
<i>Bpm</i>	confers resistance to <i>bean pod mottle</i> virus (Thomas and Zaumeyer 1950); symbol proposed by Provvidenti (1987).
<i>Bsm</i>	confers resistance to <i>bean southern mosaic</i> virus (Zaumeyer and Harter 1943); symbol proposed by Provvidenti (1987).
<i>By-1</i>	confers strain-specific resistance to pea mosaic virus, a strain of <i>bean yellow</i> mosaic virus (Schroeder and Provvidenti 1968).
<i>By-2</i>	strain-unspecific gene for temperature sensitive resistance to <i>bean yellow mosaic virus</i> (Dickson and Natti 1968).
<i>C</i>	with <i>P z j g b v</i> , sulfur-white or primrose yellow testa; no color in the hilum ring (Lamprecht 1932a, 1939, 1951a, 1951b; Tjebbes and Kooiman 1922b). According to Feenstra (1960), this <i>C</i> is the equivalent of the <i>B</i> of Tjebbes (1927), of Kooiman (1920), and of Sirks (1922), and the <i>Cm</i> of Prakken (1934). From the early 20 <sup>th</sup> century until the present, the regulation of color and pattern expression (especially in seed coats, but also in other plants organs, e.g., flowers, pods, petioles and stems) at <i>C</i> has had dual characterization as both a series of alleles at a locus and a series of very tightly linked genes in one chromosome region (Prakken, 1974). Plant introduction (PI) lines with various seed coat patterns were identified and demonstrated to be allelic (Troy and Hartman 1978). The interactions of <i>C</i> and <i>J</i> were summarized by Prakken (1972b). <i>C</i> is located on Pv08 (McClellan et al. 2002).
<i>C/c</i>	inconstant (ever-segregating) mottling with color genes (Lamprecht 1932a, 1939; Prakken 1940-1941; Shaw and Norton 1918; Tschermak 1912). According to Prakken (1974), the "complex <i>C</i> locus" includes 6 tightly linked loci, including <i>M</i> , <i>Pr</i> , <i>Acc</i> , <i>C/c</i> , <i>R</i> , and <i>C<sup>st</sup></i> .
<i>c<sup>cr</sup></i>	superscript cr, <i>completely recessive</i> : the heterozygote <i>C/c<sup>cr</sup></i> shows the pure dark pattern color <i>C/C</i> , without mottling as in <i>C/c</i> and <i>C/c<sup>u</sup></i> (Nakayama 1965).
<i>C<sup>cir</sup></i>	superscript cir, <i>circumdatus</i> (Latin): lateral accumulation of medium sized spots on the testa (Lamprecht 1947a).
<i>C<sup>ma</sup></i> ( <i>M</i> , <i>R<sup>ma</sup></i> )	responsible for constant (not heterozygosity dependent) (superscript ma) <i>marbling</i> of the seed coat; the colors depend on other genes (Emerson 1909a; Shull 1908; Smith 1939, 1947; Tschermak 1912). Later interpreted to be an allele of <i>R</i> and re-designated <i>R<sup>ma</sup></i> (Lamprecht

- 1947a). *M* was originally used by Shull (1908) for inconstant mottling. *M* with *Ro* and *V* produces marbling of the pod (Lamprecht 1940a, 1951b). According to Prakken (1974), *C*, *R*, and *M* are 3 distinct but very closely linked loci that are included in the "complex *C* locus."
- C<sup>r</sup>* indistinct, inconstant mottling of the seed coat (Lamprecht 1940a, 1947a; Smith 1939).
- C<sup>res</sup>* superscript res, *resperus* (Latin): sprinkled or speckled seed coat (Lamprecht 1940a, 1947a).
- C<sup>rho</sup>* superscript rho, *rhomboidus* (Latin): rhomboid spotting of the testa (Lamprecht 1947a; Troy and Hartman 1978).
- C<sup>st</sup>* superscript st, *striping* on seed coat and pod (Kooiman 1931; Lamprecht 1939; Sirks 1922; Smith 1939; Tjebbes and Kooiman 1919b; Tschermak 1912); considered by Lamprecht (1947a) to be due to *R<sup>st</sup>*. The *C<sup>st</sup>* allele in 'La Gaude' has the pleiotropic effect of producing blackish violet zebra-like veins on the standard petal of the flowers (Prakken 1977a).
- [*C<sup>st</sup> R Acc*] (*Aeq*) with *v*, also "darkens" the tip of the banner petal (Prakken 1972b and 1974), i.e., the otherwise white standard has a red tip; the genes *R* and *Acc* are tightly linked within the "complex *C* locus" (Prakken 1974); the *Terminalverstärkung der Blütenfarbe* character of Lamprecht (1961a) does not require his *Uc*, *Unc* genes to account for its highly variable penetrance (color intensity).
- c<sup>u</sup>* (*inh*, *i<sub>c</sub>*) superscript u, *unchangeable*: produces a creamish testa (Feenstra 1960); the modifier genes *G*, *B*, and *V* do not change the pale background color of *P J c<sup>u</sup>* (Prakken 1970). With *v<sup>lsc</sup>*, *c<sup>u</sup>* blocks production of flavonol glycosides; with *V*, *c<sup>u</sup>* blocks production of flavonol glycosides and anthocyanin (Feenstra 1960).
- [*c<sup>u</sup> Prp<sup>i</sup>*] (*Prp*, *c<sup>iii</sup>*, *Nud*) with *T P V* produces cartridge buff seed coats, with very tight genetic linkage to a syndrome of anthocyanin (superscript i) *intensification* effects: *purple* flower buds, *intense purple* flowers, *purple* pods, *purple* petioles and stems, and a blush of *purple* on leaf lamina as found in 'Royal Burgundy' (Bassett 1994a; Kooiman 1931); a series of purple pod "alleles" exist at the complex *C* locus (Bassett 1994a; Okonkwo and Clayberg 1984). The same anthocyanin intensification syndrome has been reported repeatedly (but incompletely), each time with a new gene symbol: *Nud* by Lamprecht (1935e), *c<sup>iii</sup>* by Nakayama (1964), and *Prp* by Okonkwo and Clayberg (1984).
- [*c<sup>u</sup> prp<sup>st</sup>*] (*prp<sup>st</sup>*) with *T P V* produces cartridge buff seed coats with very tight genetic linkage to green pods with *purple* (superscript st) *stripes* as found in Contender (Bassett 1994a).
- [*C Prp*] (*Prp*, *Ro*) with *T P J B V* produces black seed coats and purple pods as found in 'Preto 146' (Bassett 1994a).
- c<sup>v</sup>* a completely recessive *c* that does not show heterozygous mottling and has no effect on seed coat color except with *V*, producing a grayish brown with *G B V* (Bassett 1995b).
- [*C R*] (*R*) with *P*, produces a *red* seed coat (Emerson 1909b; Lamprecht 1935a; Tjebbes and Kooiman 1921) that has been variously described as light vinaceous (Tjebbes and Kooiman 1921), light purple vinaceous (Lamprecht 1947a), and deep oxblood red (Smith 1939), the differences possibly due to modifying genes. The flowers are red (Tjebbes and Kooiman 1922b). It does not affect the color of the hilum ring (Lamprecht 1939). *R*, *R<sup>cir</sup>*, *R<sup>r</sup>*, *R<sup>res</sup>*, *R<sup>rho</sup>*, and *r* are allelic, according to Lamprecht (1947a); but Prakken (1977b) has shown that *C<sup>st</sup>* patterns can exist without the *R* locus red color. Therefore, the striping, marbling, and other patterns are more correctly designated as properties of the *C* locus, and the bracket notation, [*C R*], is used to indicate two genes with nearly unbreakable linkage (Bassett 1991b). The interactions of [*C R*] with other genes controlling seed coat color were summarized by Prakken (1972b).
- [*C r*] (*r*) with appropriate modifier genes gives white seed coat (Emerson 1909b; Lamprecht 1940a, 1947a).
- Ca* *caruncula* (Latin): expresses a stripe pattern, originating at the caruncula and extending away from the hilum (Lamprecht 1932c and 1934a).
- Cam* confers temperature sensitive resistance to *cowpea aphid-borne mosaic virus*. Tightly linked, if not identical, to the *I* gene for resistance to bean common mosaic virus (Kyle and

- Provvidenti 1987; Provvidenti et al. 1983).
- Cav* *Caruncula verruca* (Latin): causes a wrinkling of the testa radiating from the caruncula (Lamprecht 1955). The heterozygote is less distinct.
- cc* *chlorotic cup* leaf mutation (Nagata and Bassett 1984).
- chl* pale green *chlorophyll* deficiency (Nakayama 1959a).
- cl* *circumlineatus* (Latin): in partly colored seed coats, each of the color centers and even the smallest dots are bordered by (circumlineated) a sharp precipitation-like line (Prakken 1972b).
- cml* *chlorotic moderately lanceolate* leaf mutant (Bassett 1992c).
- Co-1 (A)* an anthracnose [*Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams.-Scrib.] resistance gene discovered by McRostie (1919) and found in the Andean variety Michigan Dark Red Kidney. *Co-1* is located (Kelly et al. 2003) on Pv01 (Zuiderveen et al., 2016). The gene symbol base *Co* was proposed for all anthracnose resistance genes by Kelly and Young (1996). A comprehensive review of the genetics of anthracnose resistance in common bean is available (Kelly and Vallejo 2004; Ferreira et al., 2013).
- Co-1<sup>2</sup>* an anthracnose resistance gene discovered by Melotto and Kelly (2000) and found in ‘Kaboon’.
- Co-1<sup>3</sup>* an anthracnose resistance gene discovered by Melotto and Kelly (2000) and found in ‘Perry Marrow’.
- Co-1<sup>4</sup>* an anthracnose resistance gene discovered by Alzate-Marin et al. (2003a) and found in AND277 (Gonçalves-Vidigal et al. 2011).
- Co-1<sup>5</sup>* an anthracnose resistance gene from ‘Widusa’ discovered by Goncalves-Vidigal and Kelly (2006).
- Co-2 (Are)* an anthracnose resistance gene discovered by Mastenbroek (1960) and found in the Middle American differential variety Cornell 49242. *Co-2* is located on Pv11 (Adam-Blondon et al. 1994).
- Co-3 (Mexique 1)* an anthracnose resistance gene discovered by Bannerot (1965) and found in the Middle American variety Mexico 222. *Co-3* is located on Pv04 (Rodríguez-Suarez et al. 2004).
- Co-3<sup>2</sup>* an anthracnose resistance gene found in the Middle American variety Mexico 227 (Fouilloux 1979).
- Co-3<sup>3</sup>* an anthracnose resistance gene first described by Geffroy et al. (1999) in the variety BAT93. The *Co-3<sup>3</sup>* gene was previously named *Co-9* and subsequently found to be an allele of *Co-3* (Gonçalves-Vidigal et al., unpublished; Mendez-Vigo et al. 2005; Rodríguez-Suárez et al. 2004). *Co-3<sup>3</sup>* is also present in the differential variety PI 207262 (Alzate-Marin et al. 2003c) and is located on Pv04 (Geffroy et al. 1999).
- Co-3<sup>4</sup>* an anthracnose resistance gene previously named *Co-10* and described in the variety Ouro Negro (Alzate-Marin et al., 2003b). Tightly linked to *Phg-3* and located on Pv04 (Gonçalves-Vidigal et al. 2013).
- Co-3<sup>5</sup>* an anthracnose resistance gene previously named *Co-7* and described in the Middle American differential variety G2333 (Young et al. 1998). The allele is located on Pv04 (Sousa et al., 2014).
- Co-4 (Mexique 2)* an anthracnose resistance gene discovered by Bannerot in 1969 (Fouilloux 1976, 1979) and found in the Middle American differential variety TO. *Co-4* is located on Pv08 (Kelly et al. 2003; Oblessuc et al., 2015).
- Co-4<sup>2</sup>* an anthracnose resistance gene found in SEL 1308 and G2333 (Young et al. 1998).
- Co-4<sup>3</sup>* an anthracnose resistance gene found in PI 207262 (Alzate-Marin et al. 2002).
- Co-5 (Mexique 3)* an anthracnose resistance gene discovered by Bannerot in 1969 (Fouilloux 1976, 1979) and found in the Middle American differential variety TU and G2333, SEL 1360 (Young et al. 1998).
- Co-5<sup>2</sup>* an anthracnose resistance allele of *Co-5* found in G 2333, SEL 1360 and MSU 7-1 (Vallejo and Kelly 2009; Sousa et al. 2014).
- Co-6* an anthracnose resistance gene discovered by Schwartz et al. (1982) and found in the Middle



- American differential variety AB136. *Co-6* is located on Pv07 (Kelly et al. 2003; Mendez de Vigo 2002). It is likely that *Co-6* is located within *Co-5* gene cluster (Campa et al., 2017).
- co-8* an anthracnose resistance gene first described in differential variety AB136 (Alzate-Marin et al. 1997).
- Co-11* is an anthracnose resistance gene from ‘Michelite’ (Gonçalves-Vidigal et al. 2007).
- Co-12* an anthracnose resistance gene from cultivar ‘Jalo Vermelho’ described in Gonçalves-Vidigal et al. (2008).
- Co-13* an anthracnose resistance gene from landrace ‘Jalo Listras Pretas’ described in Gonçalves-Vidigal et al. (2009) is located on Pv03 (Lacanallo and Gonçalves-Vidigal 2015).
- Co-14* an anthracnose resistance gene from cultivar ‘Pitanga’ described in Gonçalves-Vidigal et al. (2012) was mapped to Pv01 (Gonçalves-Vidigal et al., 2016).
- Co-15* anthracnose resistance gene from Corinthiano on Pv04 linked to STS marker g2685 described in Sousa et al. (2015).
- Co-16* anthracnose resistance gene from Crioulo 159 on Pv04. Likely distinct from *Co-3* (Coimbra-Gonçalves et al. 2016).
- Co-17* anthracnose resistance gene from SEL1308 on Pv03 (Trabanco et al., 2015). Line SEL1308, derived from a backcross of cultivar G2333 (Talamanca\*2/G2333; Young and Kelly, 1996).
- cr-1 cr-2* complementary recessive genes for crippled morphology, i.e., stunted plants with small, crinkled leaves (Coyne 1965; Finke et al. 1986).
- Crg* this *complements resistance gene* is a factor necessary for the expression of *Ur-3*-mediated bean rust resistance and is located on Pv08 (Kalavacharla et al. 2000).
- cry* *crypto-dwarf*: a dwarfing gene; with *Fin* intermediate height (Nakayama 1957); with *la* produces long internodes resulting in slender type of growth in bush (*fin*) but not in tall (*Fin*) forms (Lamprecht 1947b).
- cs* *chlorotic stem* mutant (Nagata and Bassett 1984).
- Ct* for *curved pod tip* shape; *ct* for straight pod tip (Al-Muktar and Coyne 1981).
- ctv-1 ctv-2* confer resistance to beet *curly top virus* (Schultz and Dean 1947); symbol proposed by Provvidenti (1987).
- Da* straight pod (Lamprecht 1932b).
- Db* polymeric with *Da* for straight pod (Lamprecht 1932b, 1947b). [Polymeric genes have identical functions (expression) but different loci].
- dgs (gl, le)* *dark green savoy* leaf mutant (Frazier and Davis 1966b; Nagata and Bassett 1984). According to Nagata and Bassett (1984), *dgs* is synonymous with the *wrinkled leaf* mutant of Moh (1968) and the *gl (glossy)* of Motto et al. (1979); also synonymous with the *le (leathery leaf)* of Van Rheenen et al. (1984).
- dia* *diamond* leaf mutant (Nagata and Bassett 1984). Leaflets are angular, slightly chlorotic, thick, and reduced in area.
- Diap-1* structural gene for *diaphorase* enzyme (Weeden and Liang 1985).
- Diap-2* structural gene for *diaphorase* enzyme (Sprecher 1988).
- diff* *diffundere* (Latin): with *exp* gives completely colored testa except for one end of the seed; *diff* with *Bip Arc* gives maximus phenotype, with *bip Arc* gives major phenotype; extends seed coat color in partly colored seeds (Lamprecht 1940b).
- dis* *dispare* (Latin): mottled or striped flower of scarlet runner bean (Lamprecht 1951c).
- Dl-1 Dl-2 (DL<sub>1</sub> DL<sub>2</sub>)* complementary genes for *dosage-dependent lethality* and developmental abnormality; *Dl Dl Dl-2 Dl-2* is lethal, *Dl dl Dl-2 Dl-2* and *Dl Dl Dl-2 dl-2* are sublethal, *Dl dl Dl-2 dl-2* is temperature dependent abnormal, and *Dl Dl dl-2 dl-2*, *dl dl Dl-2 Dl-2*, *Dl dl dl-2 dl-2*, *dl dl Dl-2 dl-2*, and *dl dl dl-2 dl-2* are normal; *Dl* inhibits root development and *Dl-2* inhibits shoot development (Shii et al. 1980). *Dl-1* is located on linkage group 11 and *Dl-2* is located on linkage group 2 (Hannah et al., 2007).
- do* *dwarf out-crossing* mutant (Nagata and Bassett 1984). Out-crossing rates up to 56% are observed due to delayed pollen dehiscence (Nagata and Bassett 1985).
- ds (te)* *dwarf seed*: produces small seeds and short pods with deep constrictions between the seeds;

- cross pollination with *Ds* gives normal size seeds and pods on *ds/ds* plants, breaking the usual dominance of maternal genotype over embryo genotype for seed size development (Bassett 1982); the xenia effect was first described by Tschermak (1931) and the trait was named *tenuis* (Latin) for "narrow" pod by Lamprecht (1961a).
- dt-1<sup>a</sup> dt-2<sup>a</sup>* *daylength temperature*: produce early, day-length neutral flowering with complex temperature interactions (Massaya 1978).
- dt-1<sup>b</sup> dt-2<sup>b</sup>* *daylength temperature*: control flowering response to short days with complex temperature interactions; *dt-2<sup>b</sup>* causes increased production of branches (Massaya 1978).
- dw-1 dw-2* duplicate genes causing *dwarf* plant (Nakayama 1957).
- Ea Eb* polymeric genes for "flat" pod, elliptical in cross-section vs. *ea eb* round pod (Lamprecht 1932b, 1947b; Tschermak 1916).
- Est-1* structural gene for most anodal *esterase* enzyme (Weeden and Liang 1985).
- Est-2* structural gene for second most anodal *esterase* enzyme (Weeden and Liang 1985).
- exp* *expandere* (Latin): with *diff* gives solid color to seed coat except for one end of the seed, giving minimus and minor phenotypes (Lamprecht 1940b).
- F* confers resistance to the *F* strain of anthracnose found in variety Robust (McRostie 1919); 'Robust' is extinct, but it was a parent of variety Michelite, which has not been fully characterized for anthracnose resistance although close to *Co-1* type (Kelly, personal communication).
- Fa* basic gene for pod membrane (Lamprecht 1932b).
- fast* *fastigate* shape of seed (Lamprecht 1934a).
- Fb Fc* supplementary genes for pod membrane (Lamprecht 1932b).
- fa fb fc* weak pod membrane; pod may be constricted (Lamprecht 1932b); may give 9:7, 15:1, or 63:1 ratios (Lamprecht 1932b, 1947b).
- fd* *delayed flowering* response under long days (Coyne 1970).
- Fe-1 Fe-2* *Ferrum* (Latin): complementary dominant genes controlling resistance to leaf chlorosis due to iron deficiency in plants grown on calcareous soils (Coyne et al. 1982; Zaiter et al. 1987).
- Fib* *fibula* arcs, with *t*, white arcs (bows) expressed in the corona zone of seed coats, together with *expansa* partly colored pattern (Bassett 2001; Bassett and McClean 2000).
- Fin (fin)* *Finitus* (Latin): indeterminate vs. *fin* determinate plant growth (Lamprecht 1935b; Rudorf 1958); long vs. short internode; later vs. earlier flowering. *Fin* is 1 cM from *Z* (Bassett 1997c) and located on Pv01 (Koinange et al. 1996; Freyre et al. 1998).
- Fop-1* confers resistance to the Brazilian race of *Fusarium oxysporum* f. sp. *phaseoli* (Ribeiro and Hagedorn 1979).
- Fop-2* confers resistance to the U.S. race of *Fusarium oxysporum* f. sp. *phaseoli* (Ribeiro and Hagedorn 1979).
- Fr* a *fertility restoring* gene (Mackenzie and Bassett 1987) for the cytoplasmic male sterility source derived from CIAT accession line G08063 (Bassett and Shuh 1982). Restoration is partial in F<sub>1</sub>, complete and irreversible in fertile F<sub>2</sub> segregants, i.e., the gene alters the mitochondrial DNA, deleting a fragment of at least 25 kilobases in restored plants (Mackenzie et al. 1988; Mackenzie and Chase 1990).
- Fr-2* a *fertility restoring* gene that is derived from CIAT accession line G08063 and that restores fertility without deleting the same mitochondrial DNA fragment affected by *Fr* (Mackenzie 1991).
- G (Flav, Ca, Och)* The yellow-brown factor of Prakken (1970). The equivalent of *C* of Shaw and Norton (1918). Prakken (1970) believed that Lamprecht (1951a) genes *Flav*, *Ca*, and *Och* are synonyms for *G*. The interactions of *G* with other combinations of seed coat color genes are summarized by Prakken (1972b). *G* is located on Pv04 (McClean et al. 2002).
- Ga* *gametophyte* factor, which achieves complete selection for pollen carrying *Ga*, i.e., no pollen carrying *ga* achieves fertilization (Bassett et al. 1990).
- gas* *gamete-sterile*: causes both male and female sterility (Lamprecht 1952b).
- glb* *glossy bronzing* leaf mutant (Bassett 1992c).

- Gpi-c1* structural gene for *glucose phosphate isomerase* enzyme, i.e., the more anodal of the two *cytosolic* isozymes (Weeden 1986).
- Gr* in the presence of *ih*, produces *green* dry pod color; in the presence of *Ih*, produces tan dry pod color; *gr* in the presence of *ih* or *Ih*, produces tan dry pod color (Honma et al. 1968).
- gy* *greenish yellow* seed coat, usually with *P [C r]* *gy J g b v* (or *v<sup>lae</sup>*) *Rk* of the Mayocoba market class, but also expressed with *G b v* or *G B v* (Bassett et al. 2002a). A second gene (tentative symbol *Chr*) is necessary to express greenish yellow color in the corona (with *g b v<sup>lae</sup>*) and hilum ring with *g b v<sup>lae</sup>* or *g b v* (Bassett 2003c). *Gy* is either closely linked to *C* or is part of the ‘complex *C* locus’ on Pv08 (Bassett et al. 2002a).
- Hbl* (*L<sub>HB-1</sub>*) controls expression of *halo blight* tolerance in *leaves* (Hill et al. 1972).
- Hbnc* (*SC<sub>HB-1</sub>*) controls expression of *halo blight* tolerance resulting in *nonsystemic chlorosis* of leaves (Hill et al. 1972).
- Hbp* (*PD<sub>HB-1</sub>*) controls expression of *halo blight* tolerance in *pods* (Hill et al. 1972).
- hmb* controls expression of sensitivity to the *herbicide metobromuron*, where *Hmb* expresses metobromuron insensitivity (Park and Hamill 1993).
- Hss* *hypersensitivity soybean*: confers a rapid lethal necrotic response to soybean mosaic virus (SMV) that is not temperature sensitive (Kyle and Provvidenti 1993).
- Hsw* *hypersensitivity watermelon*: confers temperature sensitive resistance (lethal hypersensitivity) to watermelon mosaic virus 2. Very tightly linked, if not identical, to the *I* gene for bean common mosaic virus (Kyle and Provvidenti 1987).
- Ht-1 Ht-2* (*L-1 L-2*) genes of equal value for height of plant (Norton 1915). They also increase length of seed (Frets 1951).
- I* confers temperature sensitive resistance to bean common mosaic virus. Tightly linked, if not identical, to *Bcm*, *Cam*, *Hsw*, and *Hss* (Ali 1950; Kyle et al. 1986; Kyle and Provvidenti 1993). The *I* gene (or the complex *I* region) conditions resistance and/or lethal necrosis to a set of nine potyviruses, BCMV, WMV, BICMV, CABMV, AzMV, ThPV, SMV, PWV-K, and ZYMV (Fisher and Kyle 1994). *I* has a nearly terminal position on Pv02 (Vallejos et al. 2000).
- Ia Ib* parchmented vs. *ia* tender pod (Lamprecht 1947b). Flat or deep (elliptical cross-section) vs. round pod (Lamprecht 1932b, 1947b, 1961a).
- ian-1 ian-2* (*ia*) *indehiscent anther* where the heterozygote produces partial indehiscence (Wyatt 1984); currently, two unlinked mimic genes can produce indehiscent anther (Wyatt, personal communication).
- lbd* *leaf-bleaching dwarf* mutant (Bassett 1992c).
- ico* *internodia contracta* (Latin): internodes 4-7 cm long instead of the normal 8-11 cm (Lamprecht 1961b).
- Igr* (*Ih*) *inhibits* the action of *Gr*, conferring tan dry pod color in the presence of *Gr* or *gr* (Honma et al. 1968).
- ilo* *inflorescentia longa* (Latin): 5-7 long internodes in the inflorescence instead of the usual 2-3 (Lamprecht 1961b).
- ip* (*i<sub>1</sub>*) *inhibits* the action of *P* with respect to the color of the hypocotyl (Nakayama 1958).
- iter* *iteratus-ramifera* (Latin): with *ram* produces triple branched inflorescence (Lamprecht 1935b, 1935d).
- iv* (*i<sub>2</sub>*) *inhibits* the action of *V* with respect to the color of the hypocotyl; is lethal with *v<sup>lae</sup>* (Nakayama 1958).
- iw* *immature white* seed coat in the presence of *p* (Baggett and Kean 1984).
- J* (*Sh*) With *P*, gives light yellow-brown or pale ochraceous buff testa (Lamprecht 1933), Rohseidengelb testa (Lamprecht 1939), raw silk testa (Lamprecht 1932a, 1951a) and the same color to the hilum ring (Lamprecht 1951a; Prakken 1934). The equivalent of the *Sh* of Prakken (1934) (Lamprecht 1960; Prakken 1970). Similar to *Asp* (Lamprecht 1940c) only in seed coat shininess (Bassett 1996b). It causes seed coats to glisten and to darken with age (Lamprecht 1939). *J* is linked to *Bip* and is located on Pv10 (McClellan et al. 2002).

- j (mar)* Expresses “immature” seed coat colors, viz., paler and highly variable (seed to seed) along the ventral (darker relative to dorsal) to dorsal surface transition, for whatever combination of other seed coat color genes are present (Bassett 1996b; Prakken 1972b). *j* produces dull (mat) seed coat (Prakken 1940-41), nearly white corona with *Z*, and nearly white corona and hilum ring with *z* (Bassett 1996b; Bassett et al. 1996b). Same as *mar* of Lamprecht (1933) for a broad band of color about the hilum. With *j*, no leuco-anthocyanidins are synthesized and production of anthocyanins and flavonol glycosides is low (Feenstra 1960).
- j<sup>ers</sup> (ers-2)* The *j<sup>ers</sup>* allele (from ‘Early Wax’) differs from *j* expression: *T Z j<sup>ers</sup>* fails to express the margo pattern of *T Z j*, *T z j<sup>ers</sup>* fails to express the margo *z* pattern of *T z j*, and *t Z j<sup>ers</sup>* fails to express marginata of *t Z j*; but *t z j<sup>ers</sup>* and *t z j* express white seed coats (Bassett 1997d; Bassett et al. 2002b). *T/t z/z j/j<sup>ers</sup>* in a *P C J G B V* background expresses reverse margo pattern (Bassett et al. 2002b).
- Ke* potassium utilization efficiency (Shea et al. 1967).
- la* *Lamm*: with *cry* gives long internode; *la* with *Fin* is dwarf; *la cry fin* is slender (Lamprecht 1947b).
- Lan* lanceolate leaf mutant; *Lan/Lan* is usually a zygotic lethal, and survivors are dwarfs that do not flower; *Lan/lan* segregates 2:1 (lanceolate to normal) in selfed progeny (Bassett 1981).
- Ld* leaf distortion resembling phenoxy herbicide injury, with interveinal clearing, slight chlorosis, necrotic scarring of the midrib, altered leaf shape, and extra leaflets (Rabakoarihanta and Baggett 1983).
- Lds (Ds)* *Ld suppressor* (Rabakoarihanta and Baggett 1983).
- Lec* structural gene for the seed protein lectin or phytohemagglutinin (Osborn et al. 1986).
- Li (L)* long vs. li short internodes (Lamprecht 1947b; Norton 1915).
- lo* plants have a short inflorescence (Lamprecht 1958).
- lr-1 lr-2* the double recessive genotype produces leaf rolling of trifoliolate leaves through the third or fourth nodes, ending in stem and apical necrosis and death of the plant (Provvidenti and Schroeder 1969).
- Me* structural gene for malic enzyme (Weeden 1984).
- Mel (Me)* confers nematode resistance to *Meloidogyne incognita* (some isolates of race 1), *M. javanica*, and *M. arenaria* (Omwega et al. 1990).
- Mel-2 (Me-2)* confers nematode resistance to *Meloidogyne incognita* race 1 (isolates to which *Mel* is susceptible), race 2 and race 3, but is susceptible to *M. javanica* and *M. arenaria* (Omwega and Roberts 1992).
- mel-3 (me-3)* confers temperature sensitive nematode resistance (resistant at 26 C but susceptible at 28 C) to the same species, races, and isolates as with *Mel-2* (Omwega and Roberts 1992).
- Mf* *mancha na flor* (Portuguese): brownish-violet blotch on the base of the standard flower petal (Vieira and Shands 1969).
- mi, mia* micropylar stripe pattern (Lamprecht 1932c and 1934a); both 3:1 and 15:1 segregation were observed.
- Mic (Mip)* *micropyle inpunctata* (Latin): small dots near the micropyle (Lamprecht 1940c).
- miv* *minor intervallis* (Latin): end of seed flattened and a short distance between funicles (Lamprecht 1952a).
- Mrf* *Mosaico rugoso del frijol* (Portuguese): confers immunity to bean rugose mosaic virus (Machado and Pinchinat 1975).
- Mrf<sup>2</sup>* *Mosaico rugoso del frijol* (Portuguese): confers the localized lesion type of resistance to bean rugose mosaic virus; the order of dominance in the allelic series is *Mrf*<sup>></sup>*Mrf<sup>2</sup>*<sup>></sup>*mrf* (Machado and Pinchinat 1975).
- mrf* *mosaico rugoso del frijol* (Portuguese): confers susceptibility (systemic infection) to bean rugose mosaic virus (Machado and Pinchinat 1975).
- ms-1* an induced mutant for genic male sterility, where no pollen is produced but female fertility is unimpaired (Bassett and Silbernagel 1992).
- Mue* structural gene for methylumbelliferyl esterase (Garrido et al. 1991).

- mu* *mutator* locus that produces mutations of *us* to *Us*, thus giving normal green leaf sectors in yellow leaves due to *us mu*, where the ratio of normal to variegated plants is 15:1 (Coyne 1966).
- Nag* structural gene for *N-acetyl glucoseaminidase* enzyme (Weeden 1986).
- Nd-1 Nd-2 (D-1 D-2)* additively control the variation in *node* number on the main stem of determinate beans and additively control the number of days to flowering (Evans et al. 1975).
- nie* an induced mutation for *ineffective nodulation* by *Rhizobium* (Park and Buttery 1994).
- nnd (sym-1)* an induced mutation for *non-nodulation* by *Rhizobium*, i.e., lacking capacity for *symbiosis* (Pedalino et al. 1992).
- nnd-2* an induced mutation for *non-nodulation* by *Rhizobium* (Park and Buttery 1994).
- No* with *P v*, expresses Light *Nopal* Red (light salmon with brownish tinge) flower color and much darker reddish color of flower buds by pleiotropic action; with *P V*, expresses Pure *Nopal* Red flower; *No* action is fully dominant; *No* is linked (31 cM) to *Fin* (Lamprecht 1948b, 1961a). Allelism of *No* with *Sal* was not tested (Bassett 2003b).
- nts (nod)* *nitrogen tolerant supernodulation*: an induced mutation that permits abundant nodulation in the presence of high nitrogen (Park and Buttery 1989).
- ol* *overlapping leaflets* mutant (Bassett 1992c).
- P* basic color gene (Emerson 1909a; North and Squibbs 1952; Prakken 1934; Schreiber 1934; Shaw and Norton 1918; Shull 1908; Skoog 1952). *P* without color genes is colorless as is *p* (Lamprecht 1939; Smith 1939). According to Feenstra (1960), *P* is the equivalent of the *A* of Tschermak (1912), of Kooiman (1920), and of Sirks (1922). *P* is located on Pv07 (Erdmann et al. 2002; Koinange et al. 1996; Vallejos et al. 1992) with physical position reported by McClean et al., (2018) as a member of clade B of subclass IIIf of plant basic helix–loop–helix (bHLH) proteins.
- p<sup>sd</sup>* is an allele of the *P* (Pigment) gene with order of dominance  $P > P^{sd} > p$  (Islam et al., 2020). It replaces the *sd* gene (symbol) that conditions the slow darkening seed coat trait in pinto (Elsadr et al. 2011) and carioca beans (Alvares et al., 2019).
- p* white seed coat and flower (Emerson 1909a). Ten gene pool-specific *p* alleles conditioned the white seed phenotype, and each causative mutation affected at least one bHLH domain required for color expression (McClean et al., 2018).
- p<sup>gri</sup> (Gri, v<sup>pal</sup>)* superscript *gri*, *griseoalbus* (Latin): *p<sup>gri</sup>* with *C J B V* produces grayish white (blubber white) seed coat without a hilum ring, giving the dominance order  $P > p^{gri} > p$  (Bassett 1994b; Lamprecht 1936); *p<sup>gri</sup>* with *C J B V* produces flowers with very pale lavender wing petals and two dots of violet on the upper edge (center) of an otherwise near white standard petal (Bassett 1992b); formerly a second basic color factor like *P* (Lamprecht 1936). Lamprecht (1936) speculated that the flower color observed with *p<sup>gri</sup>* segregation must be due to an undiscovered new allele (tentatively *v<sup>pal</sup>*) at *V*. *p<sup>stp</sup>* superscript *stp*, *stippled* seed coat and white flowers with a narrow, violet banner tip and pale violet periphery (2-3 mm) on the wing petals (Bassett 1996a, 2003a).
- p<sup>hbw</sup>* stippled seed coat (different from *p<sup>stp</sup>*) and violet flowers with the lower (superscript *hbw*) half of the banner petal white (Bassett 1996a, 2003a).
- p<sup>mic</sup>* self-colored seed coat except for a white (superscript *mic*) *micropyle* stripe and violet flowers without pattern (Bassett 1998, 2003a).
- pa* *pale* green leaves (Smith 1934).
- pc* *persistant* green pod color (Dean 1968).
- pg (pa<sub>1</sub>)* *pale-green* foliage mutant (Wyatt 1981).
- Pha* structural gene for the seed protein *phaseolin* (Osborn et al. 1986).
- Phg-1* confers resistance to angular leaf spot in the common bean cultivar AND 277. *Phg-1* is linked to *Co-1<sup>4</sup>* on Pv01 (Carvalho et al. 1998; Gonçalves-Vidigal et al. 2011).
- Phg-2* confers resistance to angular leaf spot from Mexico 54 and is located on Pv08 (Sartorato et al. 2000; Namayanja et al. 2006).
- Phg-2<sup>2</sup>* confers resistance to angular leaf spot from BAT332 (Namayanja et al. 2006).

- Phg-3* confers resistance to angular leaf spot from Ouro Negro and located on Pv04 (Corrêa et al. 2001; Faleiro et al. 2003; Gonçalves-Vidigal et al. 2013).
- Phg-4* confers resistance to angular leaf spot previously reported as the major QTL ALS4.1 on Pv04, present in the common bean line G5686 (Mahuku et al. 2009; Keller et al. 2015; Souza et al. 2016).
- Phg-5* confers resistance to angular leaf spot previously reported as the major QTL ALS10.1 on Pv10, present in the common bean lines G5686 and CAL143 (Oblessuc et al. 2012, 2013; Keller et al. 2015; Souza et al. 2016).
- Pkp-1* resistance from PI 181996 to soybean rust (SBR), caused by the fungus *Phakopsora pachyrhizi* (Souza et al. 2014).
- Pmv* confers incomplete dominance for resistance to *peanut mottle virus* (Provvidenti and Chirco 1987).
- ppd (neu)* *photoperiod-insensitive* gene found in 'Redkloud' with a syndrome of effects (Wallace et al. 1993); an allele-specific associated primer is now available for *ppd* (Gu et al. 1995); probably the same locus as *Neu*<sup>+</sup> for short day vs. *neu* for day *neutral* flowering response to length of day of Rudorf (1958). The red/far-red photoreceptor gene PHYTOCHROME A3 (PHYA3) was identified as the *ppd* gene on Pv01 (Kamfwa et al., 2015; Weller et al., 2019).
- Pr* *Preventing* the "flowing out" of red color (Prakken 1972b, 1974); *pr* with pattern alleles at *C* and *R* allow the red color in the dark pattern color zones to "flow out" into the light pattern color areas, producing various light red hues such that the contrast between the dark and light pattern colors is very small; tightly linked to the *C* locus.
- Prp*<sup>i</sup>-2 a gene controlling (superscript i) *intensified* anthocyanin (*purple*) expression syndrome (not linked to *C*) in flower buds, corolla, pods, stems and leaf lamina (Bassett 2005).
- prc (pc)* *progressive chlorosis* mutant (Nagata and Bassett 1984); redesignated *prc* (Awuma and Bassett 1988).
- Prx* structural gene for *peroxidase* enzyme, i.e., the most cathodal of the peroxidase isozymes (Weeden 1986).
- Pse-1 (R1)* a halo blight resistance gene described by Walker and Patel (1964) and reported as the *R1* gene by Teverson (1991) and Taylor et al. (1996); present in the halo blight differential variety Red Mexican UI-3. *Pse-1* is located on linkage group 10 and confers resistance to pathogen races 1, 5, 7 and 9 (Miklas et al., 2009).
- Pse-2 (R2)* a halo blight resistance gene described by Teverson (1991) and Taylor et al. (1996) as present (as *R2*) in the halo blight differential variety A43 (ZAA12). Confers resistance to races 2, 3, 4, 5, 7, 8, and 9 and is located on Pv10 (Miklas et al. 2011).
- Pse-3 (R3)* a halo blight resistance gene described by Teverson (1991) and Taylor et al. (1996) as present (as *R3*) in the halo blight differential variety Tendergreen. *Pse-3* confers hypersensitive resistance response to races 3 and 4 and is completely linked with the *I* gene locus on Pv02 (Fourie et al. 2004; Teverson 1991).
- Pse-4 (R4)* a halo blight resistance gene discovered by Teverson (1991) and Taylor et al. (1996) to be present (as *R4*) in the halo blight differential variety Red Mexican UI-3.
- pse-5 (R5)* a halo blight resistance gene described by Teverson (1991) and Taylor et al. (1996) as present (as *R5*) in the halo blight differential variety A43 (ZAA12) and conditioning recessive resistance to race 8. Miklas et al. (2011) observed that this gene cosegregated with *Pse-2* on Pv10.
- Pse-6* a halo blight resistance gene identified in BelNeb-RR-1, conditioning resistance to races 1, 5, 7 and 9 and located on Pv04 (Miklas et al. 2014).
- punc* *punctatus* (Latin): causes dotting of the testa (Lamprecht 1940c).
- ram* *ramifera* (Latin): branched inflorescence (Lamprecht 1935b).
- Rbcs (rbcS)* *small* subunit of the *rubisco* enzyme (Weeden 1984).
- rf-1* *reclining foliage* due to downward slanting petioles (Bassett 1976). *Rf-1* is linked (11 cM) to *V* (Bassett 1997a), and *V* is located on Pv06 (McClellan et al. 2002).

- rf-2* *reclining foliage* mutant due to downward slanting petioles (Bassett and Awuma 1989).
- rf-3* *reclining foliage* mutant due to downward slanting petioles (Bassett and Awuma 1989).
- rfi (i)* *reclining foliage inhibitor*: recessive epistatic factor to *rf-1* and *rf-3* (Bassett 1976; Bassett and Awuma 1989).
- Rfs (m)* *reclining foliage suppressor*: dominant suppressor of *rf-1* (Bassett 1976).
- Rk* *red kidney*: the *Rk* allele does not express testaceous (pink) color of light red kidney beans (Gloyer 1928; Smith 1939) or garnet brown color of dark red kidney beans (Smith and Madsen 1948); interactions of *rk* and *rk<sup>d</sup>* with *C*, *D* (now *Z*, Bassett et al. 1999b), *J*, *B*, and *V* (using Prakken's symbols) were investigated (Smith 1961). According to Prakken (1972b), *Rk* is linked (28 cM) to *B*, which is located on Pv02 (Kyle and Dickson 1988; Vallejos et al. 2000).
- rk* *red kidney*: with *m* or *c* (now *c<sup>u</sup>*), *rk* expresses testaceous (pink) seed coat color; with *M* (red/buff marbled pattern), *rk* modifies cartridge buff expression to testaceous (Smith 1939, 1947); *rk* is dominant over *rk<sup>d</sup>* (Smith and Madsen 1948); *rk* has no expression with *j* (Lamprecht 1961c; Smith 1961).
- rk<sup>d</sup> (lin)* *red kidney* (superscript d) *dark*: with *r* (now *c<sup>u</sup>*) and *J*, *rk<sup>d</sup>* expresses garnet brown testa (Smith and Madsen 1948); *rk<sup>d</sup>* has no expression with *j* (Smith 1961). With *P v* (or *v<sup>lae</sup>*) and either *T/-* or *t/t*, *rk<sup>d</sup>* always gives red veins in the wing petals, whether clear or faint (Prakken 1972a, b); in some genetic backgrounds the red veins are "incompletely recessive", i.e., *Rk/rk<sup>cd</sup>* gives very faint red veins (Prakken 1972b). The red color of red kidney beans (all recessive alleles) is expressed by proanthocyanidins although three yellow flavonol glycosides are also present in the seed coats (Beninger and Hosfield 1999).
- rk<sup>drv</sup>* *red kidney* (superscript drv) *dark red vein*: with *P v*, a spontaneous mutant of the *rk<sup>d</sup>* gene expressing red wing petal veins that are "expanded" (larger in diameter and diffuse) compared to those of *rk<sup>d</sup>*, creating the illusion of pale pink flowers when viewed at one meter or more (Bassett 2004).
- rk<sup>cd</sup>* *red kidney* (superscript cd) *convertible dark*: *C rk<sup>cd</sup>* expresses garnet brown seed coats, whereas *c<sup>u</sup> rk<sup>cd</sup>* expresses pink (testaceous) seed coats; thus, expression at *rk<sup>cd</sup>* (from 'NW 63') is a function of interaction with *C* (Bassett and Miklas 2003).
- rk<sup>p</sup>* *red kidney* (superscript p) *pink*: *rk<sup>p</sup>* (from 'Sutter Pink') expresses consistently very weak pink color under humid growing conditions, unlike *rk* from 'Redcloud' (Bassett and Miklas 2003).
- rn-1 rn-2 (r rN)* together confer resistance to *root-knot nematode*, where 2-4 dominant alleles give susceptible reaction and 1 dominant allele gives intermediate resistance in a 11:4:1 ratio (Barrons 1940).
- rnd* *round* leaf mutant with lateral leaflet tips rounded (Nagata and Bassett 1984).
- Sal* with *P*, *Sal* expresses *salmon* red flower color and a reddish tinge to the testa; scarlet red flower is expressed with *Sal Am Beg No* (Lamprecht 1948b). *Salmon* red flower color (Fan 1, 52C or D; Royal Hort. Soc. fans) is expressed by *Sal am V<sup>wf</sup>* (or *v*), and scarlet flower (Fan 1, 43C; Royal Hort. Soc. fans) is expressed by *Sal Am V<sup>wf</sup>* (or *v*) (Bassett 2003b). *Sal Am v* expressed oxblood red seed coats (vs. mineral brown tinged with red) due either to a pleiotropic effect of *Am* or a very closely linked dominant gene (Bassett 2003b), and *Am* has no expression with *sal* (Bassett 2003b).
- sb* *spindly branch* mutant; the stems are thinner and more highly branched than normal (Awuma and Bassett 1988).
- sb<sup>ms</sup>* *spindly branch* (superscript ms) *male sterile* mutant; allelic with *sb*; anthers are atrophied and produce no viable pollen, but there is no loss of female fertility (Bassett 1991a)
- sb-2* *spindly branch* mutant; the stems are thinner and more highly branched than normal (Bassett 1990).
- sb-3* *spindly branch* mutant; the stems are thinner and more highly branched than normal (Bassett 1990).
- sil* *silver* colored leaves and severe plant stunting under high intensity light in the field; no

	stunting under glasshouse culture (Frazier and Davis 1966a; Nagata and Bassett 1984).
<i>Skdh</i>	structural gene for <i>shikimate dehydrogenase</i> enzyme (Weeden 1984).
<i>sl</i>	<i>stipelless lanceolate</i> leaf mutant (Nagata and Bassett 1984) gives a lanceolate leaf form with loss of stipels from the terminal leaflet.
<i>Smv</i>	confers incompletely dominant resistance to <i>soybean mosaic virus</i> (Provvidenti et al. 1982).
<i>St</i>	<i>stringless</i> pod; <i>st</i> gives a complete string (Prakken 1934); has modifiers.
<i>Sur</i>	<i>Sursum versus</i> (Latin): causes leaves and petioles to point downward (Lamprecht 1937) with pulvinule rotated 180E. See X <sup>su</sup> .
<i>sw-1 sw-2</i>	the double recessive genotype produces <i>seedling wilt</i> (Provvidenti and Schroeder 1969), i.e., epinasty of primary leaves, necrosis of terminal bud, and death of the plant in primary leaf stage.
<i>T</i>	self-colored seed coat and colored flowers (Emerson 1909a; Lamprecht 1934b; Shaw and Norton 1918). <i>T</i> is located Pv09 (McClellan et al. 2002).
<i>t (z-1)</i>	a seed coat pattern gene required for all partly colored seed coat patterns; has pleiotropic expression for white flowers (Schreiber 1934; Shaw and Norton 1918) and green cotyledons and hypocotyls (Prakken, 1972b). Early reports of interactions of <i>t</i> with <i>Z</i> and <i>z</i> (Lamprecht 1934b; Sax 1923; Shaw and Norton 1918) were later extended to <i>t</i> interactions with <i>Z</i> , <i>J</i> , and <i>Bip</i> (Bassett 1994c, 1996b and c, 1997c and d; Bassett et al. 2000, 2002b; Lamprecht 1940b; Schreiber 1940).
<i>t<sup>bp</sup></i>	superscript bp for <i>blue pattern</i> . A seed coat gene from G07262 that conditions blue patterned flowers in the presence of <i>Prp<sup>1</sup>-2</i> described by Bassett and Miklas (2009).
<i>t<sup>cf</sup></i>	superscript cf, <i>colored flower</i> : 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).
<i>Th-1 Th-2</i>	genes of equal value for seed <i>thickness</i> (Frets 1951).
<i>Tm</i>	confers immunity to <i>tobacco mosaic virus</i> (Thompson et al. 1952).
<i>To</i>	cell wall fiber (Prakken 1934).
<i>top</i>	<i>topiary</i> plant architecture; a spontaneous mutant with determinate habit (terminal bud is reproductive); dark green leaves on shortened rachis, petiolules, and petioles that cause overlapping leaflets held close to the stem (Guner and Myers 2000).
<i>Tor (T)</i>	<i>torquere</i> (Latin): twining habit vs. <i>tor</i> non-twining (Norton 1915; Lamprecht 1947b); confers phytochrome-controlled climbing habit in indeterminate bush bean types (Kretchner et al. 1961; Kretchmer and Wallace 1978).
<i>Tr</i>	<i>testa rupture</i> (Dickson 1969); an incompletely dominant gene with 25-30% penetrance.
<i>tri</i>	<i>tricotyledonae</i> (Latin): produces three cotyledons (Lamprecht 1961b) with 40-50% penetrance.
<i>trv</i>	confers resistance to <i>tobacco ringspot virus</i> (Tu 1983); symbol proposed by Provvidenti (1987).
<i>Ts</i>	<i>temperature-dependant string</i> formation (Drijfhout 1978a); <i>St ts</i> is without string, <i>St Ts</i> expresses incomplete string, and <i>st Ts</i> and <i>st ts</i> have complete string.
<i>tw</i>	<i>twisted</i> pod character produces pod rotation that is highly variable, from slight to more than 360 degrees in snap bean germplasm (Baggett and Kean 1995).
<i>uni</i>	<i>unifoliata</i> (Latin): unifoliolate leaves; complete sterility (Lamprecht 1935c); this material is lost, and no allelism tests were made with other unifoliolate mutants before <i>uni-1</i> was lost.
<i>Uni-2</i>	a dominant mutation for <i>unifoliolate</i> true leaves (Garrido et al. 1991).
<i>uni<sup>nde</sup></i>	induced mutation with <i>unifoliolate</i> leaves with (superscript nde) <i>node dependent expression</i> ; partial fertility and shows reversion to normal leaflet number at higher nodes (Myers and Bassett 1993).
<i>uni<sup>nie</sup></i>	<i>unifoliolate</i> leaves with (superscript nie) <i>node independent expression</i> (natural mutant); completely female sterile but male-fertile and shows consistently strong expression of the unifoliolate trait at higher nodes (Myers and Bassett 1993).
<i>Ur-1</i>	rust [ <i>Uromyces appendiculatus</i> (Pers.) Unger var. <i>appendiculatus</i> ] resistance gene



- discovered by Ballantyne (1978) and found in the Middle American source 'B1627'. Kelly et al. (1996) proposed using the *Ur* symbol as a base for all rust resistance genes.
- Ur-2* rust resistance gene discovered by Ballantyne (1978) and found in the Middle American source 'B2090'.
- Ur-2*<sup>2</sup> rust resistance allele at the *Ur-2* locus discovered by Ballantyne (1978) and found in the Middle American source 'B2055'.
- Ur-3* rust resistance gene discovered by Ballantyne (1978) (see also Ballantyne and McIntosh 1977) and found in the Middle American sources 'Aurora', 'Mex 235', 'Nep-2', and '51051', albeit with slightly different reaction profiles across a differential set of races for each source (Miklas et al, 2002). *Ur-3* is linked to the *Co-2* gene and has a nearly terminal position on Pv11 (Miklas et al. 2002; Kelly et al. 2003).
- Ur-4* (*Up-2*, *Ur-C*) rust resistance gene originally discovered by Ballantyne (1978) as *Ur-C* and rediscovered by Christ and Groth (1982) as *Up-2*. *Ur-4* is an Andean gene found in 'Early Gallatin' and is located on Pv06 (Miklas et al. 2002).
- Ur-5* (B-190) block (cluster) of eight tightly linked rust resistance genes (*Ur-5A* through *Ur-5H*) found by Stavely (1984) and present in the rust differential variety Mexico 309. *Ur-5* is located on Pv04 (Miklas et al. 2002) in the vicinity of other resistance genes (Kelly et al. 2003).
- Ur-6* (*Ur<sub>a</sub>*, *Ur-G*) rust resistance gene originally discovered by Ballantyne (1978) as *Ur-G* and rediscovered by Grafton et al. (1985) as *Ur<sub>a</sub>*. *Ur-6* is an Andean gene present in 'Olathe' and the rust differential variety Golden Gate Wax. *Ur-6* is independent of *Ur-3* and located on Pv11 (Miklas et al. 2002).
- Ur-7* (*R<sub>B11</sub>*) rust resistance gene discovered by Augustin et al. (1972) and found in the Middle American varieties GN 1140 and Pinto US-5. *Ur-7* is independent of *Ur-3* and *Ur-6* and located on Pv11 (Park et al. 2003).
- Ur-8* (*Up-1*) rust resistance gene discovered by Christ and Groth (1982) and found in the Andean variety U.S. #3.
- Ur-9* (*Ur<sub>p</sub>*) rust resistance gene discovered by Finke et al. (1986) and found in the Andean variety Pompadour Checa. *Ur-9* is located on Pv01 (Miklas et al. 2002) near the *Co-1* locus (Kelly et al. 2003).
- Ur-10* (*URPRI*) rust resistance gene discovered by Webster and Ainsworth (1988) and found in snap bean varieties Cape and Resisto.
- Ur-11* (*Ur-3*<sup>2</sup>) originally a rust resistance allele at the *Ur-3* locus discovered by Stavely (1990), but later found to be tightly linked with *Ur-3* (Stavely 1998). *Ur-11* is located on Pv11 (Miklas et al. 2002).
- Ur-12* gene conditioning adult plant resistance (APR) to bean rust discovered by Jung et al. (1998) that is initially expressed at the fourth trifoliolate leaf stage or later. *Ur-12* is found in the Andean variety Pompadour Checa and is tentatively located at a terminal position on Pv07 (Jung et al. 1998; Miklas et al. 2002).
- Ur-13* rust resistance gene discovered by Liebenberg and Pretorius (2004) and found in the Andean sugar bean variety Kranskop; however, the gene appears to be of Middle American origin and is carried by variety Redlands Pioneer (Liebenberg and Pretorius 2004). *Ur-13* is located on Pv08 (Miene et al., 2005).
- Ur-14* adominant gene in Ouro Negro on Pv04 conditioning resistance to rust, described by Souza et al. (2011).
- us* *unstable* gene that mutates to *Us* in presence of *mu* to produce green leaf sectors in a yellow leaf background due to *us mu*, resulting in variegation (Coyné 1966).
- V* (*Bl*) with *P* produces pale glaucescens testa without a hilum ring (Lamprecht 1939). The color ranges from pale violet to black depending upon other color genes present (Lamprecht 1932a; Prakken 1934, 1972b). According to Prakken (1972a) the *Bl* of Smith (1939) is the same as *V*. *Bl* with the basic color factors produces purple-violet seed coat (Smith 1939; Tjebbes and Kooiman 1921, 1922a), changes oxblood red to purple (Smith 1939), and is responsible for bluish tints to plant colors (Tjebbes and Kooiman 1921). *bl* with appropriate

genes produces red seed coat (Tjebbes and Kooiman 1922a). According to Feenstra (1960), *V* is the equivalent of the *B* of Shull (1908) and of Tschermak (1912), the *F* of Kooiman (1931), the *G* of Shaw and Norton (1918), and the *Z* of Sirks (1922). *V* is located on Pv06 (McClellan et al. 2002). *V* encodes flavonoid 3'5' hydroxylase (F3'5'H), a P450 enzyme required for the expression of dihydromyricetin-derived flavonoids in the flavonoid pathway (McClellan et al., 2022).

<i>V<sup>wf</sup></i>	a gene with the seed coat color properties of <i>V</i> but with the pleiotropic effect of (superscript wf) <i>white flower</i> color; a gene derived from <i>P. coccineus</i> (Lamprecht line M0137, now PI 527845), permitting black seed coats and scarlet or vermilion flowers in nature (Bassett 1997b).
<i>v<sup>lae</sup></i> ( <i>Cor</i> )	superscript lae, <i>laelia</i> (Latin): with <i>TP</i> gives <i>laelia</i> (pink) flowers and rose stem (Lamprecht 1935e); with <i>PCJGB</i> produces mineral brown seed coats with the black corona character; expresses dark corona (purple to black) with numerous other genotypes (Bassett 1995a). The <i>Cor</i> locus of Lamprecht (1934a, 1936) is a synonym for <i>v<sup>lae</sup></i> .
<i>v</i> <i>var</i>	<i>white flowers</i> , and with <i>PCJGB</i> , produces mineral brown seed coat (Lamprecht 1935e). <i>variegated</i> : environment-sensitive gene, in combination with <i>mu</i> and <i>us</i> produces yellow lethal plants in a ratio of 63 normal:1 variegated (Coyne 1966).
<i>vi</i> ( <i>vir<sub>f</sub></i> ) <i>wb</i>	<i>virescent foliage mutant</i> (Grafton et al. 1983). with <i>TPV</i> , gives flowers with a <i>white banner</i> petal and wings of pale violet; the gene is from the <i>P. coccineus</i> PI 273666 (Bassett 1993a).
<i>Wmv</i>	confers resistance to <i>watermelon mosaic virus 2</i> (Kyle and Provvidenti 1987; Provvidenti 1974).
<i>X<sup>su</sup></i>	<i>ex parte</i> (superscript su) <i>sursum versus</i> (Latin): causes the leaves and petals to point downward (Lamprecht 1961b); effect is similar to <i>Sur</i> , but pulvinule is rotated only 90E.
<i>Xap-1</i>	Single dominant gene resistance to <i>Xanthomonas axonopodis</i> from PR0313-58 that co-segregates with SAP6 QTL on Pv10 (Zapata et al., 2011)
<i>y</i>	with <i>Arg</i> , produces <i>yellow wax pod</i> ; with <i>arg</i> , the pod is white; <i>Y</i> with <i>Arg</i> produces green pod; <i>Y</i> with <i>arg</i> gives a greenish gray (silvery) pod (Currence 1931; Lamprecht 1947b).
<i>Z</i> ( <i>D</i> ) ( <i>ers</i> )	<i>zonal</i> partly colored seed coat patterns are expressed with <i>tz</i> (Tschermak 1912, as interpreted by Lamprecht 1934b). With <i>t</i> , the <i>Z</i> locus interacts with <i>Bip</i> to express a wide range of partly colored seed coat patterns (Lamprecht 1934b, 1940b). The <i>L</i> of Schreiber (1940) was found to be allelic with <i>J</i> (Bassett et al. 2002b); hence, all the partly colored patterns controlled by interactions (with <i>t</i> ) of <i>Z</i> and <i>L</i> (Schreiber 1940) are really interactions of <i>Z</i> with <i>J</i> . Similarly, the <i>mar</i> gene of Lamprecht (1933) was found to be allelic with <i>j</i> (Bassett 1996b); hence, the interaction of <i>t</i> with <i>j</i> expresses marginata pattern (Bassett 1994c), which is the equivalent of the <i>tZL</i> of Schreiber (1940) for marginata. Similarly, the new allele <i>t<sup>ers</sup></i> (Bassett 1997d) is now recognized to be <i>j<sup>ers</sup></i> (Bassett et al. 2002b). The <i>D</i> gene for hilum ring color was found to be allelic with <i>Z</i> (Bassett et al. 1999b). Thus, hilum ring color is controlled by the interaction of <i>J</i> and <i>Z</i> (Prakken 1970), where colorless hilum ring is expressed by <i>zj</i> . Thus, <i>Z</i> and <i>J</i> have dual roles, 1) color expression of the hilum ring and 2) major roles in the expression of partly colored seed coats. A review of partly colored seed coat patterns with illustrations and genotypes is available (Bassett and McClellan 2000). <i>Z</i> is located on Pv03 (McClellan et al. 2002).
<i>z<sup>sel</sup></i>	superscript sel, <i>sellatus</i> (Latin): with <i>t</i> , <i>z<sup>sel</sup>/z<sup>sel</sup></i> expresses <i>sellatus</i> pattern and <i>z<sup>sel</sup>/z</i> expresses piebald pattern (Bassett 1997c; Lamprecht 1934b; Tschermak 1912).
<i>z</i>	with <i>t Bip</i> , expresses virgarcus pattern; with <i>t bip</i> expresses bipunctata pattern (Bassett 1996c). For other interactions see Bassett and McClellan (2000).
<i>Znd</i>	gene found in the variety Matterhorn for resistance to soil deficiency of Zn (Singh and Westermann 2002).

## APPENDIX – Obsolete symbols removed from list

- A* basic color factor, producing yellow-brown (Kooiman 1931; Sirks 1922; Tjebbes and Kooiman 1922b; Tschermak 1912). It is the equivalent of *P*, which has priority.
- A* indeterminate versus determinate, *a*, plant habit (Emerson 1916; Norton 1915). Symbol superseded by *Fin* (Lamprecht 1935b).
- A, B, C* schematic genes contributing to the length and number of internodes (Emerson 1916). Also used as schematic genes contributing to hybrid vigor (Malinowski 1924).
- A, B, C, D* schematic genes each contributing 1 cg to a minimum seed weight (Sirks 1925).
- Aeq* *Aequicoloratus* (Latin): with *P T E Uc Unc* and *R<sup>st</sup>* or *R<sup>ma</sup>* darkens the banner petal (Lamprecht 1935e, 1948a); with *Sal* the effect is similar to *V* (Lamprecht 1948b).
- an* appears to have the functions of *P* (Hilpert 1949).
- av, sv, iv* confer resistance to bean common mosaic virus (Ali 1950; Petersen 1958).
- B* originally a "blackener", producing anthocyanin with the basic color gene *P = A* (Shull 1908; Sirks 1922; Tschermak 1912). According to Feenstra (1960) this gene is the equivalent of the *G* of Shaw and Norton (1918), the *F* of Kooiman (1920), the *Z* of Sirks (1922), and the *V* of Lamprecht (1932a) and Prakken (1934). It is the equivalent of Feenstra's *C* (1960).
- bc-u* strain-unspecific complementary gene, giving resistance to strains of bean common mosaic virus (BCMV) only when together with one or more of the strain-specific resistance genes (Drijfhout 1978b).
- bc-1<sup>2</sup>* with *bc-u* gives resistance to BCMV strains NL1, NL2, NL7, and NL8 (Drijfhout 1978b).
- bc-2<sup>2</sup>* with *bc-u* gives resistance to BCMV strains NL1, NL2, NL5, NL6, NL7, and NL8 (Drijfhout 1978b).
- B I* hypothetical genes for testa vein color and orientation (Sarafi 1974). Data not sufficient to establish new genes (Bassett, editor).
- Br* According to Prakken (1972a), the *Br* of Smith (1947, 1961) is the same as *B. Br* with *P Rk* produces brown seed coat (Smith 1947), *br* with *P Rk* green seed coat, *br* with *P rk* pink seed coat (Smith 1947).
- C R* hypothetical genes for seed coat color where *C* gives cream, *R* gives red, *C R* produces milky phenotypes, and *r c* produces pink (Sarafi 1974). The real genotypes probably involve the *Rk* locus and its modifiers (Bassett, editor).
- Ca* with color genes, *caruncula* stripe (Lamprecht 1932c). Prakken (1970) believed this gene is a synonym for *G*.
- Can* According to Prakken (1972a), *D* is the equivalent of *Can* or *Ins* of Lamprecht (1939). *Can* with color genes gives a whitish (Speckweiss) testa (Lamprecht 1939) or blubber white (Lamprecht 1951a), with a yellowish brown hilum ring (Lamprecht 1939).
- Co-7* an anthracnose resistance gene found in the Middle American differential variety G2333 (Young et al. 1998) was renamed *Co-3<sup>5</sup>*.
- Co-9* Replaced by the *Co-3<sup>3</sup>* gene symbol.
- Co-10* An anthracnose resistance gene described by Alzate-Marin et al. (2003b) in the variety Ouro Negro. It is located on linkage group 4 (Freyre et al. 1998), and has been renamed *Co-3<sup>4</sup>*.
- cyv (by-3)* Confers high level resistance to *clover yellow vein* virus, formerly known as the severe, necrotic, or pod-distorting strain of bean yellow mosaic virus (Provvidenti and Schroeder 1973; Tu 1983); symbol proposed by Provvidenti (1987). Renamed *bc-3<sup>2</sup>*.
- def* *defectus* (Latin): gene *def* is a synonym for *gy* (Bassett, editor). The hypothesis of Prakken (1972b) was that the interaction of *G/g* with *def* produced zonal variability of greenish yellow expression on seed coats. Whereas the seed coat color expression of *gy* was falsely attributed to *G b v* and *g b v*. The hypothesis of Bassett et al. (2002) is that the interaction of (*C J*) *G* or *g (b v)* with *gy* expresses greenish yellow seed coat with variable expressivity. Thus, Prakken (1972b) attributed the instability of *gy* expression to a separate and non-existent gene *def* and attributed the greenish yellow color of *gy* to *C J g b v*,

	whereas the latter genotype has only shamois expression.
<i>E</i>	intensifier with color genes (Tjebbes and Kooiman 1922b).
<i>e</i>	<i>E</i> required for complete coloring of seed coat (Emerson 1909b); the action of <i>e</i> is hypostatic on <i>t</i> , producing much reduced partial coloring of seed coat and required for the soldier series of seed coat patterns (Emerson 1909b; Lamprecht 1934b; Leakey 1988; Sax and McPhee 1923; Smith 1939). The only published data (Sax and McPhee 1923) supporting the existence of this gene is too preliminary and inadequate to establish the gene.
<i>Epi Hyp</i>	interspecific genes for <i>epigeal</i> and <i>hypogeal</i> cotyledons in <i>P. vulgaris</i> and <i>P. coccineus</i> , respectively (Lamprecht 1945, 1957). Lamprecht's model with <i>Epi</i> and <i>Hyp</i> giving 9 distinct phenotypes for cotyledon attachment position has been superseded by a quantitative model (Wall and York 1957).
<i>ers, ers-2</i>	<i>erasure</i> : genes restricting partly colored seed coat patterns, now known to be synonyms for <i>z</i> and <i>j<sup>ers</sup></i> , respectively (Bassett 1997d; Bassett and Blom 1991; Bassett et al. 2002b).
<i>Ext Int</i>	interspecific genes for <i>external</i> and <i>internal</i> stigma positions in <i>P. coccineus</i> and <i>P. vulgaris</i> , respectively (Lamprecht 1945). Lamprecht's Mendelian model with the <i>Ext</i> and <i>Int</i> loci giving 9 distinct phenotypes for stigma form has been superseded by a quantitative model (Manshardt and Bassett 1984).
<i>F</i>	was used as a color gene by Shaw and Norton (1918) with basic genes and their <i>C</i> for yellow to produce coffee-brown. It was also used similarly by Kooiman (1931) with <i>C</i> for yellow or orange-brown plus <i>E</i> , producing coffee brown, to give black ( <i>A B C E F</i> ). The combinations <i>A B F</i> , <i>A C F</i> , and <i>A D F</i> had pale lilac flowers (Tjebbes and Kooiman 1922b) perhaps the equivalent of <i>v<sup>lae</sup></i> . The gene is no longer recognized.
<i>Fcr, Fcr-2</i>	formerly (Bassett 1993b), complementary genes for <i>flower color restoration</i> with <i>t</i> ; but <i>t<sup>cf</sup></i> is now known to express flower color normally (no white flower effect) while expressing (with <i>Z</i> , <i>Bip</i> , and <i>J</i> ) partly colored seed coat patterns (Bassett et al. 1999a).
<i>Flav</i>	has a light yellow influence (Lamprecht 1951a) on seed coat color; previously considered to be recessive (Lamprecht 1939). Prakken (1970) believed this gene is a synonym for <i>G</i> .
<i>H</i>	described by Shaw and Norton (1918) as producing light brown or olive. Considered by Feenstra (1960) as the equivalent of the <i>D</i> of Shull (1908), the <i>C</i> of Tschermak (1912), the <i>E</i> of Kooiman (1931), the <i>L</i> of Sirks (1922), the <i>B</i> of Lamprecht (1939), the <i>B</i> of Prakken (1934), the <i>B</i> of Feenstra (1960), and the <i>Bl</i> of Smith (1939).
<i>ie</i>	similar to the action of <i>ip</i> ; also inhibits the action of <i>B</i> and <i>G</i> (Nakayama 1959b); considered by Lamprecht (1961c) to be equivalent of <i>c</i> .
<i>inh</i>	<i>inhibeo</i> (Latin): inhibits the action of <i>V</i> on seed coat colors (Lamprecht 1940c).
<i>Ins</i>	According to Prakken (1972a), <i>D</i> is the equivalent of <i>Can</i> or <i>Ins</i> of Lamprecht (1939). <i>Ins</i> with appropriate factors gives light buff (Lamprecht 1939) or raw silk (Lamprecht 1951a) testa; has a hilum ring.
<i>L</i>	<i>Löschungsfaktor</i> (German): inhibits (or <i>limits</i> ) the partial coloring of the testa; with <i>t</i> , producing an entirely white testa (Schreiber 1934). <i>L</i> and <i>l</i> combine with <i>Z</i> and <i>z</i> to produce several color patterns (Schreiber 1940). <i>L</i> is a synonym for <i>J</i> (Bassett et al. 2002b); Schreiber's (1940) <i>L</i> is exactly equivalent to <i>j</i> .
<i>lin</i>	<i>lineatus</i> (Latin): produces red veins in wing petals (Lamprecht 1935e). According to Prakken (1972a), red veins in wing petals are a pleiotropic effect of the testa color gene <i>rk<sup>d</sup></i> .
<i>M<sup>st</sup></i>	causes striping of the seed coat (Smith 1947); redesignated <i>R<sup>st</sup></i> (Lamprecht 1947a).
<i>mar</i>	<i>margo</i> (Latin): broad colored zone around hilum ring (Lamprecht 1933).
<i>Ms In-ms</i>	<i>Ms</i> confers <i>male sterility</i> and <i>In-ms</i> inhibits action of <i>Ms</i> , restoring pollen fertility; <i>in-ms</i> <i>Ms</i> is lethal (Mutschler and Bliss 1980). Without translocation heterozygosity to account for the semisterile class, the validity of the model is questionable (Ashraf and Bassett 1986).
<i>Nud</i>	<i>Nudus</i> (Latin): with <i>P</i> , gives purple, waxy stem and crimson flowers (Lamprecht 1935e).

<i>Och</i>	<i>Nud</i> is a synonym for [ <i>c<sup>u</sup> Prp</i> ] (Bassett 1994a; Bassett, editor). with <i>P C j</i> , gives <i>ochre</i> yellow tints such as ochraceous, Hell Lohfarben, light tawny brown, tawny olive to clay (Lamprecht 1933, 1939); has colored hilum ring (Lamprecht 1939); epistatic to <i>Vir</i> (Lamprecht 1939). Prakken (1970) believed this gene is a synonym for <i>G</i> .
<i>P</i>	(schematic) increases vigor with <i>A B C</i> (Malinowski 1924).
<i>Pur</i>	obsolete symbol for <i>V</i> (Lam-Sanchez and Vieira 1964; Okonkwo and Clayberg 1984), originally <i>Pur Ro</i> has a deep <i>purple</i> pod (Lamprecht 1951b).
<i>R</i>	(schematic) increases vigor with <i>A B C</i> (Malinowski 1924).
<i>Ro</i>	<i>Rosa</i> (German): the <i>Ro</i> of Lamprecht (1951b) and Lam-Sanchez and Vieira (1964) is synonymous with the <i>Prp</i> of Bassett (1994a) and Okonkwo and Clayberg (1984). With <i>Pur</i> ( <i>V</i> ), gives dark purple pod; with <i>pur</i> ( <i>v</i> ), gives <i>rose</i> pod color (Lamprecht 1951b). Lam-Sanchez and Vieira (1964) report <i>Ro V</i> gives dark purple pod and <i>Ro v</i> gives red pod; Okonkwo and Clayberg (1984) report <i>Ro</i> as a second locus, along with <i>Prp</i> , giving purple pods.
<i>S</i>	(schematic) increases vigor with <i>A B C</i> (Malinowski 1924).
<i>sd</i>	slow darkening seed trait. Two genes controlling slow darkening with <i>J</i> epistatic to <i>sd</i> . Presence of the dominant allele <i>J</i> results in a tendency to darken, while <i>sd</i> , is responsible for how quickly a seed coat will darken (Elsadr et al. 2011). <i>sd</i> was found to be conditioned by an allele at the <i>P<sup>sd</sup></i> locus (Islam et al., 2020).
<i>Uc Unc (I<sub>1</sub> I<sub>2</sub>)</i>	<i>uni coloris</i> (Latin): with appropriate genes, darken the banner petal (Lamprecht 1948a); either <i>Uc-uc</i> and <i>Unc-unc</i> (Lamprecht 1948a) or <i>I<sub>1</sub>-i<sub>1</sub></i> and <i>I<sub>2</sub>-i<sub>2</sub></i> (Nakayama 1958) for the presence or not of anthocyanin in hypocotyl and stem. According to Prakken (1972b), both of these gene pairs are synonyms for genes in the "complex <i>C</i> locus", e.g., <i>Unc</i> is the equivalent of <i>Str</i> .
<i>v<sup>pal</sup></i>	with <i>P</i> , gives clear light red flowers (Lamprecht 1936); later shown to be a pleiotropic effect of <i>p<sup>eri</sup></i> (Bassett 1992b, 1994b).
<i>Vir</i>	with <i>P Gri C virescens</i> or greenish shades on the testa (Lamprecht 1933); among these are Russgrun or olive black. Prakken (1970) believed that <i>Vir</i> is a synonym for <i>B</i> .
<i>Ws</i>	confers resistance to <i>Whetzelinia</i> (now <i>Sclerotinia sclerotiorum</i> ). Gene is no longer in use (Abawi et al. 1978).
<i>Xx</i>	early designation for inconstant mottling of the seed coat (Emerson 1909a); now <i>C c</i> (Lamprecht 1940a).
<i>Z</i>	constant mottling of the seed coat (Tjebbes and Kooiman 1919a); now <i>C<sup>ma</sup></i> or <i>R<sup>ma</sup></i> .
<i>Z-1</i>	self-colored seed coat (Tschermak 1912); the equivalent of <i>T</i> .
<i>Z-2</i>	pigment extender (Tschermak 1912); the equivalent of <i>Z</i> .

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## IN MEMORY OF DOUGLAS REES LAING

Douglas Rees Laing, former scientist and a director of the International Center for Tropical Agriculture, passed away peacefully in his home in Cali, Colombia on July 25, 2021.

Doug was born August 31, 1936 in Queensland, Australia, where he received his high school diploma in Agriculture from the Queensland Agricultural College (1952-1956); and a BSc in Agriculture from the University of Queensland (1957-1961). He received his PhD in Agricultural Climatology (1962-1965) from Iowa State University, with minors in Statistics and Crop Physiology. His thesis was entitled “Water Environment of Soybeans”.

Subsequent to obtaining his PhD, Doug served as lecturer at the University of Sydney (1966-1974) with short term appointments in Indonesia and the United Kingdom, and with a sabbatical leave in CIMMYT in Mexico. He joined CIAT in 1974 as the physiologist of CIAT’s Bean Program from 1974 to 1979.

His scientific career was focused on stress tolerance of common bean (*Phaseolus vulgaris*) to drought, implementing infrared temperature analysis as a technique to estimate drought stress, and identifying genotypes with deep rooting as a tolerance mechanism. He was also instrumental in designing the International Bean Yield and Adaptation Nursery (IBYAN) which for years served as a channel of improved genotypes to bean programs around the world.

He later served as Director of Crops Research from 1979 to 1984, and as Deputy Director General from 1984 to 1992. In his retirement he participated enthusiastically in environmental activities around Cali, to which he was ardently devoted. Doug was known for his boisterous personality and was not shy to express his opinions. Doug is survived by his wife Olga, daughter Kathryn, and son David.

# INTRIGUING OBSERVATIONS IN *PHASEOLUS* AND POTENTIAL FUTURE RESEARCH TRACKS

Paul Gepts

Department of Plant Sciences, University of California, Davis

Science is often divided – unjustifiably so in my humble opinion– into basic and applied realms, which contrast but rarely complement each other. In this presentation, I would like to argue that basic and applied research can be synergistic by relying on their respective strengths. In plant biology, basic research can discover new genetic, biochemical, physiological, or ecological features, whereas applied research puts these into practice. A closer relationship between applied and basic research can be established through mutual awareness and exchange of research materials, like genetic and breeding populations.

I will use *Phaseolus* beans as an experimental system to illustrate this closeness of the two research realms and suggest possible research directions for the near future in this crop genus. As grain legumes, beans (*Phaseolus* spp.) receive less funding compared to cereal and oil crops (Sinclair & Vadez 2012; Foyer et al. 2016). Nevertheless, they fulfill an important agronomic role as a N-fixing crop and dietary role as plant protein source (Gepts et al. 2008; Porch et al. 2017; Gogoi et al. 2018; Meena and Lal 2018; Vanlauwe et al. 2019; Didinger and Thompson 2021).

In the interest of brevity, I will focus primarily on evolutionary and morpho-agronomic developmental topics related to the domestication process. This is not to say that other topics are not important. For example, host-pathogen or host-herbivores (a.k.a. host-pests) remain important as they are one of the primary causes of yield limitations in crops, including beans (Cardona & Kornegay 2019). With global warming, some phytophagous insects will become more important plant predators (Lehmann et al. 2020).

A better understanding of resistance mechanisms should lead to more efficient breeding selection procedures and improved cultivars and management procedures that reduce the need for artificial insecticides. For example, harnessing the host-herbivore-hyperparasite biochemical communications in an agro-ecological context remains an elusive but promising research goal. Lima bean (*P. lunatus*) constitutes an interesting example in this regard as volatile organic compound (VOC)-mediated tri-trophic interactions, supplemented with extrafloral nectar secretions, have been well documented (Williams et al. 2005; Heil 2014). In turn, these volatiles are linked to the jasmonic acid- induced defense mechanisms of the plant. Changes in the release of VOCs as a consequence of elevated CO<sub>2</sub> may affect a plant's resistance to insects (Kost and Heil 2008).

In addition to this indirect defense mechanism, direct defense mechanisms, namely the production of cyanogenic glucosides (linamarin and lotaustralin; Ballhorn et al. 2008, 2009) or polygalacturonases (Celorio-Mancera et al. 2008, 2009), also could be harnessed. Our preliminary observations indicate that these cyanogenic glucosides are produced mainly in growing reproductive tissues, which are also the most vulnerable to damage caused by insect predators like *Lygus hesperus*, the Western tarnished plant bug (<https://www2.ipm.ucanr.edu/agriculture/dry-beans/Lygus-bugs/>), a predominant and omnivorous pest in the U.S. Southwest but also on the

U.S. East Coast. The combination of direct and indirect defense mechanisms could provide a more environmentally friendly control strategy for this insect pest.

A second topic I will only mention in passing is the need to improve culinary, nutritional, organoleptic traits, and consumer acceptability of *Phaseolus* beans. As the most important grain legume for direct human consumption (Broughton et al. 2003), these bean qualities are quite important and deserve more attention, whether in dry or snap beans, from a genetic and environmental standpoint (Kleintop et al. 2016; Moghaddam et al. 2018; Cichy et al. 2019; Winham et al. 2019; Myers et al. 2019; Bassett et al. 2021a,b; Saradadevi et al. 2021). In addition, the interest in developing new bean products should also be taken into account, whether these are culinary or medical in nature. There is a need to shorten the preparation time of beans through various forms of pre-processing to facilitate their consumption (e.g., Hooper et al. 2019; Bento et al. 2021). The medical role of *Phaseolus* beans remains an interesting and tantalizing feature. Regular consumption of grain legumes is now well established as part of a healthy diet (e.g., Thompson et al. 2008, 2017; Ganesan & Xu 2017; Chávez-Mendoza & Sánchez 2017). The search for bioactive components is under way as well as the way of administering these (e.g., Donoso-Quezada et al. 2020; Rodriguez et al. 2022).

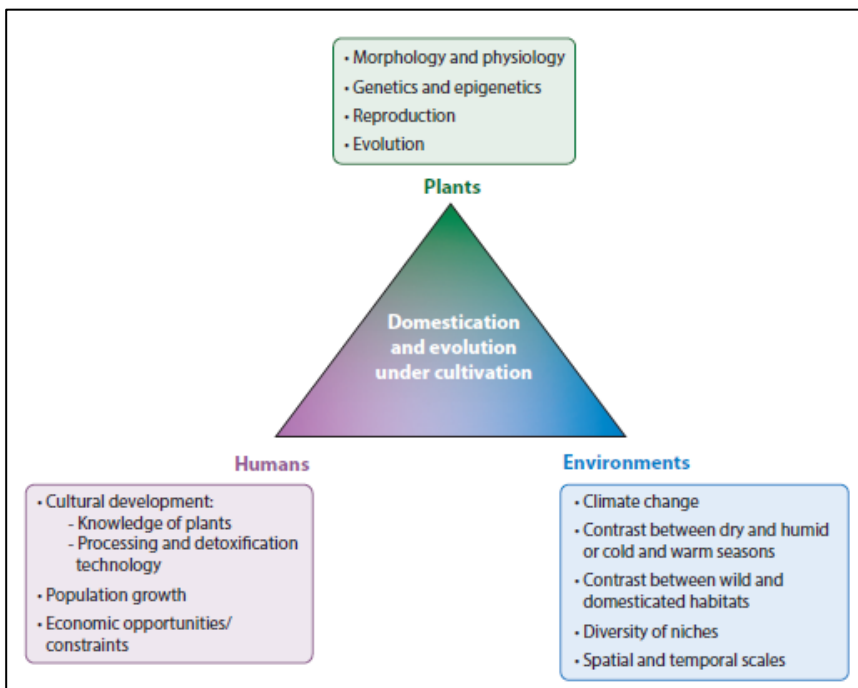
A more detailed review of the two topics is certainly warranted but in my keynote presentation at the BIC meeting, I chose to focus on the domestication process in *Phaseolus* beans and morpho-physiological traits that are related to domestication. These traits continue to be essential productivity traits but have remained unexplored until recently in the *Phaseolus* community and other crop communities in general.

### ***Phaseolus* sp. as a Domestication Hotspot**

The genus *Phaseolus* – which originated in Mesoamerica some 5-6 million years ago (Delgado Salinas et al. 2006) - consists of some 70-80 species (Freitag & Debouck 2002; Debouck 2021), five of which have been domesticated (Baudet et al. 1977; Schmit & Debouck 1991; reviewed by Parker and Gepts 2021). These include, in decreasing order of importance from a distribution and production viewpoint, common bean (*P. vulgaris* L.), lima bean (*P. lunatus* L.), runner bean (*P. coccineus* L.), tepary bean (*P. acutifolius* A. Gray), and year bean (*P. dumosus* Macfadyen). The first two of these species have been domesticated twice. In addition to domestication centers in Mesoamerica (Kwak et al. 2009; Gutiérrez-Salgado et al. 1995; Motta-Aldana et al. 2010), they were also domesticated in specific areas in the Andes. For common bean, genetic data suggest the southern Andes between southern Peru and northwestern Argentina for common bean (Kwak and Gepts 2009) and more specifically Bolivia and northwestern Argentina (Beebe et al. 2001; Rodriguez et al. 2016). For lima bean, the Andean domestication is situated in an area on the Western slope of the Andes in Ecuador and northern Peru (Motta-Aldana et al. 2010; Chacón-Sánchez and Martínez-Castillo 2017; Garcia et al. 2021). These Andean domestication centers are the result of rare, long-distance dispersal events. For common bean, the dispersal to the southern Andes has been timed at ~ 0.080 – 0.165 My based on nuclear sequences (Schmutz et al. 2014) and 0.2 My for chloroplast DNA and 0.002 My for nuclear sequences (Rendón-Anaya et al. 2017). The vector responsible for such long-distance dispersal events that bridge lowland, drier and hotter regions like the Isthmus of Panama and the Colombian Chocó region, is likely birds (Ariani et al. 2018). Similar studies should be conducted for lima bean, which presents a different geographic gene pool distribution.

It is often said the multiple domestications present a replicated experiment. The multiple *Phaseolus* domestications illustrate why this is not so. There are three categories of factors that influence the process of domestication and evolution before, during, and after domestication (**Fig. 1**): 1) Plant factors; 2) Biotic and abiotic environments; and 3) Human factors, as illustrated by the “domestication triangle” (Gepts 2004; Hufford et al. 2019). These factors vary for each domestication and result in both similarities in the domestication processes (e.g., Law of Homologous Variation of Vavilov 1922; the “Domestication syndrome” of Hammer 1984) but also differences.

One of these differences is the degree to which each is domesticated, as illustrated by the five *Phaseolus* domesticated species (**Fig. 2**). Common bean is the most domesticated species in the genus based on the number of uses (dry and green beans), the number of domestication traits, and the geographic distribution of the wild relative and the crop (Parker and Gepts 2021: Table 5 and Fig. 4). In decreasing order are, further, lima bean, runner bean, tepary bean, and year bean. It is interesting to note that the intensity of domestication and economic importance are both correlated with the size of the distribution of the respective wild relatives, perhaps signifying an environmental pre-disposition, which would have favored a dispersal of the crops outside their narrow regions of domestication.

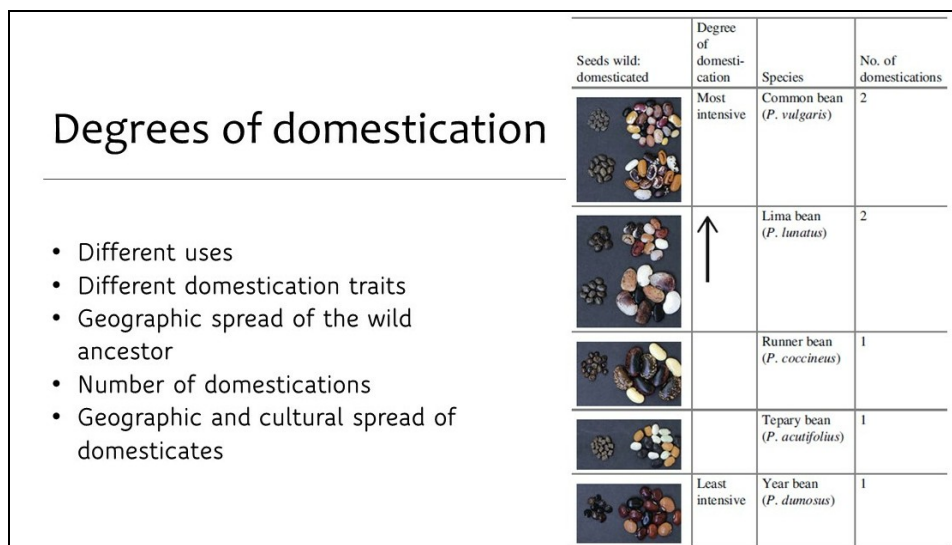


**Fig. 1.** The three main categories of factors that influence the extent of domestication and subsequent evolution under cultivation (Gepts 2004: Fig. 1.5; Hufford et al. 2019: Fig. 2).



Over the last 10 years, an impressive number of domestication genes have been isolated in common bean and their diversity in the species has been analyzed (**Fig. 3**). Some general conclusions can be drawn from this work. With one exception, they are recessive, loss-of-function alleles. The exception is the stringless mutation on Pv02 (Parker et al. 2022), which is dominant and correlated with an increased expression of the *PvIND* locus. In about equal proportions, they represent transcription factors, structural genes, and biosynthetic enzymes. All appear to have resulted from *de novo* mutations during and after the domestication phase.

**Fig. 2.** Domesticated *Phaseolus* species show different degrees of domestication, in decreasing order from the top (*P. vulgaris*) to the bottom (*P. dumosus*) (Parker & Gepts 2021).



	Year	Trait	Gene (chromosome)	Type <sup>1</sup>	Plant function	Source
	2009, 2012	Determinacy	<i>fin, PvTFL1y</i> (01)	TF (LOF)	Plant type/phenology	Kwak et al. 2008; Repinski et al. 2012; Kwak et al. 2012
	2018	Pigmentation: absence of anthocyanins	<i>P</i> (07)	TF (LOF)	Consumer preference	McClellan et al. (2018)
	2019, 2021	Photoperiodism	<i>Ppd/PhyA3</i> (01); <i>PvCo2</i> (04)	Structural (LOF); TF (LOF)	Phenology	Weller et al. 2019; González et al. 2021
	2019, 2020	Pod shattering	<i>PvPdh1</i> (03); <i>PvMyb26</i> (05)	Enzyme (LOF); TF (LOF)	Harvest	Parker et al. (2020); Di Vittori et al. (2021)
	2021	Seed dormancy	<i>Pectin acetyltransferase 8</i> (03)	Enzyme (LOF)	Planting	Soltani et al. (2021); Palmer et al. (2021)
	2021	Pigmentation: yellow pod	<i>y</i> (02)	Structural (LOF)	Consumer preference	Yang et al. (2021)

<sup>1</sup> TF: transcription factor; LOF: loss-of-function

**Fig. 3.** Domestication genes isolated in common bean (Parker & Gepts 2021).

Studying the process of domestication in crops (and animals) is not just an academic exercise. It has definite applications to crop improvement, including crop genetic diversity conservation and cultivar improvement. Past examples are the documentation of the reduction in genetic diversity (summarized in Parker & Gepts 2021: Table 3) and of co-evolution between the bean host and several pathogens or *Rhizobium* (e.g., Guzmán et al. 1995; Aguilar et al. 2004). Although the presence of the domestication genes in the domesticated gene pool may date to several 100s-1000s

years, they still represent significant diversity with an economic importance in today's production. As an example, the reduction in pod shattering is an essential trait to assure the expression of yield potential and is bound to remain so, especially in increasingly dry climates (Parker et al. 2021b). Another observation is that several genes show multiple mutations (summarized in Parker & Gepts 2021).

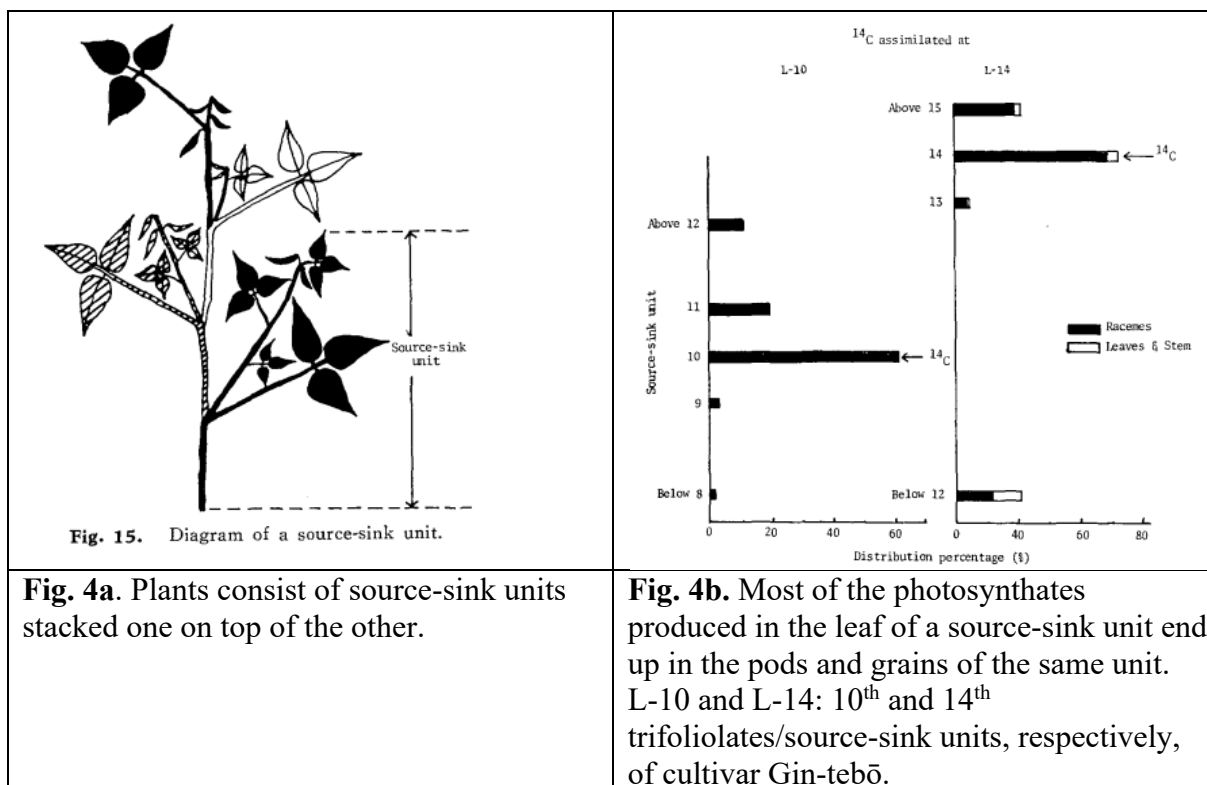
These mutations tend to be lineage-specific, characterizing either gene pools (either Andean or Mesoamerican) or eco-geographical races (Singh et al. 1991). Thus, the *fin* (*PvTFL1y*) gene has at least 8 mutations representing different mutational and geographic origin, mostly in the Andean gene pool. There are some 10 mutations in the *P* locus, responsible for the presence or absence of pigmentation in flowers and pods. In contrast with the two former traits, for which a single locus is responsible, multiple genes are responsible for the reduction of shattering, depending on gene pool and eco-geographic race. Together with the environmental effect on trait expression, this situation accounts for the persistence of pod shattering in certain market classes, like the small black/white and cranberry beans (Parker et al. 2021a). Complementation of recessive pod shattering resistance loci in progenies tested in moist harvest environments leads to the retention of pod shattering against the overall direction of bean domestication.

Finally, most of the *Phaseolus* domestication studies have been conducted in common bean. As the importance of other *Phaseolus* domesticated species increases, not only as a source of genetic diversity to improve common bean, but also as crops in their own right, further research in their domestication is warranted. Global warming will put more emphasis on tepary bean and lima bean, for example for their tolerance to terminal and intermittent drought, respectively (Medina et al. 2017). The availability of whole genome reference sequences for these two species is an important tool in that regard (Garcia et al. 2021; Moghaddam et al. 2021) in addition to those available in common bean (Assefa et al. 2019).

### **Yield Differences, Sink Strength, and Suspensors**

It is a common observation that crop genotypes, including beans, show differential yields even under favorable growth conditions and after eliminating confounding effects due to, for example, cycle length and growth habit. Although there are many factors that could account for these differences, one process that deserves more attention is the translocation, partitioning, and remobilization of photosynthates from the original photosynthesis sites to their deposition in pods (green beans) and seeds (dry beans).

To begin to understand the translocation issue, the contribution of Tanaka and Fujita (1978; <http://hdl.handle.net/2115/12922>) should be required reading for any bean scientist. They demonstrate that a bean plant (and any plant, for that matter) consists of stacks of source-sink units, each unit consisting of a trifoliolate leaf, the subtending internode, and a side branch or an inflorescence (raceme) producing flowers, pods, and then grains at the axil of each leaf (**Fig. 4a**; their Fig. 15, p. 172). Using radiolabeled CO<sub>2</sub>, they showed that most of the photosynthates produced in the leaf of a unit ends up in pods and seeds of that same unit (**Fig. 4b**; their Fig. 16, p. 172).



From Tanaka and Fujita (1978)

The translocation from the leaf mesophyll to pods and event/or grains involves several steps, through various zones of the phloem, successively the collection phloem (sieve elements and companion cells involved in phloem loading), the transport phloem (located in leaf veins, petioles, stems, peduncles, and pedicels), and the release phloem in the sinks (phloem unloading), where they are stored or metabolized (van Bel 2003; Thomas 2017). I suggest here that high yield is dependent on an efficient transfer, i.e., a timely and maximal transfer, of photosynthates across this suite of translocation and partitioning steps.

The last step in this suite is the transition from pod walls to grains inside the pods. S. Beebe and his colleagues (Assefa et al. 2013) proposed an efficiency criterion, namely the pod harvest index ( $PHI = (\text{dry weight of grains} / \text{total dry weight of pods}) \times 100$ ), reflecting how much photosynthates were translocated from pod walls to grains. One of the advantages of PHI, aside from its practicality, is that it can be applied to any treatment, i.e., in the presence or absence of stress, including drought stress. Assefa et al. (2013) proposed that PHI could be an effective selection criterion for yield under stress based on its medium high correlation with grain yield and heritability. This correlation was confirmed by Berny Mier y Teran et al. (2019b) in a larger version of the same population. The correlation between PHI and grain yields was reflected in the sharing of 3 of 8 QTLs for grain yield with those for PHI. In addition, the higher heritability of PHI may be due to the existence of a single, large PHI QTL, which accounts for 17% of the variation and is stable across environments.

Further research is needed to identify and understand the function of genes involved in the efficiency of translocation of photosynthates from the pod wall into the developing grains. It has been known for a while that developing seeds, in particular those of *Phaseolus* beans, contain a

structure – called the suspensor at the basis of the embryo. Suspensor cells contain polytene chromosomes, similar to those observed in salivary glands of *Drosophila* (Nagl 1969, Frediani et al. 1993; Chen et al. 2020). This type of chromosome, resulting from endoreduplication, is indicative of high metabolic activity; however, it remains to be determined which activities are involved. Chen et al. (2021), using laser-capture microdissection (Espina et al. 2006), showed that suspensor cells were mainly a hormone (gibberellin) factory and transported substances to the developing embryo.



**Fig. 5.** Comparison of pods of small- and large-seeded genotypes, illustrating the difference in the number of seeds (and suspensors) (from Guy Dirix)

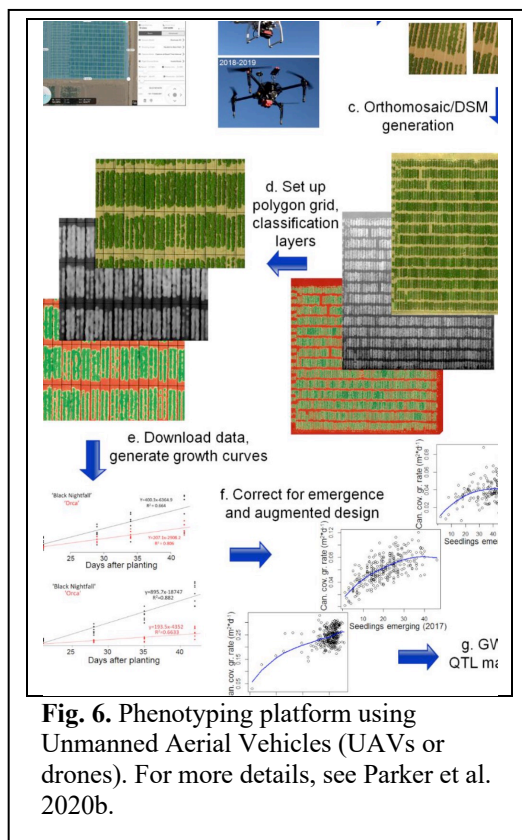
One can speculate about the nature of the substances being transported by the suspensors, but photosynthates may be included among these substances. Furthermore, one can ask whether the number of suspensors in a pod is correlated with sink strength and, ultimately, yield. This may account for the higher yield of small-seeded genotypes, which contain generally more seeds (and, therefore, more suspensors per pod) (**Fig. 5**). It remains to be seen how one can select for increased sink strength.

### **Vigor of Germination and Early Growth**

Germination and early growth vigor are rarely primary breeding objectives in *Phaseolus* beans, and grain legumes in general, in contrast with cereals (e.g., Mwendwa et al. 2020). As long as the germination percentage is adequate for the locally relevant agronomic practices, no further attention is paid to this trait in contrast with later agronomically important traits, like disease and pest resistance, and consumer quality attributes. Likewise, early growth, defined here as the growth stage between emergence (V1) and the first trifoliolate (V3; Fernández et al. 1983), is generally considered as a transitory phase on the way to bigger and better events to happen in the vegetative and reproductive phases of the bean plant. Yet, these early stages can play important roles in the overall performance of the bean crop. For example, a dense and vigorous seedling population helps establish a dense canopy that can outcompete weeds through shading.

A dense canopy can also help conserve soil moisture, especially in arid environments. It may not be a coincidence that two eco-geographic races of common bean with a traditionally prostrate bush growth habit (type III of Singh 1982) originated in arid environments (races Durango and Chile; Singh et al. 1991). Whereas an erect bush growth habit (type II) is highly desirable in environments that are moist, especially at harvest, in order to facilitate grain harvest, a prostrate, ground-covering growth habit should be acceptable in arid areas, especially when harvest is accomplished by wind-rowing.

Effective development of breeding lines with vigorous germination requires genetic variation for this trait (e.g., Sexton et al. 1997) and an efficient, high-precision selection method. Parker et al. (2020b) combined a genome-wide association study (GWAS) of the Middle American Diversity Panel (MDP; Moghaddam et al. 2016) and a biparental recombinant inbred population to study the

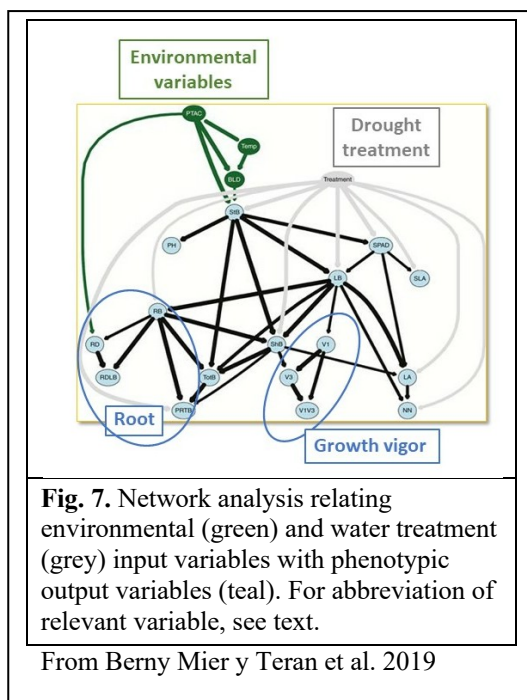


inheritance of early-growth vigor. Their results showed that this trait is the result of complex interactions between genotype and environmental factors. Unmanned Aerial Vehicles (UAVs or drones) provided high-throughput, precise data acquisition through weekly flights over bean fields and the first quantitative trait loci (QTLs) for any trait in *Phaseolus* beans (**Fig. 6**). The recombinant inbred population resulted from the cross between two Mesoamerican genotypes, Black Nightfall (fast early grower) and Orca (slow grower). QTL analysis of this population in each of three years revealed QTLs on chromosomes Pv06, Pv07, and Pv09. The Pv07 QTL in the biparental population was found on the same chromosome arm as the most significant SNP from the GWAS analysis.

Early growth vigor appeared to play a role in drought stress tolerance of wild common bean as well. Berny Mier y Teran et al. (2019a) were interested in determining the tolerance to drought of wild beans from environments with contrasting aridity. They conducted a greenhouse-based comparison of wild and domesticated Mesoamerican genotypes in a setting where root and aerial growth could be followed under

well-watered and intermittent drought conditions from emergence (V1) to the first trifoliolate leaf (V3). A network analysis allowed the combination of environmental variables (PTAC: Priestley–Taylor  $\alpha$  coefficient, which is a measure of actual over potential evapotranspiration, integrating soil-water availability; Temp: annual mean temperature; and BLD: soil bulk density), water treatment (well-watered vs. drought), and phenotypic response variables (**Fig. 7**). The result of their study was that two sets of variables represented the ultimate differences among wild accessions depending on their origin. The first set represented – not surprisingly - different expressions of root biomass (RB), root depth (RD), root depth length per biomass (RDLB), and the proportion of roots to total biomass (PRTB). This observation confirmed our hypothesis that wild bean from arid areas tended to have deeper roots. The second set reflects early growth vigor, including time to emergence (V1), time from planting to V3 (V3), and time from emergence to V3 (V1V3). Under drought stress, the growth of domesticated plants tended to slow down, in contrast to growth of wild plants.

The importance of wild accessions from arid environments in conferring tolerance to drought was further confirmed by Berny Mier y Teran et al. (2020) who compared the yield benefit obtained by a



domesticated breeding line introgressed with wild beans from arid (central and northern Mexico) and moist areas (Guatemala). None of the wild accessions, regardless of their original environment, contributed a yield increase under well-watered conditions. However, only the accessions from arid areas were able to increase the yield of the introgressed domesticated line. A corollary of this result is that it provides a way to pre-select wild accessions for introgression into the domesticated gene pool, which we call the GAMA approach. This approach combines Geographic, Agronomic or Adaptation, and Molecular data to pre-select germplasm for screening and introgression. The Parker et al. (2019) and Berny Mier y Teran et al. (2019a) suggested the value in making more precise measurements of specific growth traits, either by adopting novel instrumentation (like UAVs) or conducting experiments in more controlled environments like a greenhouse.

### **Beans Are No Potted Plants: Tropism and Non-Tropism Movements**

Plants are sessile. Unlike animals they do not move around. However, this does not mean that they do not exhibit movements. Actually, the movement of plant organs is extremely important as they provide a form of adaptation and defense against adverse conditions. The observation of plant movements has been facilitated by the use of time-lapse photography (Mahlandt & Goedhart 2021). Perhaps the best known of the plant movements is the sudden and rapid leaflet movement of *Mimosa pudica* (the sensitive plant), a leguminous plant. The mechanical stimulus of a landing insect may cause this rapid movement, which then drives the potential herbivore away: <https://www.youtube.com/watch?v=BLTcVNYOhUc> (Tran et al. 2021). In grain legumes, the engine of these movements are the pulvini, in various locations of the petiole and leaflet attachments.

Movements in which the response of the plant is independent of the direction of the stimulus are called *non-tropisms* or *nastic movements*. Other non-tropisms include circadian movements, in which no external stimuli are recorded. Beans provide an excellent example of such circadian rhythms, best seen with primary leaves of the plant (<https://www.youtube.com/watch?v=jXbPJEyWwCA> : see segment from 1:08 to 1:55). These leaves are pointed downward during the night. At daybreak, they gradually lift themselves up until they point upward at midday. In the second half of day, the movement in opposite direction takes place. There are also stress-induced movements, caused, for example, by heat or drought. They limit temperature increases in the leaf tissues and protect the photosynthetic apparatus.

In addition to these non-tropisms, there are also tropisms, movements in which the direction of the plant's movement depends directly on the direction of the stimulus. Thus, plants exhibit gravi- or geotropism, in response to gravity, and phototropism, in response to light. Both the aerial and root tissues show tropisms. In beans, stems show *circumnutation*, a circular or helical movement of the long main stems or side branches in pursuit of a physical support (Stolarz 2009). This movement is followed by a *thigmotropism*, in response to a mechanical contact, and *twining*, a tight coiling around the physical support. It is this sequence of circumnutation – thigmotropism – twining that allows a climbing bean plant to search for and take advantage of physical support, such as for wild beans in their native environment in which they have to compete for light and other resources. Another example are domesticated climbing beans in the *milpa* agroecosystem, in which they are grown in association with maize as a physical support.

Lesser known, but likely more important, are root movements. Roots also show circumnutation, demonstrated by beans: <https://www.youtube.com/watch?v=w77zPAtVTuI>. Root circumnutation allows plants to penetrate the soil profile and find their way through open space among soil

particles and other obstacles. If it were not for circumnutation, the growing root tips would bump against soil obstacles and be prevented from penetrating soils (<https://www.youtube.com/watch?v=uXwYKymTiQg>). A further important function of root circumnutation is the search for nutrients.

Research is needed to better understand the molecular and biochemical mechanisms of plant movements (Tran et al. 2021; Taylor et al. 2021). It remains to be determined how domestication has affected these movements and whether there has been an increase or decrease in the intensity of these movements during and after domestication. For example, it may well be that the sequence circumnutation-thigmotropism-twinning has been lost wholly or partially during the evolution post-domestication from climbing types (growth habit IV; Singh 1982) to various bush types, but especially the determinate, erect growth habit (type I). In contrast, the root circumnutation may have increased to intensify the search for soil nutrients, as suggested by the increase of root whorls in domesticated beans, which builds up phosphorus uptake (Miguel et al. 2013). This intensification of resource acquisition would be consistent with the domestication syndrome in other crops, like cereals (Wright et al. 2004; Roucou et al. 2018).

### **Epilogue**

In this presentation, I have attempted to present various potential research topics. For agricultural production – and especially that of beans, which plays such an important complementary role as a source of nitrogen in agro-ecosystems and protein and other nutrients in our food systems – to keep up with the increase in human population, we have to be imaginative and innovative in three areas:

- a) the search for genetic resources, including other domesticated *Phaseolus* species, not only as a source of genetic diversity to improve common bean but also as crops for direct human consumption;
- b) the adoption of tools that make our selections more efficient, such as UAVs (drones), genomic tools, and experimental designs; and
- c) the identification of novel traits that have an impact on productivity and quality traits.

It was a pleasure to prepare and present this keynote address. I thank the Bean Improvement Cooperative and especially its president, Dr. Timothy Porch for giving me this opportunity. I wish the BIC and its members many years of productive and fascinating research on beans!

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## INCREASING BEAN PRODUCTIVITY THROUGH APPLICATION OF ZINC (ZN) AND MAGNESIUM (MG) FOLIAR FERTILIZERS

Yeboah, S.<sup>1</sup>, Oteng-Darko, P.<sup>1</sup>, Asibuo, J.<sup>1</sup>, Adomako, J.<sup>1</sup>, Acheampong, P.P.<sup>1</sup> and Butare, L.<sup>2</sup>

<sup>1</sup>CSIR-Crops Research Institute, P. O. Box 3785, Kumasi-Ghana; <sup>2</sup>International Center for Tropical Agriculture- Nairobi, Kenya

**INTRODUCTION:** Africa needs innovative plant nutrition strategies to address the multiple nutrient deficiencies that limit food production. Soil degradation is a major hindrance to the performance of agriculture production in sub-Saharan Africa (SSA). Inadequate application of nutrients to agricultural soils, extensive soil erosion, bush burning, and other factors have resulted in about 65% soil degradation (Rahman et al., 2014). Soil degradation is a major contributing factor to low agricultural productivity and rising malnutrition in SSA, including Ghana (Asiedu-Amoako et al., 2016).

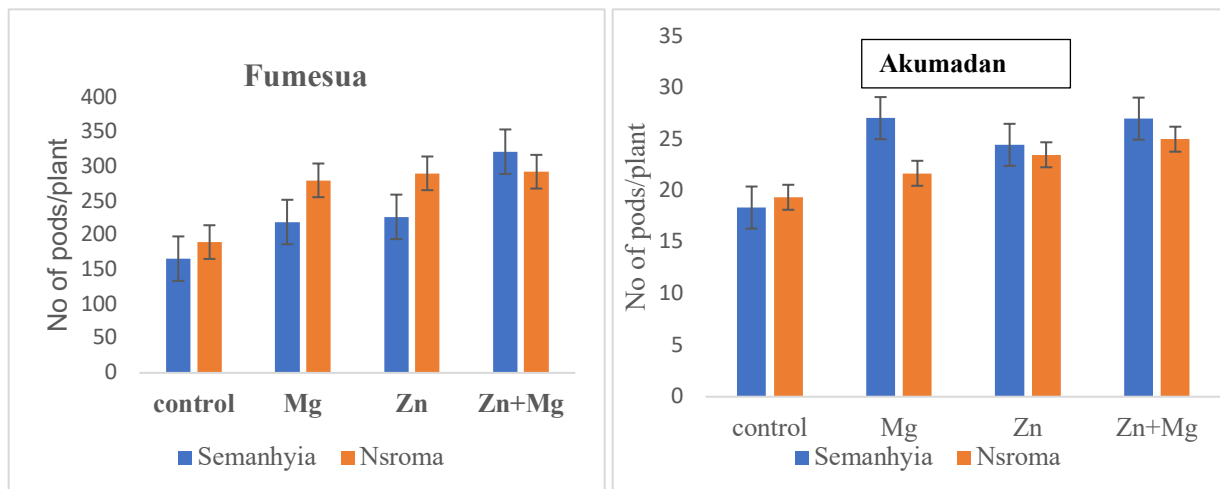
Among the micro-nutrients, iron and magnesium has been noted to be highly deficient in Ghanaian soils (Elias et al., 2017). Foliar application of fertilizers can guarantee nutrient availability to beans, leading to higher yield and seed quality. Different approaches including glycine have been used to improve mineral nutrient status of plants toward safer products and improved human health. This study was conducted to investigate the effect of zinc, magnesium, and combined zinc and magnesium foliar fertilizer on two improved common bean (*Phaseolus vulgaris* L.) varieties locally referred to as Adoye and Nsroma in the forest (Fumesua) and forest-savannah transition (Akumadan) agroecological zones of Ghana during the 2018 and 2019 cropping seasons.

Among fertilizer application methods, foliar nutrition facilitates easy and quick consumption of nutrients by the plant. It is therefore envisaged that adequate foliar supply of zinc (Zn) and magnesium (Mg) to common bean (*Phaseolus vulgaris* L.) will increase the productivity of the crop and nutritional value of the seeds.

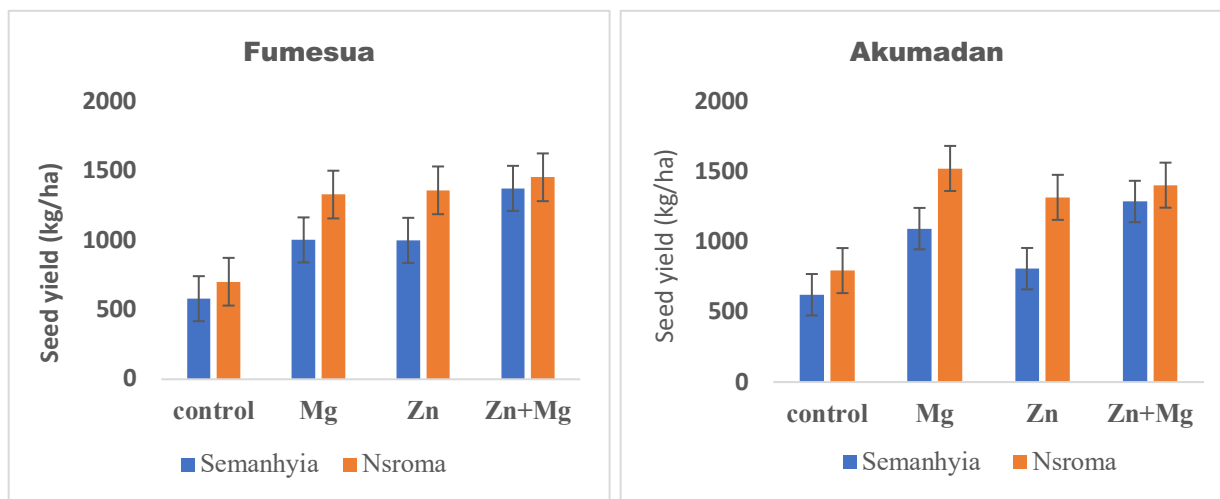
**MATERIALS AND METHODS:** The study was conducted at Fumesua and Akumadan in the forest and forest-savanna agro-ecological zones of Ghana. The experimental design utilized for this study was split-plot with common bean varieties released by CSIR-Crops Research Institute as the main plot (*Ennepa*, *Semanhya*). The sub-plots were zinc (0, 100 and 200 g ha<sup>-1</sup>) and magnesium (0, 112 and 224 g ha<sup>-1</sup>) foliar fertilizers plus control (water spray) with three replications. The concentration of micronutrients was considered as 6:1000. Zinc and magnesium foliar fertilizers were applied at vegetative and flowering stages after crop emergence. Common bean was planted at 0.2 m within rows and 0.5 m between rows with 1 m between plots and 1.5 m between replications. All the treatments received a blanket application of urea at 14 days after planting at the rate of 75 kg N/ha. Statistical analyses were undertaken using the Univariate model of the statistical package SPSS 22.0 (IBM Corporation, Chicago, IL, USA) at a probability level of 5% (P < 0.05). The differences between the means were determined using Standard Error of Difference (SED) at P < 0.05. The data from the different fertilizer rates were averaged before statistical analysis to obtain the average values.

**RESULTS AND DISCUSSION:** Irrespective of variety, the treatment performance followed a similar trend. No amendment recorded the lowest number of pods per plant irrespective of variety in all locations studied, whilst combined application of zinc (Zn) and magnesium (Mg) consistently performed better than the control (Fig. 1 and 2). Foliar application of zinc and magnesium

increased the number of pods by 23-30% compared to the control. However, differences between combined amended plots and sole zinc and magnesium amended plots were not significant in most cases. Similarly, foliar application of zinc and magnesium enhanced seed yield, which was observed in both locations (Fig. 3 and 4). The results imply that the attributes which play an essential and key role in yield production are number of pods per plant. Consistently *Nsroma* responded better to foliar application compared to *Semanhya*, indicating its potential to achieve higher production with appropriate fertilizer management. Irrespective of variety, the magnesium and zinc had comparative performance. From this, combined application of zinc and magnesium appears to have potential as a cost-effective amendment for common bean production in tropical soils environments of Ghana.



Figures 1 and 2. Number of pods/plant measured under foliar fertilization in Fumesua and Akumadan



Figures 3 and 4: Seed yield of common beans measured under foliar fertilization in Fumesua and Akumadan

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# GENOME-WIDE ASSOCIATION STUDY OF CHLOROPHYLL CONTENT AND AGRONOMIC TRAITS IN COMMON BEAN (*PHASEOLUS VULGARIS* L.)

Henry Cordoba-Novoa<sup>1</sup>, Valerio Hoyos-Villegas<sup>1\*</sup>

<sup>1</sup>Pulse Breeding and Genetics Laboratory, Department of Plant Science, McGill University, Montreal, QC, Canada. \*Corresponding author

## INTRODUCTION

Drought is one of the main abiotic stresses affecting crop productivity worldwide (Lamaoui et al., 2018). In climate change scenarios, water availability is expected to decrease and temperatures to rise. There is an urgent need to develop drought-tolerant bean varieties with improved adaptation. Identifying candidate genes involved in the control of physiological responses to water deficit is a priority in bean breeding. This study was aimed at performing GWAS analyses for lodging, pigments, and the Normalized difference vegetation index (NDVI) under natural conditions in the field.

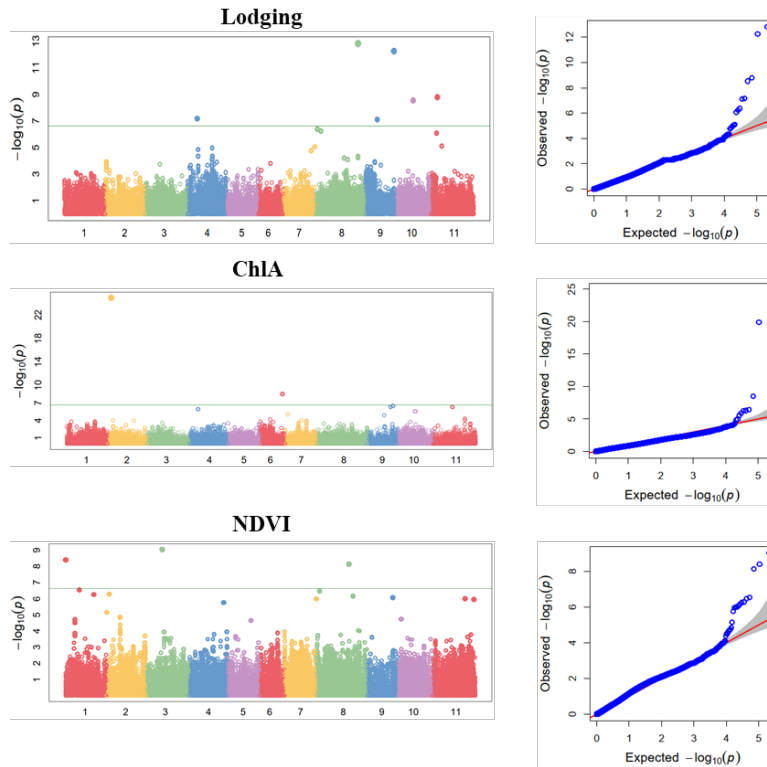
## MATERIALS AND METHODS

A set of 300 accessions of common bean from the Mesoamerican Diversity Panel (MDP) were evaluated in the summer of 2021 at the Lods Research Center of McGill University. A randomized complete block design (RCBD) with three replicates was carried out. Field phenotyping included the evaluation of lodging on a scale from 1 – 10, pigments content as previously described (Dhanapal et al., 2016), and NDVI using the Crop Circle Multispectral Sensor ACS-435 (Holland Scientific, USA). All the accessions were previously genotyped (Moghaddam et al., 2016) and a set of around 200K SNPs were available. GWAS analysis were conducted in GAPIT (Tang et al., 2016) using multiple models and the BLUP-adjusted means for the experiment. Based on the model fit, FarmCPU was selected for further analyses. Candidate genes were identified using a window of  $\pm 100$  Kb from the significant SNPs (adjusted  $p$ -value  $< 0.05$ ) by browsing the common bean reference genome v2.1 (Schmutz et al., 2014) in Phytozome.

## RESULTS

Six significant SNPs were identified for lodging on Chr04, and Chr08 – Chr11, ss54054394, ss35943424, ss7997584, ss21681250, ss12188488, ss15022480 (Figure 1). Previously, in a subset of the MDP, Hoyos-Villegas et al. (2017) identified candidate genes on Chr02 and Chr07. Our results add new candidate QTLs for lodging improvement. For pigments content, two (ss27458214 and ss9555176), eight (ss57089719, ss8011150, ss28854757, ss48231536, ss25662221, ss50893830, ss16854668, and ss26877247), four (ss35400054, ss1696489, ss48231536, and ss20435750), and six (ss8129890, ss33563948, ss16041140, ss14626484, ss32729733, and ss3329188) significant SNPs were detected for chlorophyll a (ChlA), Chl B, total Chl, and carotenoids, respectively. These SNPs were located across the bean genome. Under water deficit, Leitão et al., (2021) identified SNPs associated with pigment content on different chromosomes. NDVI is correlated with plant development and can be used to estimate above ground biomass (Peng et al., 2019). Three significant SNPs were identified for NDVI on Chr01, Chr03, and Chr08 corresponding to ss361963, ss20133620, and ss40526484.





**Figure 1.** Manhattan and QQ plots for GWAS analyses for lodging, Chlorophyll A, and NDVI. The green line represents the threshold to consider a SNP significant after FDR correction for multiple testing.

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## GENOTYPE BY ENVIRONMENT INTERACTION EFFECT AND STABILITY ANALYSIS FOR COMMON BEAN (*PHASEOLUS VULGARIS* L.) IN BURUNDI

Eric Nduwarugira<sup>1</sup> and Susan Nchimbi-Msolla<sup>2</sup>, Mukankusi Clare Mugisha<sup>3</sup>, Rowland Chirwa<sup>4</sup> and Jean Berchmans Bizimana<sup>5</sup>

<sup>1</sup> Institut des Sciences Agronomiques du Burundi -ISABU-, Bujumbura-Burundi; <sup>2</sup>Sokoine University of Agriculture -SUA-, Morogoro, Tanzania; <sup>3</sup> International Center for Tropical Agriculture, CIAT Uganda, Kampala, Uganda, <sup>4</sup> International Center for Tropical Agriculture - CIAT Malawi-. <sup>5</sup>International Rice Research Institute, Bujumbura-Burundi.

**INTRODUCTION:** Bean is among the most essential subsistence food crops in Burundi as it forms an important part of the daily diet providing 20% of required calories and 50% of proteins. The crop is playing a significant role in the smallholder economy as an imperative source of proteins and income as a cash crop. Despite its importance and role, its productivity is constrained by biotic and abiotic stresses such as diseases, declining soil fertility, poor crop management practices, including low inputs and poor access to improved germplasm, and unreliable climatic conditions. This has led to low agricultural productivity by the smallholder farmers. The variability in environment, namely locations, seasonal fluctuations and their interactions, highly influence the performance of genotypes in relation to yield potential. The objective of this study was to assess GxE interaction effect and stability analysis.

**MATERIALS AND METHODS:** The study was conducted in Burundi at the ISABU research institute with 8 lines during the second (2013B) cropping season. Experimental material comprised 8 biofortified genotypes of common bean (*Phaseolus vulgaris* L.). The experiments were laid out in a split-plot design in each location and replicated three times. The Location was considered as the main plot (factor A); while the spacing was considered as sub-plot (factor B) and genotypes were taken as sub-sub plot (factor C). Three types of spacings were used at each location in order to create nine micro environments for stability analysis. The collected data included grain yield (GY) in ton/ha, days to 50% flowering (DFL), days to physiological maturity (DPM), plant height in cm (PH), number of pods per plant (P/P), number of seeds per pod (S/P), one hundred seed weight (HSW) in grams.

**RESULTS AND CONCLUSIONS:** The G x E interaction was observed and results of the combined analysis of variance revealed that the studied genotypes differed significantly for all of the traits. It has been concluded that environmental factors significantly affected the performance of the bean genotypes. Based on good performance, each site presented genotypes which were comparable to the check. However, since the check was the latest to mature, the genotypes which performed almost the same to the check variety across seasons, namely KAB06F2-8-24, KAB06F2-8-78, KAB06F2-8-53 and KAB06F2-8-13, were recommended for other stages of selection due to their early maturity trait (Table 1). Results also revealed the complete absence of genotypes that were widely adapted and stable in seed yield (Table 2). However, genotypes KAB06F2-8-24 and KAB06F2-8-78 were adapted to low yielding environments whereas KAB06F2-8-53 and KAB06F2-8-13 were found suitable to fertile environmental conditions. Based on the results of this study, future studies should focus on more seasons and locations in different agro-ecological zones in order to support the results obtained in this study.

**Table 1.** Main effect of genotypes on yield and yields components in combined sites during the second cropping seasons of the 2013.

Genotypes	DFL	PH	DPM	P/P	S/P	HSW (gr)	GY (t)
1	38.0bc	65.9b	79.0b	11.0ab	3.0b	44.5b	2.3ab
2	39.0b	62.5bc	79.0b	11.0ab	3.0b	39.8e	2.2cde
3	38.0bc	55.5d	79.0b	12.0a	4.0a	38.3e	2.2cde
4	38.0bc	63.6bc	79.0b	10.0ab	4.0a	43.2cd	2.1cde
5	38.0bc	59.7cd	79.0b	9.0b	3.0b	46.6a	2.1de
6	38.0bc	61.5bc	79.0b	9.0b	3.0b	41.8de	2.0e
7	38.0bc	66.8ab	79.0b	10.0ab	3.0b	42.2cde	2.3abc
8	42.0a	71.4a	87.0a	9.0b	4.0a	43.6bc	2.5a
Mean	39.0	63.4	80.0	10.0	4.0	42.5	2.2
S.E.	0.1	1.5	0.9	0.3	0.1	0.3	0.0
C.V. (%)	6.2	34.3	15.8	47.7	41.5	10.8	28.1
Spac1 (15cm x 40cm)	39.0a	60.9b	80.0a	9.0b	4.0a	41.8b	2.4a
Spac2 (20cm x 40cm)	39.0a	64.6a	80.0a	10.0b	4.0a	42.0b	2.2ab
Spac3 (40cm x 40cm)	39.0a	64.6a	81.0a	12.0a	4.0a	43.7a	2.0b
Mean	39	63.4	80.0	10.0	4.0	42.5	2.2
S.E.	0.3	2.6	1.0	0.5	0.2	0.5	0.1
C.V. (%)	6.2	34.4	10.1	49.5	38.9	10.5	27.3
S1: Gisozi	40.0a	40.9c	91.0a	9.0b	3.0a	39.2b	1.9a
S2: Murongwe	40.0a	63.8b	77.0b	9.0b	3.0	44.4a	2.5a
S3: Moso	36.0b	85.4a	74.0c	13.0a	4.0a	43.9a	2.2a
Mean	42.0	63.4	81.0	10.0	3.0	42.4	2.2
S.E.	0.2	1.4	0.4	0.5	0.2	0.5	0.1
C.V. (%)	4.4	20.3	3.9	36.7	36.2	9.2	25.1

**Key:** Spac= Spacing, Geno= Genotype, S. E=Standard Error, CV= Coefficient of Variation, t= tons, gr=grams, S= Site, 1= KAB06F2-8-53, 2= KAB06F2-8-24, 3= KAB06F2-8-78, 4= KAB06F2-8-135, 5= KAB06F2-8-22, 6= KAB10F2-8-136, 7= KAB06F2-8-13, 8= GLP2

**Table 2.** Relationship among stability parameters with seed yield and its components during the second cropping season of 2013.

Genotypes	P/P			S/P			100 SW (g)			SY (t/ha)		
	Mean	$\beta_i$	S <sup>2</sup> d	Mea	$\beta_i$	S <sup>2</sup> d	Mea	$\beta_i$	S <sup>2</sup> d	Mea	$\beta_i$	S <sup>2</sup> d
1	11	1.35	-5.07	3	0.69	-0.67	44.52	1.32*	-1.75	2.31	1.2	0.024
2	11	0.44*	-4.89	3	0.44	-0.61	39.75	0.85	-0.67	2.19	0.74	-0.055
3	12	2.04*	-1.96	4	2.95	0.47	38.33	1.18	1.89	2.2	0.58	-0.048
4	10	0.9	-3.60	4	0.9	1.23*	43.22	1.4	0.08	2.14	0.262	-0.059
5	9	0.52*	-5.21	3	0.7	-0.66	46.55	1.23	0.74	2.06	0.318	-0.07
6	9	1.06	-4.83	3	0.65	-0.58	41.8	1.13	-0.74	2.02	1.18	-0.03
7	10	0.89	-0.95	3	0.42	-0.6	42.2	1.12	-1.94	2.28	1.2	-0.09

# COMPARATIVE ANALYSIS OF DIFFERENT PHENOTYPIC AND GENOTYPIC SELECTION STRATEGIES TO INCREASE THE GENETIC GAIN FOR YIELD FROM A USING NESTED ASSOCIATION MAPPING POPULATION IN DRY BEAN

Maryam Vazin and K. Peter Pauls<sup>1</sup>

<sup>1</sup>University of Guelph Bean Breeding Program

## INTRODUCTION

Understanding the genetic basis of complex traits, including yield in crops such as common bean (*Phaseolus vulgaris* L.), is the most sustainable way to address the growing global demand for food. However, yield and its related traits are controlled by multiple genes with major and minor allele effects. The aim of plant breeding is to continually improve advantageous traits in order to make the crops more efficient. Several selection strategies can be implemented to increase the annual gain in yield potential in common bean breeding programs. The use of Nested Association Mapping (NAM) populations combines the advantages of linkage mapping and association mapping and is the choice for mapping the effects of rare alleles in germplasm collections.

## MATERIAL AND METHODS

A Nested Association Mapping (NAM) population of 600 F<sub>4:5</sub> recombinant inbred lines (RIL) were created with the cultivar Ex Rico 23 as the common parent, and 10 founder lines that span the genetic diversity of Ontario Mesoamerican germplasm. F<sub>1</sub>S of the population were self-pollinated to produce recombinant inbred lines. 600 RILs from the NAM population (approximately 60 /cross) were evaluated for two years (2016, 2017) at two field sites: Guelph Elora Research Station (ERS) and Woodstock Research Station (WRS) in Ontario, Canada. The field trials were planted as a 6 x (10 x 10) unbalanced square lattice design. The experimental unit for all field locations was a 36 cm x 1.9 m plot with 135 seeds planted at 2.5 cm and they were characterized for different agronomic traits including yield, days to 50% flowering, and days to maturity in the field in four environments.

A phenotypic selection experiment was conducted to determine the theoretical limit for gain from selection from the NAM population. In order to test the effectiveness of phenotypic selection, two populations were created, based on twenty-five RILs with the lowest and highest productivity in 2016 and 2017. In addition, a random sample of RILS was selected as an environmental control population in replicated field trails in 2019 at ERS and WRS, in Ontario, Canada in 6 environments. The field trials were planted as a 9 x 10 unbalanced square lattice design. All experimental designs were designed with Agrobase®99 software.

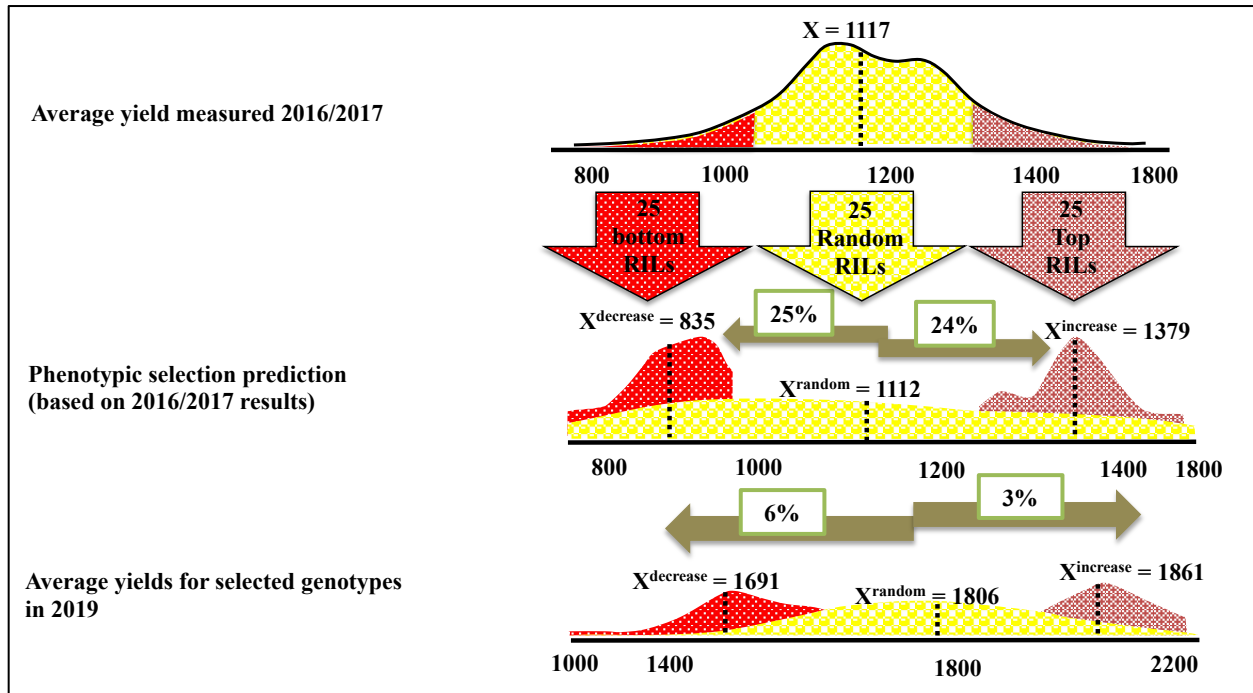
## RESULTS AND DISCUSSION

The distribution of all the traits, days to 50% flowering, days to maturity, and yield was continuous and showed some transgressive segregation compared to the parental lines for each environment. The results indicate that higher genetic gain for yield in the population was created by selective population in comparison with random selection.

The distribution of average yield for 2016 and 2017, followed by the predicted and actual yield selection comparison data from 2019 is shown in Figure 1. The average yields of the NAM population, represented by the average for 2016/17 and the random selection in 2019, increased by 61% from 2016/17 to 2019 illustrating the effect that the environment can have on quantitative traits, like yield (Figure1).

The predicted yield gain from selection for the two populations was 24% for the high selection and 25% for the low population, when compared to the population of randomly selected RILs. The actual effect of selection was only 3% for the high yielding selection and 6% for the low yielding population (Figure 1).

Molecular markers, linked to genes controlling quantitative traits might be more efficient in selecting for superior genotypes because they are not influenced by environmental factors. In the future, genomics and QTL index selection strategies will be compared with other tested selection methods to evaluate their efficiency for increasing the overall genetic gain for yield in the tested NAM population.



**Figure 1.** Predicted and actual yields ( $\text{kg ha}^{-1}$ ) for populations selected from 600  $F_{4:5}$  RILs in the navy bean NAM population in two years (2016 and 2017) compared to 2019 at the Elora Research Station (ERS) and the Woodstock Research Station (WRS).

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# RESISTANCE OF COMMON BEAN LINES TO ROOT ROT CAUSED BY *FUSARIUM SOLANI*

Yohari E. Torres-González<sup>1</sup>, Consuelo Estévez De Jensen<sup>1</sup>, James S. Beaver<sup>1</sup>,  
Timothy G. Porch<sup>2</sup>

<sup>1</sup>Department of Agroenvironmental Sciences, University of Puerto Rico-Mayagüez

<sup>2</sup>USDA-ARS-TARS, Mayagüez, PR

## INTRODUCTION

*Fusarium* root rot (FRR), caused by *Fusarium solani* is a common soil-borne disease affecting common bean production in the tropics. Yield losses associated with FRR may surpass 80% in susceptible cultivars (Schneider et al., 2001). The *Fusarium solani* species complex may cause foot and root rot; symptoms such as wilting and chlorosis on the aerial portion of the plant depend on genotype susceptibility and the crop growth. The severity of root rot caused by *F. solani* may increase due to environmental factors that stress the plant (Cichy et al., 2007). The development of resistant common bean varieties is the most effective strategy to control the disease, however sources of resistance to *F. solani* are limited. The objective of this study was to identify common bean lines from the BASE 120 nursery and advanced lines developed by USDA- Puerto Rico with resistance to *F. solani*.

## MATERIALS AND METHODS

The response of 189 common bean lines to *Fusarium* root rot (FRR) was evaluated in a root rot field planted continuously with beans for more than 50 years in Isabela, PR. Plants were removed from the soil and disease severity was assessed using a CIAT Scale (1-9) (Van Schoonhoven & Pastor-Corrales, 1987). Root fragments were surface sterilized and placed on aPDA media for fungal isolate recovery from the roots of each line. *Fusarium* spp. isolates were identified based on morphological and cultural characteristics, as well as a PCR assay using primers corresponding to the ITS, Elongation Factor and B- tubuline regions. For both experiments, mean disease severity was compared using an ANOVA and LSD Fisher test ( $P < 0.05$ ).

Under screen house conditions, in Fortuna Station, Juana Diaz, the reaction of 26 common bean lines to *Fusarium solani* was assessed after inoculation with isolate 19-00514. Plants were inoculated eight and fourteen days after planting at a concentration of  $5 \times 10^6$  macroconidia/ ml/ plant. Disease severity was evaluated 21 days after planting using the CIAT scale (1-9). For isolate recovery, root fragments were surface sterilized with bleach (10%), rinsed three times and placed on aPDA media.

## RESULTS AND DISCUSSION

In the screen house trial, mean disease severity of two white beans “Bella” (1.5) and “Beníquez” (1.5) were significantly less severe ( $P > 0.05$ ) when compared to TARS-LH1 (6) (Table 1.). Montcalm and VAX 1 mean disease severity values were 4.5 and 4, respectively. In the field trial, the response to *Fusarium* root rot of the 189 common bean lines varied. The mean disease severity values ranged between 3.1- 4.6. Isolates identified were *F. brachygibbosum*, *F. oxysporum* *F. chlamydosporum* and *Fusarium* spp. Outstanding lines from the 189 lines were: 20IS-9319 (DS 1), 20IS-9347 (DS 1) and 20IS-9416 (DS 1) (data not shown). The results obtained from the field trial were confirmed using controlled inoculations in the screen house evaluation. We recommend an evaluation of diverse common bean lines across locations and strains for the development of a differential set of lines for *Fusarium* root rot.

**Table 1. *Fusarium* root rot disease severity (DS) on 26 common bean lines.**

Susceptible Lines	DS <sup>1</sup> (1-9)	Resistant Lines	DS (1-9)
TARS-LH1	6	BAT-477	3
Amadeus	5	TARS-MST1	3
USRM-20	4.5	G21212	3
VAX 1	4.5	PR0443-151	3
Montcalm	4	TARS-LFR1	2.5
SEF-10	4	MEN-2201-04	2.5
TARS-HT1	4	PR1165-3	2.5
BIOF-470	4	TARS-10IS-2421	2.5
Redhawk	3.5	BK-92	2.5
Matterhorn	3.5	A686	2.5
Matambú	3.5	NCB-280	2
TARS-VCI-4B	3.5	Bella	1.5
Zorro	3	Beníquez	1.5

<sup>1</sup> Rated on the CIAT 1-9 scale where 1=no symptoms and 9=very severe symptoms.

\*\*P<0.05; LSD= 2.70

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## FIELD AND MOLECULAR SCREENING OF BACTERIAL DISEASES IN ONTARIO-ADAPTED DRY BEAN GENOTYPES

Caio Rodrigues Correa<sup>1</sup>, Emily Morneau<sup>2</sup>, BaiLing Zhang<sup>2</sup>, Alyssa Thibodeau<sup>2</sup>, Owen Wally<sup>2</sup>, Jamie Larsen<sup>2</sup>, Chris Gillard<sup>1</sup>

<sup>1</sup>Department of Plant Agriculture, University of Guelph, Guelph, ON, Canada N1G 2W1;

<sup>2</sup>Harrow Research and Development Centre AAFC, Harrow, ON, Canada N0R 1G0.

**INTRODUCTION:** Bacterial blights are a recurring constraint for dry bean production in Ontario, causing yield losses of 20-45% (Boersma et al. 2015), and increasing costs due to importation of certified disease-free seed from the US. Control measures, such as intercropping, crop rotation, and copper-based bactericides provide erratic results, and the development of resistant cultivars is considered the most sustainable and cost-effective strategy. Common bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) and *X. fuscans* subsp. *fuscans* (*Xff*) is the most widespread and well-known bacterial disease of common bean, while studies on bacterial brown spot (BBS), caused by *Pseudomonas syringae* pv. *syringae* (*Pss*), are scarce, despite potential yield losses of 55% (Muedi et al. 2015). Several molecular markers associated with CBB resistance have been developed, but it is unclear if these markers can also predict disease response to BBS. Thus, the present study aims to evaluate the resistance levels of Ontario-adapted common bean germplasm to CBB and BBS in the field, and to test the efficacy of three molecular markers in predicting disease response in the field.

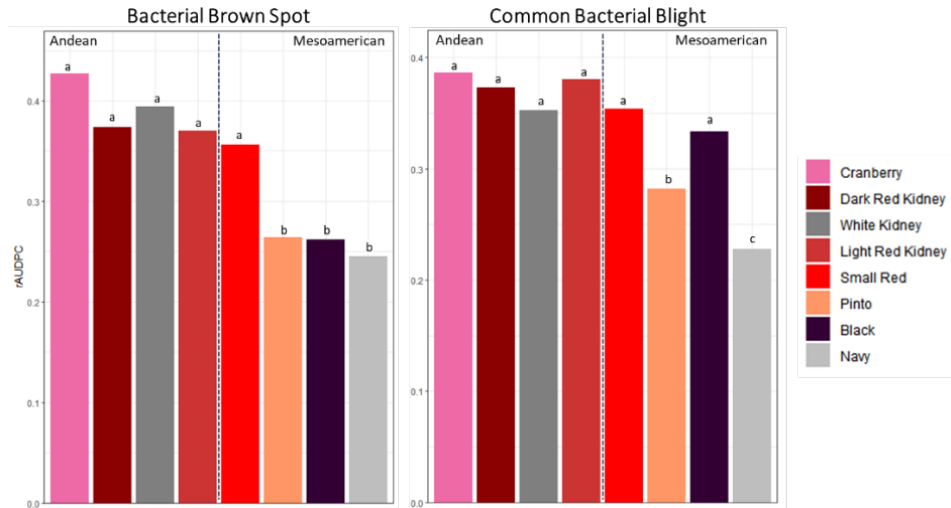
**MATERIAL AND METHODS:** A total of 85 Ontario-adapted genotypes of common bean, comprising eight market classes were evaluated under field conditions in shared Agriculture and Agri-Food Canada (AAFC) and University of Guelph-Ridgetown disease nurseries. The BBS nurseries were planted in Exeter and London, ON, while the CBB nurseries were planted in Harrow and London, ON. The experimental design at all locations was a randomized block design with four replications. The genotypes were planted as a ‘hill plot’ of seven seeds with 75 cm between plots and 61 cm between rows. Plants were artificially inoculated using a mechanical sprayer at 40-50 days after planting. The BBS inoculum was prepared by mixing equal parts of *Pss* isolates 39 and 40 (provided by S. Chatterton, AAFC-Lethbridge) at a concentration of 10<sup>8</sup> CFU/mL. Similarly, the CBB inoculum was prepared using two *Xap* isolates (18 and 98) and two *Xff* isolates (12 and 118). Plants were rated twice for disease symptoms using a 0-5 scale to calculate the relative area under the disease progress curve (rAUDPC) values. The genotypes were screened via PCR for the presence of three molecular markers previously identified to be associated with CBB resistance: BC420-CG9, G7-NPP (also known as SU91; Morneau et al. 2019), and SAP6. Analysis of variance was performed to detect differences in disease ratings between BBS and CBB, among market classes, and marker states.

**RESULTS AND DISCUSSION:** Mesoamerican beans exhibited higher resistance levels than Andean beans for both bacterial brown spot and common bacterial blight (Fig. 1, Fig. 2). Ratings for both diseases varied among market classes (Fig. 1). There was a strong positive correlation ( $r = 0.73$ ,  $p < 0.001$ ) between BBS and CBB ratings (Fig. 2), which suggests that there are common loci controlling the disease response against both pathogen species. This finding is further supported by the single marker analysis results, which showed significant differences ( $p < 0.05$ ) between marker states for all markers, for both CBB and BBS (Table 1). Our results indicate that CBB markers BC420-CG9, G7-NPP, and SAP6 can also be used to select lines resistant to BBS.

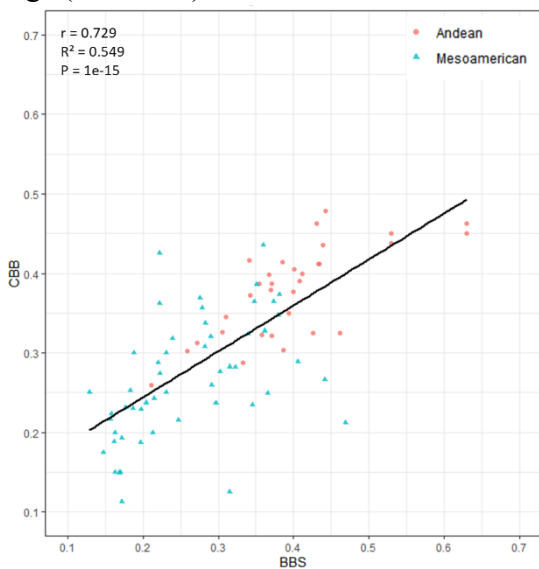


Further research should focus on identifying resistance loci specific to each disease, and breeding efforts should aim to improve resistance for Andean classes, which are particularly susceptible to bacterial diseases.

**Figure 1:** Bacterial brown spot and common bacterial blight mean ratings (rAUDPC) by market class. Means followed by the same letter were statistically identical according to Scott-Knott test.



**Figure 2:** Correlation between BBS and CBB ratings (rAUDPC).



**Table 1:** BBS and CBB mean ratings by DNA marker.

Marker	Band size	Ratings (rAUDPC)	
		BBS	CBB
BC420	375 bp	0.286	0.306
	415 bp	0.226	0.209
G7-NPP	535 bp	0.340	0.341
	956 bp	0.185	0.242
SAP6	no band	0.306	0.324
	820 bp	0.284	0.307

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# PHENOTYPING INSECT RESISTANCE WITH AUTONOMOUS FIELD-BASED INSECT SENSORS

KJ Gibson<sup>1</sup>, EN Bick<sup>2,3</sup>, A Palkovic<sup>1</sup>, and P Gepts<sup>1</sup>

<sup>1</sup>Department of Plant Sciences, University of California, Davis, Davis, California, USA

<sup>2</sup>Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg, DK &

<sup>3</sup>FaunaPhotonics APS, Støberigade 14, 2450, Copenhagen, SV, DK

## INTRODUCTION

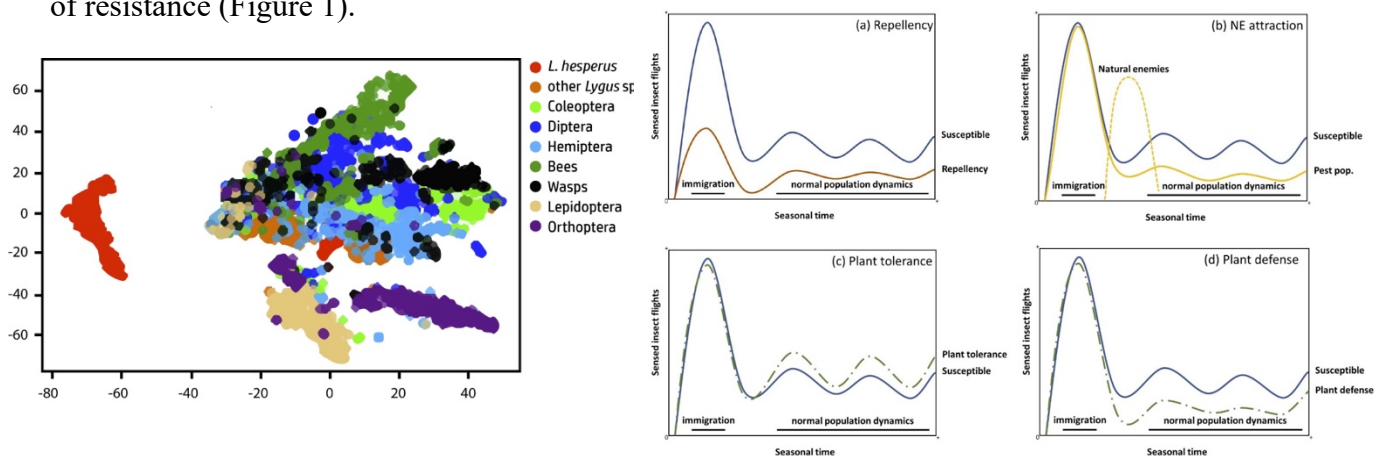
Breeding programs predominantly identify insect resistance with yield trials, manual sampling, and visual scoring. Manually collected insect data typically includes discrete temporal counts from sweep netting, taping, and vacuuming or cumulative measurements from traps and sticky cards (Hillhouse and Pitre, 1974; McCravy, 2018). These labor-intensive methods lack the precision and detail to identify the mechanisms of resistance. With more precise phenotype data, breeders would be able to apply advanced breeding techniques including marker-assisted selection, genomic selection, and interspecific hybridization (Rubiales *et al* 2015). Autonomous field-based insect sensors may provide researchers with the quantity and quality of data needed to more precisely and efficiently breed for insect resistance. In this study, we tested the use of these sensors in Lima bean for phenotyping resistance to the Western Tarnished Plant Bug, *Lygus hesperus* Knight (Hemiptera: Miridae).

## MATERIALS AND METHODS

On May 15, 2021, three commercial varieties of Lima beans (UC 92: *Lygus* susceptible; UC Haskell and UC Beija Flor: *Lygus* resistant) were planted at the Plant Sciences Field Facility of the University of California, Davis (38°32'18.8"N 121°47'19.9"W). The planting was 42 m long, 4 rows wide with 5 cm spacing within rows. The field was conventionally managed with drip irrigation and no insecticides. A Volito insect sensor (FaunaPhotonics Aps., Copenhagen, Denmark) was placed above each variety. The sensor functions by emitting infrared light that is backscattered to a photodiode receiver when interrupted by an insect flight, resulting in a timeseries of light intensity (Rydmer *et al.*, 2022). The sensor was placed on the edge of the planting 28 m from the end of the strip. The sensor's measurement volume was placed above canopy height, over the 4 rows of the planting. The sensor was raised weekly to maintain consistent height above canopy. The sensors measured insect flights continuously from June 17th to September 1<sup>st</sup>, 2021. Flowering starting on June 25th, 2021 for UC 92, June 30<sup>th</sup>, 2021 for UC Beija Flor, and July 11<sup>th</sup>, 2021 for UC Haskell. To verify the sensor data, each plot contained two water traps which were emptied every three days. These samples were analyzed for total number of insects and number of adult *L. hesperus*.

Neural networks applied to optical insect sensor data can classify signal to species (Kirkeby *et al.*, 2021). A multiclass convolutional neural network (as specified in Bick, Edwards, and De Fine Licht, 2021) was trained, validated, and tested on sensor-recorded events from 10 insect groups. Specifically, we trained the model to compare signal from *L. hesperus* flights to European *Lygus* spp., Coleoptera, Diptera, other Hemiptera, Bees, Wasps, Lepidoptera, and Orthoptera flights. This algorithm was applied to field data to identify sensed *L. hesperus* (Figure 1).

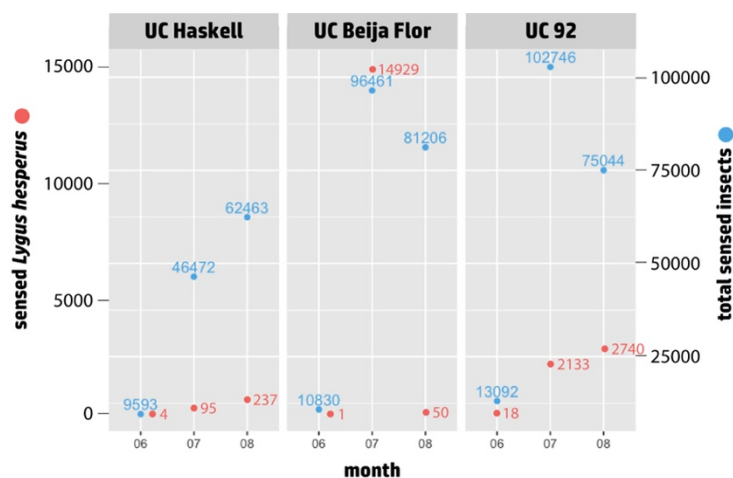
We hypothesize that a susceptible variety, like UC 92, would have a high peak of sensed flights early in the season as insects migrate into the field followed by stable but fluctuating levels of sensed insect flights throughout the season. Resistant varieties like UC Haskell and UC Beija Flor would be expected to have different patterns of sensed flights based on the specific mechanism of resistance (Figure 1).



**Figure 1: (left) TSN-e Plot showing validation of machine learning classifier (right) Graphical representation of hypothesized patterns of sensed insect flights in resistant varieties**

## RESULTS AND DISCUSSION

As shown in Figure 2, we found that UC 92 (yield = 729g/15ft plot) was the more consistently attractive host variety with flights steadily increasing over the course of the season, possibly due to a combination of immigration into and reproduction within the plot. UC Beija Flor (yield = 1,231g/15ft plot) exhibits a tolerance strategy, with high *Lygus* numbers in the economically critical July flowering window. UC Haskell (yield = 1,891g/15ft plot) exhibits a repellency strategy with low numbers of *Lygus* present throughout the season with moderate growth in numbers that can be attributed to reproduction within the plot.



**Figure 2: Total sensed insects (blue) and sensed *Lygus hesperus* (red) for each variety by month, note only the last 13 days in June were measured.**

## ACKNOWLEDGEMENTS

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# A CASE FOR BREEDING ORGANIC SNAP BEANS IN AN ORGANIC SELECTION ENVIRONMENT

Hayley E. Park<sup>1</sup>, Ryan M. King<sup>2</sup>, James R. Myers<sup>1</sup>

<sup>1</sup>Department of Horticulture, Oregon State University, Corvallis, OR

<sup>2</sup>USDA-Agricultural Research Service, Corvallis, OR

## INTRODUCTION

Oregon ranked 4<sup>th</sup> nationwide in 2019 for both conventional and organic snap bean production, and 2<sup>nd</sup> nationwide for production of organic snap beans intended for processing (USDA - National Agricultural Statistics Service, n.d., 2020). If the increasing acreage of organic crops across the United States can be assumed to be true within the state, there is a need for an increased focus on developing systems of support for Oregon's organic farms. Among the requisite supports are improved snap bean varieties that are suited to organic farming in the Pacific Northwest regions. Snap bean production in western Oregon is consistently marked by cool, wet spring conditions that hamper stand establishment and early season performance. Factors such as seed color and testa thickness are favorable for improved germination, however, this conflicts with the processing industry's preference for white-seeded cultivars.

To identify breeding objectives for organic snap beans, we created and utilized 4 populations of recombinant inbred families. These populations are a result of two initial crosses made in the winter of 2015. Following the F<sub>1</sub> generation of these crosses, each of the two recombinant inbred populations was separated into two groups and grown in parallel under conventional and organic conditions. This process resulted in four populations, which are summarized in Table 1. The parents used in crosses were selected in a manner that pairs a modern, elite snap bean to an older variety with documented performance in organic and low-input conditions. The seed color in the elite parental cultivars, OR5630 and Hystyle, ranges from white to light green (persistent color), respectively. The seed color in the traditionally organic parental lines, Provider and Black Valentine, ranges from purple to black, respectively.

**Table 1.** Four snap bean populations were created following the F<sub>1</sub> generation of two crosses, providing a means of comparing the role of breeding environment during inbreeding.

Population	Cross	Selection System
HYPR-O	Hystyle x Provider	Organic
HYPR-C	Hystyle x Provider	Conventional
ORBV-O	OR5630 x Black Valentine	Organic
ORBV-C	OR5630 x Black Valentine	Conventional

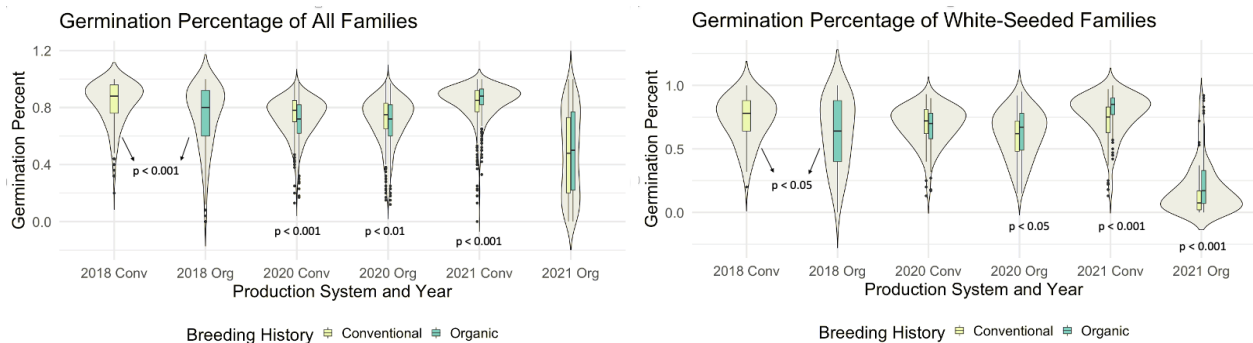
Over three years of multi-environment studies, under both conventional and organic management, we explored the following questions: How does seed color relate to early-season performance? Does breeding in an organic selection environment alter seed color or other early season performance factors?

## RESULTS

### Germination Rate

Our trials indicated a broad trend towards a lower germination rate among white-seeded accessions. As seen in Figure 1, the germination percentage overall was lower for white-seeded families in every season and environment, when compared to data for the combined colored and white seeded families.

When evaluating germination rate for all families, both colored and white seeded, a significant trend towards higher germination among conventionally bred accessions was observed in both conventional and organic production environments. This trend was reversed, however, when white seeded accessions were considered independently. In the latter case, the white seeded accessions bred under organic field management had a higher germination rate in comparison with their conventionally bred counterparts.



**Figure 1.** Germination rates were reported for each year, with all four populations grown in both a conventional and organic production environment.

## DISCUSSION

These results bolster our hypothesis that breeding under organic management is key to developing crop varieties that perform well on organic farms. In evaluating the snap bean germination data, we saw a significant trend towards higher germination among white seeded accessions that were bred under organic management. This finding is particularly relevant to the organic processing market, which must operate without the assistance of fungicides to boost germination. Previous findings have indicated that seed coat thickness and durability is tied to both the presence of the persistent color (*pc*) trait and seed coat pigment (Cirak & Myers, 2021). Considering this, our results lead to questions regarding the role of the *pc* trait in our populations and how it relates to selection under conventional or organic management.

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# EVALUATING THE EFFECT OF FUNGICIDE ON ENDOPHYTES IN SOYBEAN (*GLYCINE MAX L.*)

**Bijula M. Sureshabu, Nathan Braun, Brian Kontz, Senthil Subramanian and Febina M. Mathew**

Department of Agronomy, Horticulture and Plant Science,  
South Dakota State University, Brookings, SD, USA

## INTRODUCTION

Endophytes are the organisms inhabit within the plant, and they can be saprophytes, mutualistic, or pathogens (Redman et al. 2001). Among the endophytes such as *Diaporthe* sp. (Sinclair 1993), *Fusarium* sp. (Diaz Arias et al. 2011), and *Alternaria* sp. (Kunwar et al. 1986) have been reported to cause disease in soybean (*Glycine max L.*). To manage diseases, soybean farmers use foliar fungicides (Wise and Mueller 2011), however, it has been reported that certain fungicides, such as those belonging to quinone outside inhibitor fungicides (QoI), increased the incidence of endophytic *Diaporthe*, although, the fungicide decreased the incidence of *Alternaria* sp. in soybean (Batzer and Mueller 2020).

The objective of this study is to determine the effect of soybean labelled fungicides; pyraclostrobin (FRAC 11), boscalid (FRAC 7) and tebuconazole (FRAC 3) on the pathogenic fungal endophytes in the soybean seeds.

## MATERIALS AND METHODS

A field trial was conducted in Felt Farm, Brookings, South Dakota in 2021. The variety used for planting belonged to maturity group 1.5, in four rows plots with 30 inches spacing between each row. Treatments were designed in a randomized complete block design with four replications and non-treated control. Fungicides, pyraclostrobin (Headline), boscalid (Endura), and tebuconazole (Folicur), were sprayed at a rate of 0.44L/ha at R3 growth stage, when the pods began to fill. At R7 growth stage, when the pods were fully filled and matured, 10 plants were randomly selected from the outer rows of each plot, leaving the middle rows for harvest. Seed samples (n=16) were surface sterilized with 0.4% sodium hypochlorite, 70% ethanol and rinsed twice with sterile water and placed in full strength PDA media amended with streptomycin in 9-cm diameter Petri plates. Seed germination and fungal growth were observed after 2 to 3 weeks of incubation. New hyphal tips were transferred to new plates to obtain pure cultures. Statistical analyses were done using a multivariate community analysis model in R software (v2.11.1; R core team 2012; “mvabund” package), to compare the composition of endophytes belonging to the dominant fungal genera between the fungicide treated and the non-treated control plots.

## RESULTS

Based on the morphological features of the fungal isolates (colony growth, spore production), the dominant genera were *Diaporthe*, *Alternaria* and *Fusarium* among the seven genera (*Alternaria*, *Diaporthe*, *Fusarium*, *Cercospora*, *Gibellulopsis*, *Peniphora* and *Phaeosphaeriopsis*).

Multi-variate analyses showed that pyraclostrobin significantly decreased the isolation frequency of *Fusarium* sp. (p=0.001) compared to the non-treated control (Table 1). Also, application of pyraclostrobin, tebuconazole and boscalid did not significantly affect the isolation frequency of *Diaporthe* or *Alternaria* sp. (Table 1, 2 and 3).

**Table 1.** Effect of pyraclostrobin (Headline) on isolation frequency of *Diaporthe*, *Alternaria* and *Fusarium* ( $\alpha=0.05$ )

	<i>Diaporthe</i>	<i>Alternaria</i>	<i>Fusarium</i>
Wald stat	1.76	1.46	0.029
<i>p</i> -value	0.065	0.18	0.001
Coefficient	-1.65	0.78	-14.51

**Table 2.** Effect of boscalid (Endura) on isolation frequency of *Diaporthe*, *Alternaria* and *Fusarium* ( $\alpha=0.05$ )

	<i>Diaporthe</i>	<i>Alternaria</i>	<i>Fusarium</i>
Wald stat	1.03	1.824	0.000
<i>p</i> -value	0.40	0.07	0.87
Coefficient	-1.50	-0.55	-14.51

**Table 3.** Effect of tebuconazole (Folicur) on isolation frequency of *Diaporthe*, *Alternaria* and *Fusarium* ( $\alpha=0.05$ )

	<i>Diaporthe</i>	<i>Alternaria</i>	<i>Fusarium</i>
Wald stat	1.170	0.893	0.000
<i>p</i> -value	0.372	0.335	0.86
Coefficient	-0.844	0.826	<0.000014

## CONCLUSION

Our preliminary study suggests that foliar fungicides may not affect the incidence of endophytic *Alternaria* and *Diaporthe* in soybean seeds.

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## HEAVY DUTY LEGUMES: GETTING TO THE ROOT OF ABIOTIC STRESS TOLERANCE IN BEANS

Olga Khmel'nitsky<sup>1</sup>, Hayley Sussman<sup>1</sup>, Li'ang Yu<sup>1</sup>, Andrew D.L. Nelson<sup>1</sup>,  
Magdalena M. Julkowska<sup>1</sup>

<sup>1</sup>Boyce Thompson Institute, 533 Tower Road, NY 14853 Ithaca, United States of America

**INTRODUCTION:** Cowpea (*Vigna unguiculata*) and tepary bean (*Phaseolus acutifolius*) are important crops in dry-land agriculture and a source of protein for many subsistence farmers. Both cowpea and tepary bean exhibit an ability to adapt to abiotic stresses like drought, salt and heat, yet the molecular mechanisms underlying these adaptations remain to be unveiled. While natural diversity panels of both species are available to the community or are currently being developed, the phenotyping methods to study abiotic stress responses under a controlled environment have not been established. In this study, we present a cost-efficient phenotyping setup for legume phenotyping, and examine physiological responses to drought stress in tepary and five cowpea accessions by comparing them to the drought-sensitive common bean plant (*Phaseolus vulgaris*). We also examined responses to heat and salt stress in the five cowpea accessions.

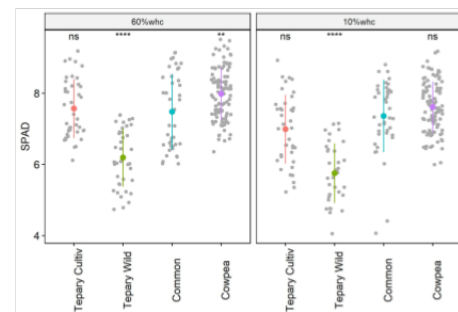
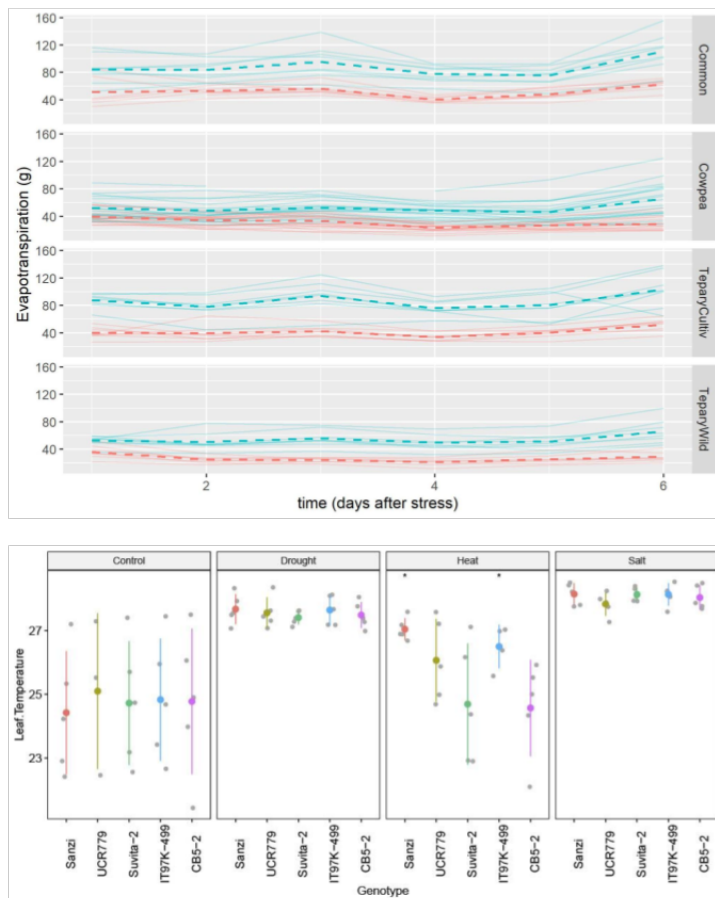
**MATERIALS AND METHODS:** All plants were germinated in Cornell soil mix in 4-inch pots. All the pots were filled with soil, and left for 2 days to dry out. The measured pot weight represented 0% soil water-holding capacity (SWHC). Subsequently, the pots were soaked overnight in trays filled with tap water, and left to drip the excess water for 1 hour. The measured pot weights after soaking represented 100% soil water-holding capacity. For each pot, we calculated a target weight of 60% (control treatment), 20% (drought stress cowpea), 10% (drought stress tepary), 50% (salt stress cowpea and tepary) SWHC. We germinated five cowpea accessions (Sanzi, UCR779, IT97K-499, Suvita-2, and CB5-2) and 2 wild (San Catalina and San Pedro) and 2 cultivated (Yellow and Black) tepary bean accessions. All plants were germinated in a growth chamber (12/12h day/night cycle, with 26/24°C and 60% humidity, light intensity 400  $\mu\text{mol} \times \text{m}^{-2} \text{s}^{-1}$ ). At 18 days after sowing, all the pots were measured daily and watered to the target weight. Plants undergoing heat stress were put in a growth chamber with increased temperature (34/24°C day/night), and all other settings kept the same as the germination growth chamber. Salt stress was applied by soaking pots watered to 50% SWHC, and subsequently soaking them in 200 mM NaCl solution - resulting in the final 100 mM NaCl treatment of the plants.

The evapotranspiration rates were calculated from daily measurements of the weight of each pot, except for the plants that underwent heat stress, watered every day to maximum capacity. The plant chlorophyll content, leaf temperature and photosynthetic efficiency were measured with PhotosynQ MultispeQ before and after applying stress. The plant growth was monitored using the in-house developed phenotyping setup, where the pots are placed on the rotating platform and a RaspberryPi operated camera took 7 images of the rotating plant. The images were analyzed using the PlantCV pipeline to estimate plant growth rates. The design of the PhenoCage, as well as the underlying code, will be made available at <https://github.com/mmjulkowska>

We developed an Arduino-based tool, to automatically weigh the plants, water each pot to the reference weight, and record the pot weight before and after watering. The design of the Arduino-based scale, as well as the 3D printed housing for the device, will be made available at <https://github.com/mmjulkowska>



**RESULTS AND CONCLUSIONS:** We observed that both drought and salt stress reduced evapotranspiration in all studied species. The difference in daily evapotranspiration rate was the highest for common bean and cultivated tepary, and the lowest for cowpea and wild tepary bean (Fig. 1A). While none of our treatments had a significant effect on chlorophyll content, we did observe significant differences between the studied legume species (Fig. 1B). Heat and drought stress increased the leaf temperature across all cowpea accessions, thereby reducing the variation between studied accessions (Fig. 1C). Heat stress treatment of cowpea increased the variation between the accessions (Fig. 1C), suggesting extensive natural variation in responses to heat stress within the cowpea natural diversity panel. These results suggest that tepary bean and cowpea are tolerant to drought stress, and possibly salinity, partially due to their efficient use of available water even under non-stress conditions. In the future, we aim to better understand the mechanism behind these strategies through screening natural diversity panels of both cowpea and tepary beans, and using Genome-Wide Association Studies. The developed system and preliminary data serve as a primer for future efforts in examining legumes' responses to abiotic stresses during early vegetative development. The slides and full presentation can be accessed at <https://doi.org/10.6084/m9.figshare.19341254.v1>



**Figure 1. Phenotypic difference in response to abiotic stress between and within cowpea, tepary beans and common bean. (A)** Five cowpea accessions, two wild tepary bean accessions, two cultivated tepary bean accessions and one common bean accessions were grown under control (aqua-blue lines, 60% soil water-holding capacity) and drought stress (red lines, 10% soil water-holding capacity) and their evapotranspiration was measured daily. Each line represent one plant, while dashed lines represent species average evapotranspiration. **(B)** Chlorophyll content (SPAD) was measured for all plants at 6 days after stress using PhotosynQ MultiSpeq. The significant differences between Common bean and other species are indicated with \*, \*\*, \*\*\* and \*\*\*\* for t-test derived p-value below 0.05, 0.01, 0.001 and 0.0001 respectively. **(C)** Leaf temperature was measured for 5 cowpea accessions at 6 days after stress using PhotosynQ MultiSpeq. The significant differences between CB5-2 and other accessions are indicated with \*, \*\*, \*\*\* and \*\*\*\* for t-test derived p-value below 0.05, 0.01, 0.001 and 0.0001 respectively.

# FARMER PARTICIPATORY VARIETY SELECTION FOR YELLOW BEAN GENOTYPES IN ZAMBIA

**Kelvin Kamfwa<sup>1</sup>, Swivia Hamabwe<sup>1</sup>, Kuwabo Kuwabo<sup>1</sup>, Maria Mazala<sup>1</sup>, Mwiinga Mulube<sup>2</sup>, Kennedy Muimui<sup>2</sup> and Karen Cichy<sup>3</sup>**

<sup>1</sup>University of Zambia, Dept. of Plant Sciences, P.O Box 32379, Lusaka, Zambia; <sup>2</sup>Zambia Agricultural Research Institute, Misanfu, Kasama; <sup>3</sup>USDA-ARS, Sugarbeet and Bean Research Unit, 1066 Bogue St., East Lansing, MI 48824, USA

## INTRODUCTION

Common bean (*Phaseolus vulgaris*) is a major source of dietary protein, fiber, carbohydrates, and minerals. Sustained breeding efforts at both national and international levels has resulted in the development of higher yielding bean varieties resistant to biotic and abiotic stresses. Despite the obvious agronomic superiority of newly developed and released varieties, their adoption remains low in some countries. One of the contributing factors is lack of awareness about the availability of improved varieties. The other contributing factor is that some of the new varieties do not possess traits considered important by the farmers. One strategy that could potentially enhance variety adoption by farmers is participatory Variety Selection (PVS). PVS refers to participation by the farmers in the selection of finished or nearly finished varieties. In PVS, varietal traits that farmers consider to be important in their production systems are selected. In the case of common bean, these traits may include growth habit, seed shape, seed color, seed size, pest and disease resistance, and maturity. Because farmer's preference for particular traits is considered in PVS, there is rapid adoption of the new varieties by farmers (Witcombe et al., 1996). During PVS, farmers use an integrated selection criterion, which considers several traits simultaneously. PVS provides an opportunity for a rapid appraisal of the demand by farmers for specific traits. This information is vital for adjustments to the product profile and objectives of a breeding program to quickly respond to farmer's needs. The impact of PVS is likely to be high in common bean because it has very diverse in plant architecture and seed traits, and furthermore it tends to have a narrow market and ecological adaptation. The objective of this study was to identify yellow bean genotypes with traits preferred by farmers using farmer participatory variety selection.

## MATERIALS AND METHODS

A yellow bean collection (YBC) comprised of 289 yellow bean genotypes was evaluated. The YBC is comprised of elite breeding lines and varieties from several countries (Sadohara et al., 2022). A total of 289 YBC genotypes were grown in 2020 and 2021 growing seasons as a rainfed crop at the Malashi Research Station in Mpika, Zambia. The 300 genotypes were planted in a randomized complete block design with three replications. The experimental plot was comprised of two rows that were 4 m long each. The spacing between rows was 0.6 m. When 90% of the genotypes in the YBC had reached harvest maturity, a group of 40 (20 female and 20 male) farmers were selected to take part in PVS. The farmers were selected from farming blocks of the Mpika district, which is located in a major bean-producing region of Zambia. The same group of farmers was used in both 2020 and 2021 growing seasons. Before the start of PVS, farmers were briefed on the procedure. A black plastic bag was placed at each of the experimental plots. Also, freshly shelled seed from a plant in a row was displayed at the front of the experimental plot so that farmers could consider the seed traits in their selections. Then each farmer was given 10 labels. Each farmer was allowed to walk individually through each replication and then select the 10 best genotypes out of the 300 genotypes that were in each replication. Discussions were held with individual

farmers to obtain insights into the traits that they considered important in their selections. A ranking of the 300 genotypes was done based on the number of farmers that selected a genotype.

## RESULTS AND DISCUSSION

In 2020, a total of 12 top-ranked genotypes were selected. Then in 2021 another 12 top-ranked genotypes were selected. Out of these two selections in 2020 and 2021, four genotypes were consistently selected (Table 1). The major traits that farmers considered in their selections were productivity, seed color and seed size. All farmers wanted a higher yielding genotype. Also, most farmers preferred genotypes with large seed size with Manteca or Njano seed color. Interestingly, of the five selected genotypes, two have Njano seed color. There is currently no variety in Zambia with a Njano seed color. The selection of a Njano seed color may suggest that Zambian farmers may be ready to adopt varieties with the Njano seed type. There were no obvious differences between males and females in terms of the traits that they considered important in their selections.

In this study, five yellow genotypes with variable seed colors were selected using PVS. These five genotypes could be used in Zambia for genetic improvement of yellow beans. PVS also provided important insights into the traits that Zambian farmers considered important for a variety. This information could help in the development of a product profile to meet the needs of the farmers and to enhance the adoption of the yellow bean varieties to be developed in the future.

**Table 1.** Seed yield and description of yellow beans selected by farmers in participatory variety selection conducted in 2020 and 2021 at the Malashi Research Station, Mpika, Zambia.

Genotype	Market Class	Source	Seed Yield (Kg ha <sup>-1</sup> )	
			2020	2021
Y1609-1	Manteca	Breeding line from MSU	1,642	1,570
Ervilha	Manteca	Landrace from Angola	987	2,050
Masindi	Njano	Landrace from East Africa	1,029	1,529
UGK 93	Njano	Variety from CIAT - Uganda	1,182	1,075

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# DEVELOPING NON-DARKENING CRANBERRY BEANS BY MARKER-ASSISTED BACKCROSSING

Sajida Noor, K. Peter Pauls

Department of Plant Agriculture, University of Guelph, Canada

## INTRODUCTION

Post-harvest seed coat darkening is a highly undesirable trait that results in a significant loss in several market classes of common beans (*Phaseolus vulgaris*) as it is associated with lower nutritional quality, increased cooking time, and decreased palatability. A single nucleotide polymorphism (SNP) found in the coding region of the Myb gene *Phvul.010G130600* in a nondarkening genotype (Wit-rood) close to the *J* locus for seed coat colour has been developed into a gene-based dominant marker for the dominant allele of *Phvul.010G130600* (Erfatpour and Pauls, 2020). A codominant marker is needed to allow identification of heterozygous (*Jj*), homozygous recessive (*jj*) and homozygous dominant individuals (*JJ*) in breeding populations. The objectives of this study are to develop a co-dominant marker for the *J* locus controlling the nondarkening phenotype, as well as genome-wide background markers, to facilitate marker-assisted backcross introgression of the nondarkening trait into an elite cranberry background.

## MATERIALS AND METHODS

High yielding, large seeded and disease resistant darkening cranberry beans lines; OAC Firestripe, and OAC Navabi and non-darkening cranberry bean; Wit-rood boontje, were used for the study. OAC Firestripe and OAC Navabi were both used as recurrent parents (*JJ*, females) in backcross populations established from crosses with Wit-rood (donor



**Figure 3. Progeny [F<sub>1</sub>(Jj)] from the initial parental cross**

parent *jj*, male). Progeny from the initial crosses [F<sub>1</sub>(*Jj*)] (Fig.1) and subsequent backcross progeny carrying a nondarkening *j* allele will be used as pollen parents in backcrosses with OAC Firestripe and OAC Navabi to create backcross (BC) populations with the nondarkening trait. A codominant KASP marker for *J* and *j* was developed with an Applied Biosystems QuantStudio 6 & 7 instrument. Whole genome sequencing of the parents was done with an Illumina MiSeq to identify polymorphic SNPs between recurrent and donor parents and the sequence data was analyzed with QIAGEN CLC genomics workbench.

## RESULTS AND DISCUSSION

A Kompetitive Allele Specific PCR (KASP) co-dominant marker was developed from the SNP marker (G/-) for the darkening allele of *Phvul.010G130600*. In the KASP assay fluorophore VIC was used to report the non-darkening allele and FAM was used to report the darkening allele (Fig.2). DNA samples from F<sub>1</sub>s gave intermediate fluorescent values for VIC and FAM indicating the ability of the assay to identify heterozygotes.

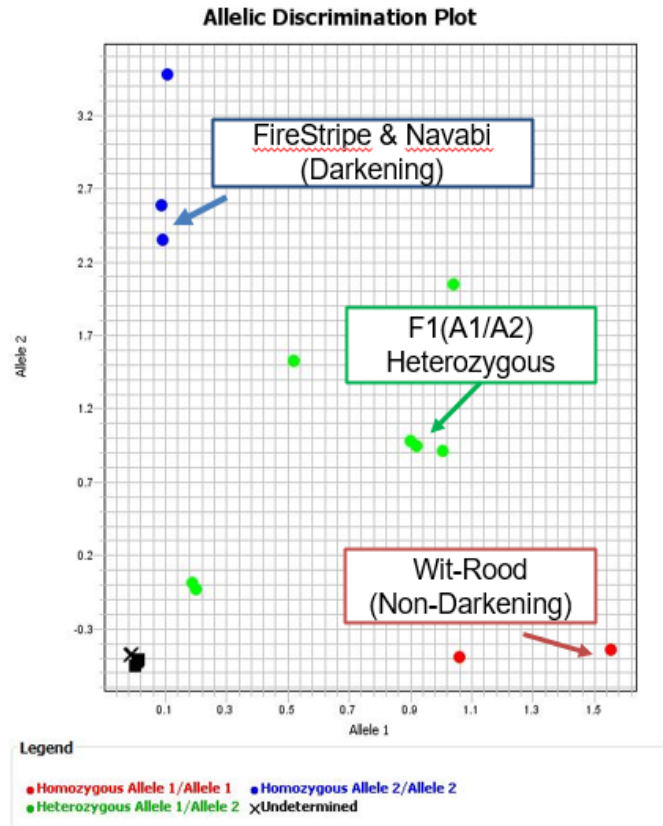
The whole genome sequences of the parental genotypes were assembled from the Illumina sequences using a reference [*P. vulgaris*v2.1 reference assembly available in Phytozome v12.1 (<https://phytozome.jgi.doe.gov>)] The sequences had 77%, 72%, and 81% coverage for Wit-Rood, OAC Firestripe and OAC Navabi, respectively. SNP calling of the aligned sequences identified 780 and 778 SNPs, between OAC Navabi and Wit-rood and OAC Firestripe and Wit-rood, respectively. Of these, 684 were common between the recurrent parents and 685 were polymorphic between the recurrent parents and Wit-rood. The SNPs will be used to select the recurrent parent genomes in marker assisted introgression of the nondarkening trait into OAC Navabi and OAC Firestripe genomes in future.

## CONCLUSION

A codominant marker for identifying individuals that are heterozygous for the gene associated with the nondarkening trait in Wit-rood was identified that can be used to introgress this trait into elite cranberry bean germplasm by marker assisted backcrossing.

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**Figure 4. Allelic discrimination plot for KASP marker**

# PHENOTYPIC AND MOLECULAR ANALYSIS OF THE GREEN COTYLEDON TYPES AMONG THE PHASEOLOID GRAIN LEGUMES LIMA BEAN, COMMON BEAN, AND COWPEA

Kimberly Gibson, Antonia Palkovic, Paul Gepts, R. Varma Penmetsta

Department of Plant Sciences, University of California at Davis, Davis, CA 95616

**INTRODUCTION:** Beans whose dry mature seeds are naturally green colored are found in both cool-season (garden pea, lentil, and chickpea) and warm-season (common bean, lima bean, and cowpea) pulse crops. Genetic studies indicate that the green cotyledon trait is conditioned as a single recessive (loss-of-function) gene, with the white or yellow cotyledon trait being dominant. The green-cotyledon types of garden pea and chickpea have higher levels of carotenoids including of provitaminA (Ashokkumar et al 2014; Sivasakthi et al 2019), offering an avenue for the genetic biofortification for carotenoid content.

Molecular genetic studies indicate that the 'staygreen' (SGR) gene conditions the green cotyledon trait in garden pea (Sato et al 2007, Armstead et al 2007), common bean (Davis et al 2010), and chickpea (Sivasakthi et al 2019). SGR is involved in senescence processes whose loss-of-function results in the failure to degrade chlorophyll in leaves and seeds, resulting in a so called 'stay-green' phenotype in a wide range of plants (Hortensteiner 2009).

These observations suggest that a SGR type of gene might also condition the green cotyledon trait in lima bean and cowpea. To test this hypothesis, we analyzed sets of common bean, lima bean, and cowpea genotypes with either white or green cotyledons.

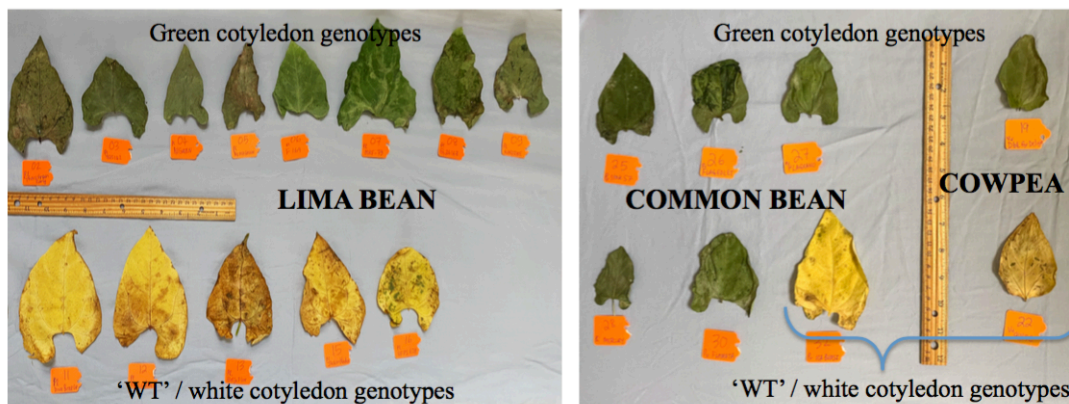
**MATERIALS AND METHODS:** Plant lines used in this study were obtained from the US germplasm system (<https://npgsweb.ars-grin.gov/gringlobal/search>), supplemented with material from within our bean breeding program. Study plants were grown in greenhouses and phenotypes were observed visually and recorded. To assay leaf senescence, leaves or leaflets of similar developmental age were enclosed in aluminum foil for 5-7 days after which they were detached from the plants, examined for leaf color, and an image was collected.

To identify SGR-like candidate genes in lima bean and cowpea, we performed homology searches of publicly accessible cowpea and lima bean transcript and genome data with the previously published SGR gene orthologs from common bean (Davis et al 2010) and chickpea (Sivasakti et al 2019). In both lima bean and cowpea we identified two sequence homologs located on the canonical chromosomes 02 and 09, respectively, with bean and chickpea probes.

To examine nucleotide sequence variation in these candidate genes, genomic and cDNA of the white and green cotyledon plant lines were used as templates for PCR amplification. Amplified PCR products were examined using agarose gel electrophoresis, purified with standard PCR product clean-up methods, and sequenced using the Sanger sequencing method at an on-campus service facility. Individual sequence trace files were manually curated with the 4Peaks software and high-quality sequences were exported for further analysis. Variant calling relied on their occurrence in at least two independent PCR and sequencing reactions.

**RESULTS AND DISCUSSION:** As observed previously in chickpea (Sivasakti et al 2019), dark treatment of intact leaves (by foil-wrapping) of the white cotyledon lines in lima bean, common bean and cowpea led to their yellowing, reflecting the normal de-greening or leaf senescence that is accelerated by darkness [Figure 1]. By contrast, dark treatment of leaves of the green cotyledon

types in the three species was associated with delayed de-greening, with leaves remaining green [Figure 1].



**Figure 1.** Degreening in white vs green cotyledon genotypes in dark treated unifoliolate leaves.

Sequence analyses of the first SGR-like candidate found on Pv/Pl/Vu chromosome 09 did not find functional variation between white and green cotyledon types in the three species. By contrast, the second SGR-like gene tested, from chromosome 02, identified predicted loss-of-function changes exclusively among the green-seeded lines. A single nucleotide change (non-sense mutation) was found in most green cotyledon lima beans (Bridgeton, Thorogreen Improved Early, G25142 Early Thorogreen, Allgreen, Nemagreen, F-169, Mrf-79, G26163 IC109623) and a 9-basepair coding region deletion was found in a green cotyledon lima (G26557 Kingstone). In common bean, all six green cotyledon lines examined (Flageolet, Flagrano, Mercury, Shade, Flamata, Flaveol, stock 57) shared an apparently large deletion within the SGR-like gene on chromosome 02, similar to that previously described by Davis et al (2010). In cowpea, sequence changes were not found though additional work is needed here.

That deleterious loss-of-function alleles characterizing all green cotyledon lines strongly suggests that SGR orthologs are the likely causal gene for the trait in lima and common beans. The green cotyledon trait represents a Vavilovian “homologous series of variation” with shared phenotypes and underlying genes across several cultivated grain legumes.

**ACKNOWLEDGEMENTS:** Supported by a US National Institute of Food and Agriculture grant #2018-67013-27619

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# MULTIPLE GENOMIC REGIONS GOVERN TOLERANCE TO SULFENTRAZONE IN SNAP BEAN (*PHASEOLUS VULGARIS* L.)

**Ana Saballos<sup>1</sup>, Alvaro Soler-Garzón<sup>2</sup>, Matthew Brooks<sup>1</sup>, John Patrick Hart<sup>3</sup>, Alexander Edward Lipka<sup>4</sup>, Philip Miklas<sup>2</sup>, Ronald Edward Peachey<sup>2</sup>, Patrick J Tranel<sup>4</sup>, Martin M. Williams II<sup>1\*</sup>**

<sup>1</sup>Global Change and Photosynthesis Research Unit, United States Department of Agriculture–Agricultural Research Service, Urbana, Illinois, United States of America; <sup>2</sup>Grain Legume Research Unit, United States Department of Agriculture–Agricultural Research Service, Prosser, Washington, United State of America; <sup>3</sup>Earthwork Seeds, Davis, California, United States of America; <sup>4</sup>Department of Crop Sciences, University of Illinois, Urbana, Illinois, United States of America

**INTRODUCTION:** Waterhemp (*Amaranthus tuberculatus*) contamination of the packed product in mechanically harvested snap bean (*Phaseolus vulgaris*) is a major challenge for snap bean producers. Expanding availability and use of herbicides with different modes of action allows herbicide rotation to improve weed control and delay herbicide resistance in weed populations. Sulfentrazone effectively controls waterhemp and multiple other broadleaf weeds; however, it is not labeled for use in snap bean. The extent of naturally occurring tolerance to sulfentrazone in snap bean cultivars and its genetic basis are unknown. In this study, we used a snap bean diversity panel combined with a GWAS approach to investigate the genetic basis of sulfentrazone tolerance.

**MATERIALS AND METHODS:** A subset of the SNAp bean Association Panel (SNAP) comprising 277 genotypes representing the diversity of snap beans grown in the US over the last century was used in this study. Genotypic information was obtained by genotyping by sequencing. Polymorphic SNPs with at least 5% minimum allele frequency were used to perform genome-wide association analysis for the traits measured in the field experiments. Field trials were conducted at the University of Illinois Vegetable Crop Farm near Urbana, IL, in 2019 and 2020. The experimental design was a strip plot with three blocks (replications). Each block consisted of vertical strips of an herbicide treatment factor and horizontal strips of a genotype treatment factor. Plots consisted of single rows (76-cm spacing) of individual genotypes transecting both herbicide treatment strips. Each genotype by herbicide subplot was 2.4 m in length planted with 30 seeds. Plots received one of two levels within 24 hours after planting: sulfentrazone at 860 g a.i. ha<sup>-1</sup> or a nontreated control. Before planting, we measured the 100-seed weight of the seed lots used to plant the trials. We evaluated the numbers of established plants out of the 30 seeds (PD) and the average dry biomass (PB) in treated and control plots three weeks after planting. The percentage of PD and BP of the treated compared to the control plots was calculated to determine the tolerance of each genotype to sulfentrazone.

**RESULTS AND CONCLUSIONS:** The traits had moderate to high heritability (broad-based), and the genotyped genetic markers were able to explain a significant proportion of the variability (chip-based heritability). Correlations between seed weight and tolerance traits, and between tolerance traits and PD and BP under control conditions were observed. Multiple genomic regions were associated with the traits. Genes with possible functions in xenobiotic detoxification and oxidative stress tolerance are located near the significant SNPs. No association with SNPs near the location of the PPO enzymes (the target for sulfentrazone) and tolerance traits was detected. Taken together, the results point to a non-target site resistance mechanism for the tolerance to sulfentrazone observed in the panel. The tolerance is heritable, and selection of tolerant cultivars is possible using the associated SNPs.



**Table 1.** Genes located in the significant GWAS intervals independent of seed weight whose putative functions suggest plausible roles in the mechanisms mediating tolerance to sulfentrazone.

Chr	Representative SNP	MAF	Allele	Interval position	Candidate genes	GO/KOG/InterPro Description
1	S01_5474118	0.06	T/C	5474118-6699374	Phvul.001G051100	G-type lectin S-receptor-like serine/threonine protein kinase.
	S01_5800008	0.07	C/T		Phvul.001G052100	Cytochrome P450 protein CYP2 subfamily.
	S01_6218687	0.06	G/C		Phvul.001G054800	Iron/ascorbate family oxidoreductase.
	S01_50676731	0.06	A/G	50593075-50793332	Phvul.001G257500	Regulator of G-protein1
	S01_50677029	0.06	G/C		Phvul.001G258000	Golden2-like transcription factor, response to ABA
	S01_50754135	0.06	A/C		Phvul.001G258200	Heat shock protein
					Phvul.001G258300	Chloroplast protein
					Phvul.001G258700	MYB Transcription factor
					Phvul.001G259000	Cytosolic GADPH (C subunit)
4	S04_1593169	0.09	A/T	1593169-3102077	Phvul.004G014200	2-methylene-furan-3-one reductase / Enone oxidoreductase
	S04_1627881	0.07	A/T		Phvul.004G014500	Protein farnesyltransferase subunit beta
	S04_1840022	0.07	A/G		Phvul.004G014600	2-hydroxyacid dehydrogenase-related
	S04_1858494	0.09	C/A		Phvul.004G014666	2-hydroxyacid dehydrogenase-related
	S04_2121679	0.16	G/T		Phvul.004G014732	2-hydroxyacid dehydrogenase-related
	S04_2617000	0.06	C/A		Phvul.004G014800	2-hydroxyacid dehydrogenase-related
	S04_2720823	0.18	T/G		Phvul.004G018000	Aldehyde dehydrogenase
	S04_2829965	0.17	T/G		Phvul.004G018900	Alpha-dioxygenase (DOX1)
					Phvul.004G020100	Plant peroxidase
					Phvul.004G020500	Ferredoxin-thioredoxin reductase catalytic chain (FTR-c)
					Phvul.004G021100	Cytochrome P450 CYP2 subfamily
					Phvul.004G021200	Cytochrome P450 CYP2 subfamily
					Phvul.004G021300	Cytochrome P450 CYP2 subfamily
					Phvul.004G021400	Cytochrome P450 CYP2 subfamily
					Phvul.004G021500	Cytochrome P450 CYP2 subfamily
					Phvul.004G021600	Cytochrome P450 CYP2 subfamily
					Phvul.004G021700	Cytochrome P450 CYP2 subfamily
					Phvul.004G021800	Cytochrome P450 CYP2 subfamily
					Phvul.004G021900	Cytochrome P450 CYP2 subfamily
					Phvul.004G022000	Cytochrome P450 CYP2 subfamily
					Phvul.004G022400	Mannose-1-phosphate guanylyltransferase
					Phvul.004G022700	Polygalacturonate 4-alpha-galacturonosyltransferase
					Phvul.004G023066	Multi-Drug Resistance exporter
			Phvul.004G023132	Multi-Drug Resistance exporter		
			Phvul.004G025200	NAD(P)H-Quinone oxidoreductase subunit 2		
8	S08_52971932	0.20	A/G	52620196-53099410	Phvul.008G190100	RING/U-box domain-containing protein
	Phvul.008G190300				Integrin-Linked Kinase (ILK)	
	Phvul.008G190500				HVA22-like protein	
	Phvul.008G191000				Cytochrome P450, family 26, subfamily A (CYP26A)	

# GENE EXPRESSION PATTERNS IN DRY BEAN SEEDS RELATED TO SOAKING TIME AND COOKING TIME

Hannah Jeffery<sup>1</sup>, Nyasha Mudukuti<sup>1</sup>, Dr. Robin Buell<sup>1</sup>, Dr. Kevin Childs<sup>1</sup>, Dr. Karen Cichy<sup>2</sup>

<sup>1</sup>Michigan State University and <sup>2</sup>USDA-ARS, East Lansing, MI

**ABSTRACT:** Dry beans are a nutritious food, but their lengthy cooking requirements are barriers to consumption. Pre-soaking beans from 3 up to 18 hrs dramatically reduce cooking times. While soaking allows hydration to occur prior to cooking, there is evidence that enzymatic changes to pectic polysaccharides also occur during soaking that shorten bean cooking time (Martinez-Manrique *et al.*, 2011). Little is known about gene expression changes that are taking place during soaking that influence cooking time. Significant genetic variability for cooking time is present in the species, and numerous fast-cooking genotypes have been identified with the potential to appeal to consumers. Fast-cooking genotypes exhibit some compositional and physiological characteristics, including less insoluble fiber and thinner cotyledon cell walls. The goal of this study was to compare gene expression in fast cooking and slow cooking bean genotypes. RNA was extracted from beans at five soaking time points (0, 3, 6, 12, and 18 hrs). The reads were subjected to DESeq2, weighted gene co-expression network analysis (WGCNA), alignment to QTL for cooking time and water uptake, and GO enrichment analysis. Modules correlated with cooking time and soaking time were discovered in soaking seeds. Significant differences in gene expression between the fast and slow cooking genotypes were observed at hour six of soaking relative to hour zero of soaking (raw beans). Candidate genes for slow-cooking time were identified and their relevance to current theories for the control of cooking time is discussed.

**INTRODUCTION:** The genetic mechanisms controlling cooking time are not yet fully understood, but it is known that soaking can decrease the cooking time of dry bean and that there are multiple genes involved in the genetic control of cooking time. QTL analyses have been conducted for cooking time. Two populations have been developed, the TTRIL (Berry *et al.*, 2020) which is a cross of brown beans, TZ-27 (slower cooking) and TZ-37 (faster cooking), and the YYRIL (Bassett *et al.*, 2021a) which is a cross of two yellow beans, PI527538 (slower cooking) and Ervilha (faster cooking). Genetic regions associated with water uptake and cooking time were found using these recombinant inbred populations (Bassett *et al.*, 2021; Berry *et al.*, 2020). Water uptake QTL were investigated because increased water uptake generally lowers the cooking time of fresh beans, and different genotypes respond differently to soaking. For example, PI527538 has a faster cooking time than Ervilha without soaking. However, after soaking for 12 hrs, Ervilha has a faster cooking time than PI527538. The genotypes that cook faster following soaking tend to have thinner cotyledon cell walls and less insoluble fiber in their cotyledons. The genotypes that cook faster when unsoaked tend to have thinner seed coats (Bassett *et al.*, 2021b).

**MATERIALS AND METHODS:** To prepare this experiment, the four genotypes: TZ-27, TZ-37, PI527538 and Ervilha were soaked for 0, 3, 6, 12, and 18 hours. RNA was extracted for RNA-sequencing in triplicate from seeds. The genotypes were cooked and soaked at each time point. The weight of the genotypes was determined at each soaking time as well to calculate the water uptake percentage of the genotypes. The RNA-sequencing data was analyzed using the following pipeline: the data quality was checked with FastQC. The mRNA reads were trimmed with CutAdapt, then their quality was checked again using FastQC. HiSat2 and HTSeq were used to

align and count the transcripts, respectively. To identify genes that were expressed synchronously, WGCNA, or weighted gene co-expression network analysis was employed (Langfelder and Horvath, 2008). DESeq2 was used to discover significantly differentially expressed genes between different genotypes. To verify that these genes are related to cooking time, genes from the DESeq2 analysis were filtered using the WGCNA pathway data and the QTL from the TTRIL and YYRIL populations for cooking time and soaking time. Lastly, functional annotation was performed on the WGCNA modules using topGO. The data quality was sufficient for this analysis.

**RESULTS AND DISCUSSION:** Water uptake during soaking leveled out at around 12 hours of soaking for all four genotypes. Cooking times also reached their lowest levels for all four genotypes after 12 hr soaking. When comparing differential gene expression within genotypes, it was found that more unique genes were expressed in the slower cooking beans compared to the faster cooking beans at hour six of soaking. Not only were more unique genes expressed in each genotype at hour six, but also the kinds of genes being expressed were completely different between fast and slow cooking genotypes. In terms of gene expression, the slow-cooking beans were 5.6 more like each other than the fast-cooking beans were. Thirty genes were found by filtering the DESeq2 data with WGCNA and QTL data. Of those, 93% were differentially expressed at hour six of soaking. In the yellow beans, 42 genes, 57% of which were differentially expressed at hour six, were found. One gene was differentially expressed in both brown and yellow beans, that being NAD(P)-binding Rossmann fold superfamily protein. It was upregulated in fast cooking beans at an early soaking stage. It is possible that this gene has a universal regulatory role in cooking time. In WGCNA, terms related to the response of auxin, macromolecule localization, translation, and nucleic acid metabolism were identified, indicating that the beans undergo cellular modification during soaking (Borrego-Benjumea *et al.*, 2019). This seems to trigger a reaction within the beans that leads to differences in cooking time and occurs within six hours of soaking. Stress and hypoxia may reference oxidative damage that takes place in the seeds (Nakayama *et al.*, 2017).

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# EFFECT OF LIMA BEAN LEAFLET SHAPE ON CANOPY TEMPERATURE AND HUMIDITY IN A HUMID CLIMATE

Emmalea G. Ernest\*<sup>1</sup>, Adam Ginn<sup>2</sup> and Thomas A. Evans<sup>2</sup>

<sup>1</sup>Cooperative Extension, University of Delaware, Georgetown, DE

<sup>2</sup>Department of Plant and Soil Sciences, University of Delaware, Newark, DE

## INTRODUCTION

Leaflet shape in wild and cultivated lima bean (*Phaseolus lunatus*) ranges from lanceolate to ovate. Leaflet shape could affect agronomic traits, such as yield and maturity, as well as disease avoidance and abiotic stress tolerance. To test the impacts of leaflet shape, pairs of near isogenic lines (NILs) with lanceolate and ovate leaflet shapes were developed. Inoculated lima bean downy mildew (*Phytophthora phaseoli*) trials conducted in 2019 and 2020, with five such pairs, suggested some disease avoidance benefit of lanceolate leaflet shape. In both years, the lanceolate member of the two pairs had a lower severity rating. Separate field trials were conducted in 2020 and 2021 to better understand how leaflet shape affects agronomic traits and canopy microclimate, a major factor in disease avoidance.

## MATERIALS AND METHODS

Split plots of four NIL pairs were planted in late June of 2020 and 2021. Plots were three rows wide and Onset temperature and humidity loggers were placed in the canopy for three of four replications at the early flowering stage (early August). The center rows of each plot were harvested to evaluate plant size and yield. Maturity was evaluated in 2020 by categorizing the pods from five plants from each plot as succulent, immature or dry.

## RESULTS AND DISCUSSION

Leaflet shape did not significantly affect yield in 2020 but in 2021 ovate lines had higher yield (Table 1). In both years ovate lines had higher biomass production (Table 1). Maturity was earlier in the lanceolate lines with significantly fewer immature pods and significantly more dry pods at harvest (Table 1). Lower yields for lanceolate lines in 2021 may have been due to harvest at a later than optimal time, resulting in a high percent dry seed.

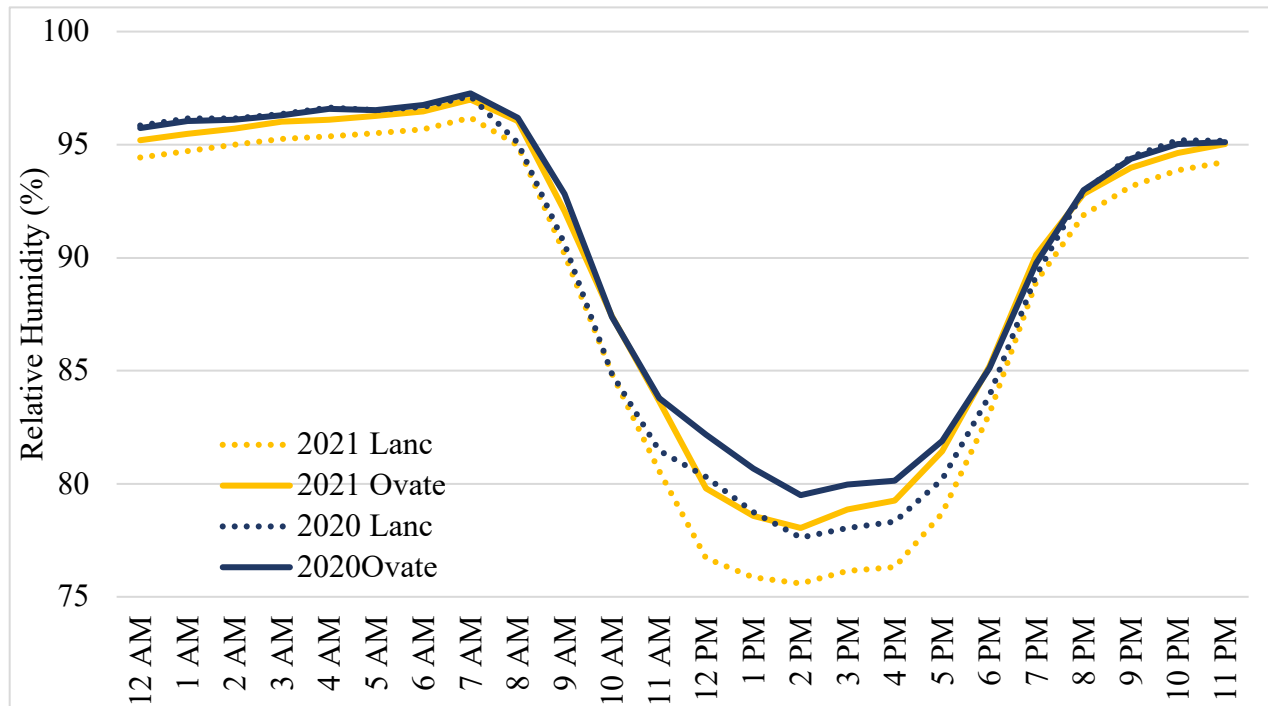
**Table 1.** Yield, Biomass and Pod Maturity Averaged for all Ovate and Lanceolate Lines

	Yield (kg/ha)		Biomass (kg/ha)		2020 Pod Maturity (%)*		
	2020	2021	2020	2021	Succulent	Immature	Dry
<b>Ovate</b>	4,311	3,468	29,238	21,278	67.4	19.8	12.8
<b>Lanceolate</b>	4,453	3,000	24,859	18,392	69.8	8.1	22.1
<i>p-value</i>	<i>0.3539</i>	<i>0.0031</i>	<i>&lt;0.0001</i>	<i>0.0016</i>	<i>0.5683</i>	<i>0.0061</i>	<i>0.0053</i>

\*Note that target harvest is 10% dry pods.

Lanceolate lines had significantly lower canopy humidity during the day from 8:00 a.m. to 6:00 p.m., but canopy humidity at other times was not significantly different between lanceolate and ovate lines (Figure 1). This effect was more pronounced in 2021.

**Figure 1.** Average Canopy Temperature by Hour in Aug. and Sept.



Lanceolate lines had significantly higher daytime canopy temperatures, except during dry periods, when lanceolate NILs had lower daytime canopy temperature than their ovate counterparts. Lanceolate lines had lower canopy temperature at night but this difference was only significant in 2020.

Plant architecture traits beside leaflet shape (e.g. plant size, sprawling vs upright growth habit) also impacted canopy temperature and humidity.

## CONCLUSIONS

There are no obvious agronomic drawbacks to lanceolate leaves, although under certain conditions ovate lines produce higher yields. The lanceolate leaflet shape may beneficially lower canopy humidity and inhibit disease development, especially in larger plants. Lower nighttime canopy temperatures observed in lanceolate lines is probably not enough to reduce nighttime heat stress damage to pollen and anthers. The lower canopy temperature in lanceolate lines on hot, dry days might indicate more efficient water use/drought tolerance conferred by lanceolate leaves. Other plant traits also have a significant effect on canopy temperature and humidity, such as plant size, plant architecture, stomatal control. Combining lanceolate leaflet shape with upright architecture, moderate plant size and environmentally responsive stomatal control could result in genotypes with disease avoidant canopy conditions and abiotic stress tolerance.

# GENOME-WIDE ASSOCIATION MAPPING REVEALS CANDIDATE GENES AND NEW ADJUSTMENTS TO THE HOST-PATHOGEN INTERACTION FOR RESISTANCE TO BEAN COMMON MOSAIC AND NECROSIS VIRUSES IN COMMON BEAN

Alvaro Soler-Garzón<sup>1</sup>, Phillip E. McClean<sup>2</sup> and Phillip N. Miklas<sup>3</sup>

<sup>1</sup>Irrigated Agriculture Research and Extension Center, Washington State University, Prosser, WA, United States, <sup>2</sup>Department of Plant Sciences, North Dakota State University, Fargo, ND, United States, <sup>3</sup>Grain Legume Genetics and Physiology Research Unit, United States Department of Agriculture - Agricultural Research Service (USDA-ARS), Prosser, WA, United States

## INTRODUCTION

Bean common mosaic (BCMV) and necrosis (BCMNV) viruses are related positive-stranded RNA viruses in the Potyvirus genus that plague common bean (*Phaseolus vulgaris* L.) worldwide. Host plant resistance is the primary disease control method. Resistance is regulated by the dominant *I* gene and six recessive alleles (*bc-1*, *bc-1<sup>2</sup>*, *bc-2*, *bc-2<sup>2</sup>*, *bc-3*, and *bc-u*) distributed across four loci (Drijfhout, 1978). Strain diversity is classified into eight Patho-Groups (PG) based on interactions with 12 Host-Groups (HG) (Drijfhout, 1978; Feng et al., 2015). We sought to identify candidate genes conferring resistance to BCMV and BCMNV, examine genetic interactions among them, and exploit polymorphisms for MAS.

## MATERIALS AND METHODS

182 dry bean lines from the Durango Diversity Panel (DDP), were genotyped with 1.2M biallelic SNPs obtained by whole-genome resequencing. Phenotypic reactions from greenhouse inoculations with US-6 (PG-VII), NL-8 (PG-III), and NL-3 (PG-VI) strains and the genotypic SNP data for the DDP were integrated for GWAS using a multi-locus random-SNP-effect mixed linear model. 217 F<sub>2</sub> and 2,629 F<sub>2:3</sub> plants, and the OV3 (Othello/VAX 3) RIL population, were evaluated for BCMV and BCMNV reactions and genotyped for SNP markers with the resistant candidate genes (based on the reference genome G19883 v2.1).

## RESULTS AND DISCUSSION

GWAS revealed significant peak regions on chromosomes Pv03 (*bc-1*), Pv05 (*bc-u* and the new *bc-4*), and Pv11 (*bc-2*). Side-by-side Receptor-Like protein Kinases, Phvul.003G038700 and Phvul.003G038800, were candidate genes for *bc-1*. A basic Leucine Zipper transcription factor protein gene, Phvul.005G124100, is the candidate gene for *bc-u*, which carries a stop-gain mutation resulting in a nonfunctional protein (Soler-Garzón et al., 2021a). In *bc-2*, a genomic interval was identified, Phvul.011G092700, a Vps4 AAA+ ATPase protein, as the *bc-2* candidate gene, and in UI-111 and Robust lines carry two different mutations, which introduce a premature stop codon. Phvul.005G125100, another Vps4 AAA+ ATPase protein, was identified as the candidate gene for the new recessive *bc-4* gene, and the recessive allele is likely an amino acid substitution in the MIT domain (Soler-Garzón et al., 2021b). SNP markers for *bc-1*, *bc-u*, *bc-4* and *bc-2* (Table 1) and new markers for the *I* and *bc-3* genes were used to genotype the resistance genes underpinning BCMV/BCMNV phenotypes in the DDP, host-group (HG) differentials, and segregating F<sub>3</sub> families.

Results revealed major adjustments to the current host-pathogen interaction model: (i) there is only one resistance allele for *bc-1*, and differential expression is based on the presence vs. absence of

*bc-u*; (ii) there is only one *bc-2* allele with a differential reaction based on whether it is combined with *bc-4* or *bc-u*; (iii) *bc-4* and *bc-u* are tightly linked in repulsion on Pv05. These candidate genes, markers, and adjustments to the host-pathogen interaction will facilitate breeding for resistance to BCMV and related BCMNV in common bean.

**Table 1.** Summary candidate genes and mutations detected by GWAS and sequencing. For each mutation, a SNP or indel marker was developed.

Recessive gene	Candidate gene models (G19833 v2.1)	Annotation	Chr	SNP or Indel Position (G19833 v2.1)	Type of Mutation
<i>bc-1</i>	Phvul.003G038700 Phvul.003G038800	Receptor-Like Protein Kinase	Pv03	4,203,361	Missense
<i>bc-u</i> <sup>d</sup>	Phvul.005G124100	bZIP transcription factor family protein	Pv05	36,114,516	Stop-gain
<i>bc-4</i>	Phvul.005G125100	Vps4 AAA+ ATPase ESCRT protein	Pv05	36,225,550	Missense
<i>bc-2</i> <sup>[UI 111]</sup>	Phvul.011G092700	Vps4 AAA+ ATPase ESCRT protein	Pv11	9,272,542- 9,262,459	3' end truncated
<i>bc-2</i> <sup>[Robust]</sup>				9,278,765	deletion-frameshift

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# MAPPING AN ANTHRACNOSE RESISTANCE LOCUS IN ANDEAN COMMON BEAN LANDRACE BEIJA FLOR

Larissa F.S. Xavier<sup>1,2</sup>, Marcial A. Pastor-Corrales<sup>1</sup>, Giseli Valentini<sup>1</sup>, Oscar P. Hurtado-Gonzales<sup>3</sup>, Qijian Song<sup>1</sup>, Juliana P. Poletine<sup>2</sup>, Maria Celeste Gonçalves-Vidigal<sup>2</sup>

<sup>1</sup>Soybean Genomics and Improvement Laboratory, USDA-ARS, Beltsville, MD, 20705, USA.

<sup>2</sup>Department of Agronomy, Maringa State University, Maringa, PR, 87020-900, Brazil; <sup>3</sup>Plant Germplasm Quarantine Program, USDA-APHIS, Beltsville, MD, 20705, USA.

## INTRODUCTION

Anthracnose is a major disease of common bean worldwide. The regular appearance of new virulent strains of *Colletotrichum lindemuthianum* (CL) threaten common bean production especially of varieties with single resistance genes. The hundreds of reported races of CL segregate into two groups, one Andean and another Mesoamerican (Pastor-Corrales, 1996). Anthracnose resistance genes of Andean origin often confer broad resistance to highly virulent Mesoamerican races of CL. Beija Flor (BF), an Andean landrace, has been reported as resistant to broadly virulent Mesoamerican races of CL. In this study, we aimed to confirm and expand the knowledge about the resistance spectrum of BF and to perform fine mapping to position the resistance locus of BF in the genome of common bean.

## MATERIALS AND METHODS

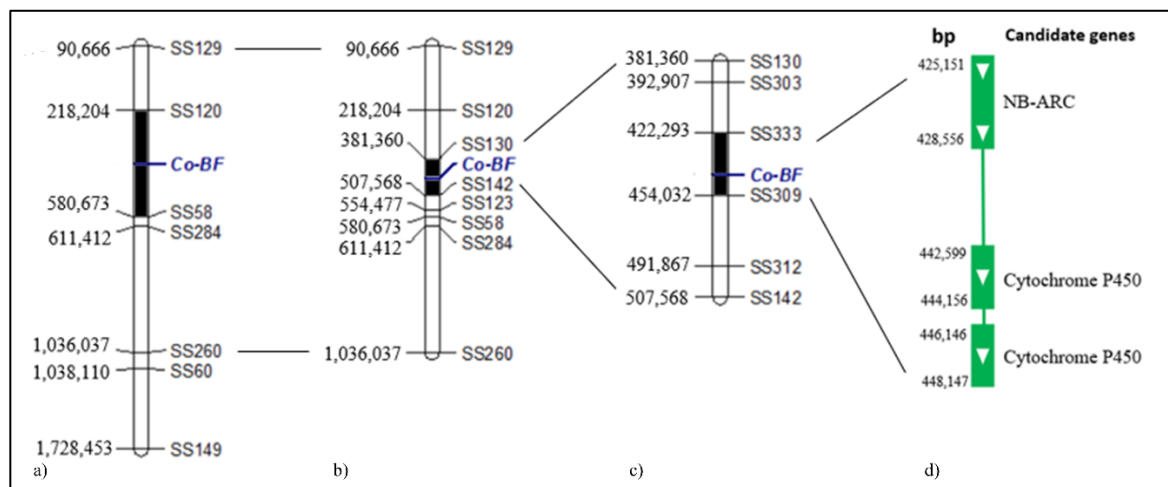
We evaluated the reaction of BF to 17 (9, 17, 64, 65, 73, 89, 109, 127, 321, 449, 453, 469, 1545, 1601, 1993, 2047, 3481) Mesoamerican and seven (4, 7, 19, 23, 31, 39, 55) Andean races of CL. The inheritance of resistance and the mapping of the resistance locus in BF was performed using two different populations. The first population included 157 F<sub>2:3</sub> families from the Beija Flor × Cornell 49242 cross that was inoculated with race 1545. The second population comprised 390 F<sub>2</sub> plants from the Beija Flor × Crioulo 159 cross. This population was divided into two groups. The first group with 182 F<sub>2</sub> plants was inoculated with races 321 and 1545, while the second group with 208 F<sub>2</sub> plants was inoculated with races 4, 321, 453 and 1545. These crosses were also used to perform bulked segregant analysis which were genotyped with the BARCBEAN12K\_BeadChip containing 11,292 SNPs. The genomic region containing the anthracnose resistance gene in BF was targeted for the development of specific KASP markers to be used to advance the mapping of the resistance locus. Fine mapping was performed using 1,261 F<sub>3</sub> plants from the Beija Flor × Crioulo 159 cross. Selected new SNPs were used to develop KASP markers using haplotype analysis based on whole genome sequencing of Beija Flor and other bean cultivars.

## RESULTS AND DISCUSSION

Beija Flor was resistant to 15 (80.24 %) of the 17 Mesoamerican races of CL, including races 2047 and 1993 that infected 11 and 8 of the 12 differential cultivars respectively. BF was also resistant to four (51.14 %) of the seven Andean races. These results showed that BF conferred broad resistance to Mesoamerican races of CL but it also was resistant to more than half of the Andean races used in this study. Inoculating the 157 F<sub>2:3</sub> families with race 1545, 182 F<sub>2</sub> plants with races 321 and 1545, and 208 F<sub>2</sub> plants with races 4, 321, 453 and 1545 of CL, revealed that the resistance of BF to 17 Mesoamerican and 4 Andean races of CL is conferred by a single and dominant gene. Bulk segregant analysis and high throughput genotyping with the BARCBean\_12k BeadChip indicated that the resistance locus in Beija Flor was on the upper end of chromosome Pv04. The KASP markers used to genotype the populations Beija Flor × Cornell 49242 and Beija Flor ×



Crioulo 159 crosses positioned the BF resistance locus in two genomic regions of 295 kb and 362 kb, respectively. The fine mapping on 1,261 F<sub>3</sub> plants from the Beija Flor × Crioulo 159 cross using KASP markers positioned the Beija Flor resistance locus in much smaller (31.7 kb) genomic region flanked by KASP markers SS333 (422,293 bp) and SS309 (454,032 bp) (RefGen V1.0). Three candidate genes were identified within this region in the *P. vulgaris* reference genome of G19833 (Fig. 1). In conclusion, the resistance in BF to broadly virulent Mesoamerican but also to Andean races of CL, is conferred by a single and dominant gene positioned in a 31.7 kb genomic region of chromosome Pv04 containing three candidate genes.



**Figure 1.** Fine mapping of the anthracnose resistance locus present in the Andean landrace Beija Flor (provisionally designated as *Co-BF*) on chromosome Pv04 of common bean using the BF × Crioulo 159 cross. a) Physical map of the resistance locus constructed using 390 F<sub>2</sub> plants. b) Physical map of the resistance locus constructed using 96 F<sub>3</sub> recombinant plants; c) Physical map of the resistance locus constructed using 52 F<sub>3</sub> recombinant plants; d) The 31.7 kb genomic region contained three candidate genes in the *P. vulgaris* reference genome of G19833.

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# CHARACTERIZATION OF ECONOMIC IMPORTANCE OF COMMON BEAN PATHOGENS (*PSEUDOCERCOSPORA GRISEOLA* AND *COLLETOTRICHUM LINDEMUTHIANUM*)

Papias H. Binagwa

Tanzania Agricultural Research Institute – Selian Center, P.O. Box 6024 Arusha, Tanzania

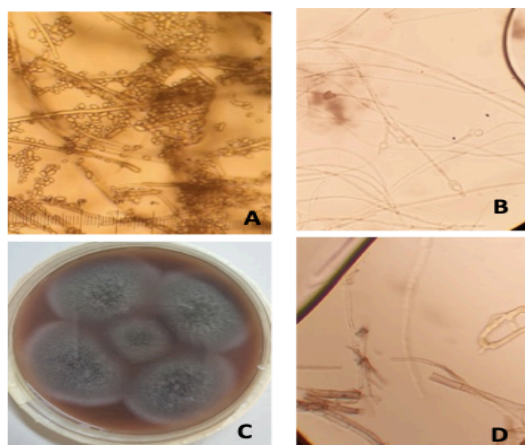
**INTRODUCTION:** Disease development is affected by various factors including production system, agronomic management, growing environment, and the dry or green bean cultivars utilized (Singh & Schwartz, 2010). Average yield losses may be up to 100% depending on the occurrence and severity of the individual and collective diseases occurring in the same field, production systems and management practices used. Based on economic importance, fungal diseases cause higher losses followed by bacterial and viral diseases (Mahuku & Riascos, 2004; Kelly & Vallejo, 2004). In the study area, *P. griseola* and *C. lindemuthianum* disease pathogens were found to be more devastating on the red mottled varieties, specifically ‘Lyamungo 85’ and ‘Lyamungo 90’ with 36 and 31 years after release, respectively, as well as ‘Calima Uyole.’ Disease has resulted in some of the seed farms being rejected by seed quality regulators due to high rates of infection. Consequently, these were found to be economical diseases since they reduce income to the farmers, seed companies and cause food insecurity. For instance at TARI – Selian centre and seed companies in Arusha, Lyamungo 90 was found to be more susceptible to these diseases. Therefore, the TARI – Selian centre took initiatives to characterize the pathogens and determine the reaction of various breeding lines of common bean with similar market seed classes. The objective of this study was i) to characterize the diseases with economic impact ii) to identify the pathogens morphologically and molecularly.

**MATERIALS AND METHODS:** (1) *Exploration diseases of focus:* Literature review and direct communication with bean value chain actors were conducted. Two common bean diseases were found to cause huge losses to specific red mottled bean varieties, namely Lyamungo 85 and Lyamungo 90. (2) *Sampling of disease samples:* Disease samples were collected from TARI – Selian farms. Samples were kept in paper bags and taken to the pathology laboratory for further pathological procedures. (3) *Preparation of media for disease isolation:* The pathogen growth media were prepared by aliquoting 100ml of V-8 juice in 500mls glass bottles. About 10g of agar was added in the bottle followed with 1.5g of Calcium carbonate. The glass bottles were filled with distilled water to make 500mls. This mixture was autoclaved at 121 degrees Celsius for 15 minutes and left to cool down prior to pouring the media in petri dishes and were kept in a refrigerator at 8 degrees Celsius until the isolation assay (4) *Pathogen isolation:* The collected plant samples were cut into small pieces (1-2cm), the pieces were washed with distilled water for 30 seconds and sterilized with 70%vv ethanol and then rinsed with distilled water. The pieces were placed on paper towels until dried. Then, 3-4 pieces were placed on the growth media and sealed with parafilm to prevent contamination. The sealed samples were kept in an incubator at 27 degrees Celsius for 4-5 days (5) *Morphological characterization:* Pathogen growth features (spores, mycelium, conidia, hyphae, and others) were used to determine whether the grown pathogen was a typical pathogen of focus. Both direct and microscopic observations (at 10x, 40x and 100x) were used to determine these features.

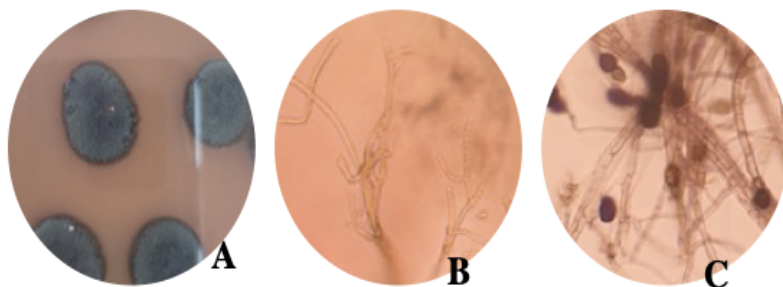
**RESULTS:** More than twenty stakeholders raised concerns about disease susceptibility in Red mottled bean varieties, particularly Lyamungo 90. From 2017, the infection of anthracnose and

angular leaf spot became severe at TARI – Selian centre farms. Three companies (BEULA, ALSSEM and ASA) also were affected by these pathogens. Through field survey, two fungal diseases were observed at these sites (anthracnose and angular leaf spot). The infection was higher on four varieties: Lyamungo 85, Lyamungo 90, Calima Uyole (red mottled) and Selian 13 (yellow and round).

In this study, four samples collected from two distant locations (Ndorobo and bean trial). Using pathogen isolation, both samples were positive for both *P. griseola* and *C. lindemuthianum* (Figures 1&2). Using a microscope, various morphological features were observed including conidia (figure 1A), appressoria (Figure 1B), darkish mycelium at the upper surface (Figure 1C) and conidiophores (Figure 1D). Also, for *P. griseola*, the samples were consistent of angular spot leaf symptoms. Features like hyphae/conidiophores, cylindrical conidia with various septa and blackish mycelium were observed under the microscope (Figure 2).



**Figure 1: Morphological features of the *C. Lindemuthianum*.** (A) Conidia - germinate, produce new generation but also function in dispersal mechanisms. (B) Appressoria - specialized cell that used to infect host plants (C) Mycelium - fibrous structure that function as feeding structure of the fungus (D) Conidiophore - specialized hyphae which conidia develop.



**Figure 2: Morphological features of the *P. griseola*** (A) blackish mycelium (B) hyphae/conidiophore (C) cylindrical conidia with various septa.

**CONCLUSIONS:** Integrative characterization of disease-causing pathogens by engaging commodity value chain actors is an effective approach and a way forward for developing resistant crop varieties with a better understanding of the actor’s needs.

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# DIFFERENTIALLY EXPRESSED GENES IN RESPONSE TO ANTHRACNOSE RACE 38 LOCATED IN THE Co-2 CLUSTER

María Jurado, Ana Campa, Juan José Ferreira

Agri-Food Research and Development Regional Service (SERIDA), Asturias, Spain

## INTRODUCTION

Resistance to anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus), is controlled by major race-specific genes (*Co*- genes) organized in clusters as the Co-2 cluster located at the end of chromosome Pv11. In this study, RNA-seq was used to investigate the differentially expressed genes (DEGs) in response to race 38 of *C. lindemuthianum* in an isogenic line (NIL) carrying a resistant locus in the Co-2 cluster.

## MATERIALS AND METHODS

**Plant Material.** Two common bean genotypes were used in this study, A25 and A4804. Line A25 is a selection in the market class fabada, a genotype susceptible to race 38. Line A4804 is a resistant genotype to race 38 which was obtained from the cross A2806 x X4562. These lines had an anthracnose resistance gene located in the Co-2 cluster, mapped in the physical position 46.65 – 48.65 Mbp [1]. The resistance present in originally derived from Cornell 49242.

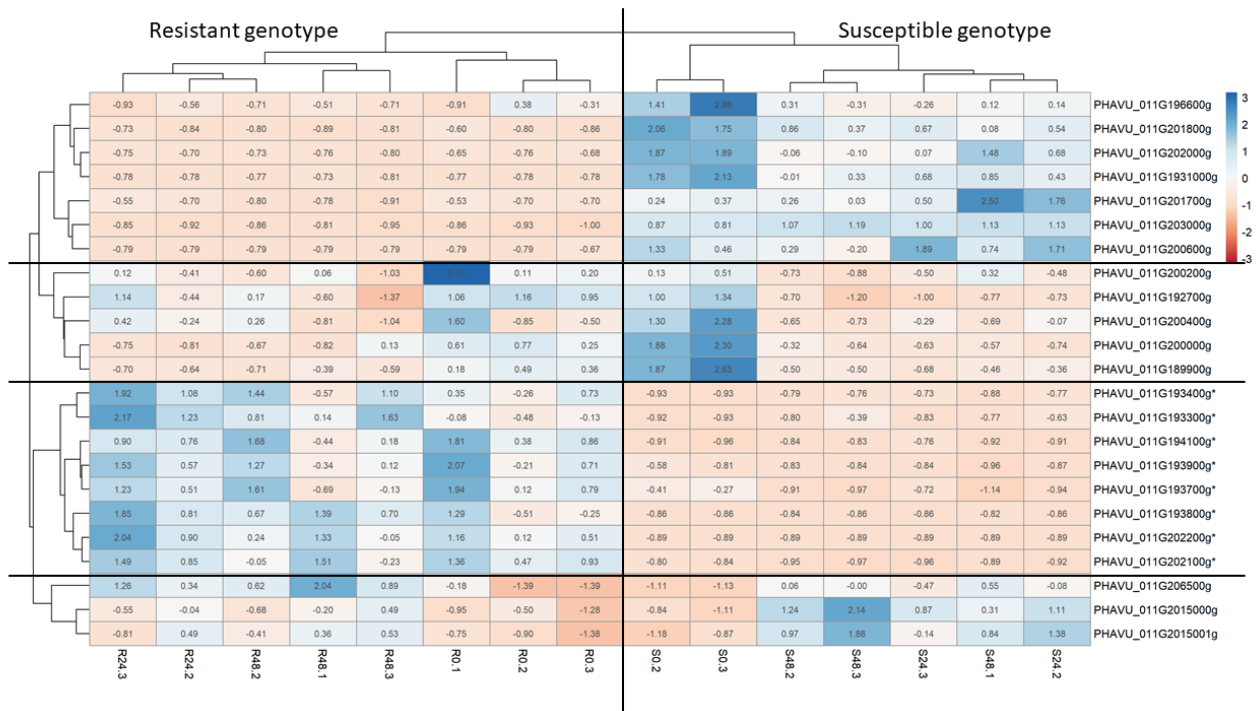
**Inoculation procedure and RNA isolation.** Seedlings of both lines were inoculated with race 38 ( $1.5 \times 10^6$  conidia/mL) and maintained under controlled conditions (24°C, 100 % RH, and 12 hours of photoperiod). The experimental design had three replicates corresponding to three resistance tests (1, 2, 3), two genotypes per replicate (A25 and A4804), and three treatments corresponding to three hours post-inoculation (hpi): 0 (just before inoculation), 24 and 48 hpi. The leaf tissues were harvested, flash-frozen in liquid nitrogen, and stored at -80°C before RNA extraction. RNA isolation, sequencing and reads assembly were carried out by Macrogen Inc. (Seoul, Rep. of Korea) using the G19833 v1.0 genome (<https://www.ncbi.nlm.nih.gov/> [2]).

**Comparative Transcriptome Analysis.** The NOISeq package (2.38.0) in R [3] and pheatmap 1.0.12 package in R platform [4] were used to explore the quality of the samples and to detect DEGs in the following comparisons: genotypes at different hpi (0-24, 0-48, 24-48) and between genotypes at the same hpi. FPKM (Fragments Per Kilobase of transcript per Million Mapped reads) normalized data of the 18 samples were used in the evaluation through a principal component analysis (PCA) and a hierarchical clustering analysis (HCA) to detect the possible source of noise in the experiment. The DEGs were investigated in the nine comparisons considering  $q > 0.80$  with the function `noiseq()`. DEGs found in the Co-2 region were used to build a clustered heatmap and functional annotation of these genes was investigated in the reference bean genome [2].

## RESULTS AND DISCUSSION

Seven days after inoculation with race 38, the susceptible genotype was dead while the resistant genotype did not exhibit symptoms. A quality analysis of the RNA-seq sequencing reads showed 3 samples (S0.1, S24.1 and R24.1; where S0.1 is the susceptible genotype at 0 hpi in test 1) with low RIN values and with a separation from the other samples of their group in the PCA and HCA analysis. These samples were removed. In total, 2850 unique DEGs were identified in the different comparisons.

In the region containing the Co-2 cluster in A4804, there were 165 annotated genes, among them 23 were differentially expressed in response to the pathogen. The representation of the FPKM values of these genes in a clustered heatmap revealed a separation between the resistant and susceptible genotypes (Figure 1). Also, the 23 DEGs were grouped in four main groups: i) 7 genes with higher expression in the susceptible genotypes, and no changes in the resistant genotypes; ii) 5 genes which tend to decrease their expression after inoculation in both genotypes; iii) 8 genes with higher expression in the resistant genotypes, and no-changes in the susceptible genotype after inoculation, all them with Serin/threonine protein kinases or leucin rich repeat domains; iv) 3 genes which tend to increase expression after inoculation in both genotypes. These results provide a closer approximation to the genes conferring the resistance reaction to race 38 in the Co-2 cluster present in the bean genotype A4804.



**Figure 5.** Clustered heatmap with the 23 DEGs in the Co-2 region (46.65-48.65 Mbp)

## ACKNOWLEDGMENTS

This work was supported, in part, by the European Union’s Horizon 2020 Research and Innovation Programme under grant agreement no. 774244 (Breeding for resilient, efficient and sustainable organic vegetable production; BRESOV). M. Jurado is supported by the FPI Grant PRE2019-091249 financed by MCIN/AEI/10.13039/501100011033 and FSE “El FSE invierte en tu futuro”.

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# EVALUATION OF THE RESPONSE TO POWDERY MILDEW AND WHITE MOLD IN THE SNAP BEAN PANEL

A. Campa<sup>1</sup>, S.A. Sheikahmadi<sup>1</sup>, E. Bitocchi<sup>2</sup>, R. Papa<sup>2</sup>, J.J. Ferreira<sup>1</sup>

<sup>1</sup>Agri-Food Research and Development Regional Service (SERIDA), Asturias, Spain

<sup>2</sup>Università Politecnica delle Marche, Ancona, Italy

## INTRODUCTION

Genetic resistance to diseases is a relevant trait for adaptation to organic production in which the use of chemical pesticides is limited. Powdery mildew (PM, *Erysiphe diffusa*) and white mold (WM, *Sclerotinia sclerotiorum*), are two of the main diseases affecting European common bean (*Phaseolus vulgaris* L.) crops. In this work, the response to local isolates of these fungi was evaluated in the Snap Bean Panel (SBP) to identify the most promising materials for organic farming.

## MATERIALS AND METHODS

**Plant Material.** SBP consists of 311 lines derived from European gene bank accessions, working collections, and seed companies (García-Fernández et al. 2022).

**Experimental design.** Two resistance tests in controlled conditions using a randomized incomplete block design were carried out for each pathogen. A test contained two replicates (pots)/line, with 4 seedlings/pot. Common checks (lines Porrillo Sintetico, Xana, Cornell49242, and A195) were included in each test. Resistant lines were confirmed in a third evaluation.

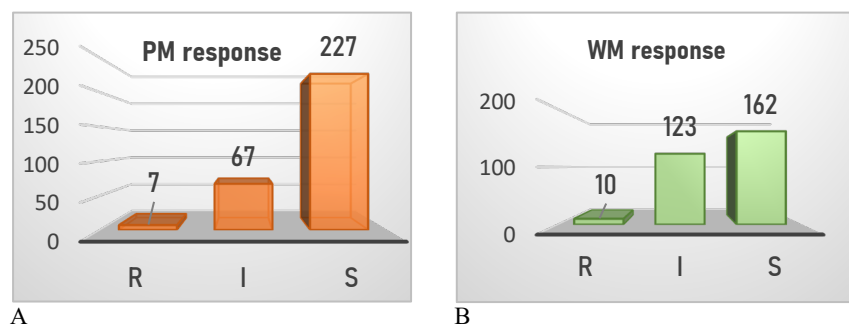
**PM resistance test.** A total of 301 SBP lines were evaluated for response to a local PM isolate according to Trabanco et al. (2012). Responses were recorded as infection type (IT) on a scale of 0 to 4, where IT0 was classified as resistant (R), IT1- IT3 as intermediate (I), and IT4 as susceptible (S).

**WM resistance test.** A total of 295 lines were evaluated against a local WM isolate using the straw test (Petzoldt and Dickson 1996). Disease progression was evaluated based on the level of infection on the main stem on a 1–9 severity scale. Response 1-3 was classified as R, 4-6 as I, and 7-9 as S.

## RESULTS AND DISCUSSION

Figure 1A shows the results of the PM evaluation. Most lines (227) were classified as S, with clear symptoms and conidia production, 67 lines were classified as I, and 7 lines as R. Figure 1B shows the results of the WM evaluation. Most lines (162) were classified as S; 123 lines as I, and 10 lines as R. The R lines to PM or WM are indicated in Table 1, as well as a morpho-agronomic description based on basic traits such as growth habit, and pod and seed traits (data taken from García-Fernández et al. 2022). All resistance sources against PM showed determinate growth habits, green pods, and white small seeds. In contrast, the lines with high resistance to WM exhibited indeterminate growth habits, yellow or green pod colors, and different seed sizes and colors. Considering the reaction against both diseases, 5 lines showed an intermediate reaction. The most promising materials identified for organic farming were SBP240 and SBP160, with resistance to PM and with an intermediate response to WM. Both lines are morphologically similar, with determinate growth habits, green pods, and white small seeds.

This work allowed the identification of two promising materials for organic farming, the identification of resistance sources against PM and WM, and also highlights the need for breeding in some snap bean market classes.



**Figure 1.** Histograms show the distribution for the reaction against a local isolate of powdery mildew (A) and white mold (B) in the SBP. R, resistant; I, intermediate; S, susceptible.

**Table 1.** Morphological description of the main resistance sources identified against PM and/or WM. R, resistant; I, intermediate; S, susceptible.

Line	PM	WM	Growth habit	Pod color <sup>(1)</sup>	Pod length <sup>(1)</sup>	Pod section <sup>(1)</sup>	Seed color <sup>(2)</sup>	Seed size <sup>(2)</sup>
SBP005	R	S	determinate	green	short	flat	white	small
SBP145	R	S	determinate	green	medium	rounded	white	very small
SBP160	R	S	determinate	green	medium	rounded	white	very small
SBP273	R	S	determinate	green	long	rounded	white	very small
SBP301	R	-	determinate	green	medium	rounded	white	small
SBP302	R	-	determinate	green	medium	rounded	white	small
SBP037	S	R	indeterminate	yellow	medium	flat	brown	small
SBP038	S	R	indeterminate	yellow	short	flat	brown	small
SBP082	S	R	indeterminate	green	long	flat	red	large
SBP118	S	R	indeterminate	yellow	short	flat	mottle	medium
SBP303	S	R	indeterminate	yellow	short	flat	brown	small
SBP305	S	R	indeterminate	yellow	medium	flat	brown	small
SBP306	S	R	indeterminate	green	short	flat	brown	small
SBP312	S	R	indeterminate	green	short	flat	brown	small
SBP320	S	R	indeterminate	green	medium	flat	black	small
SBP334	S	R	indeterminate	yellow	medium	flat	black	small
SBP092	I	I	indeterminate	yellow	long	flat	brown	medium
SBP033	I	I	determinate	yellow	medium	rounded	white	medium
SBP093	I	I	indeterminate	yellow	short	flat	purple	medium
SBP091	I	I	determinate	green	medium	rounded	brown	medium
SBP123	I	I	indeterminate	green	long	flat	white	medium
SBP240	R	I	determinate	green	short	pear	white	small
SBP160	R	I	determinate	green	medium	rounded	white	very small

(1) Pod phenotypic variation in the Snap Bean Panel, <https://zenodo.org/record/5557139#.YgTZBN9ByUk>

(2) Seed diversity catalog of the Snap Bean Panel, <https://zenodo.org/record/5557174#.YgTYrt9ByUk>

## ACKNOWLEDGMENTS

This work was supported by the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement no. 774244 (BRESOV)

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# FINE MAPPING OF *Ur-11*, A GENE CONFERRING RESISTANCE TO ALL BUT ONE OF THE RACES OF *Uromyces appendiculatus* MAINTAINED AT BELTSVILLE

Giseli Valentini<sup>1,2</sup>, Marcial A. Pastor-Corrales<sup>1</sup>, Larissa F.S. Xavier<sup>1</sup>, Oscar P. Hurtado-Gonzales<sup>3</sup>, Qijian Song<sup>1</sup>

<sup>1</sup>Soybean Genomics and Improvement Laboratory, USDA-ARS, Beltsville, MD 20705. <sup>2</sup>Oak Ridge Institute for Science and Education, ARS Research Participation Program, Oak Ridge, TN 37830; <sup>3</sup>Plant Germplasm Quarantine Program, USDA-APHIS, Beltsville, MD, 20705.

## INTRODUCTION

The *Ur-11* gene present in the Guatemalan common bean landrace PI 181996 confers resistance to all but one of the 90 races of *Uromyces appendiculatus* maintained at the USDA-ARS-Beltsville, Maryland. PI 181996 is only susceptible to race 22-52 (108) from Honduras. The recurrent production of new virulent strains by *U. appendiculatus* has resulted in major yield losses, principally on varieties with single rust resistance genes. Thus, development of cultivars combining two or more rust resistance genes is an effective strategy to manage the broadly virulent *U. appendiculatus* (Pastor-Corrales et al. 2003). The broad-spectrum resistance of *Ur-11* is a critical component of gene pyramiding for durable resistance. Recently, the *Ur-11* gene has been combined with other rust resistance genes, for the development of pinto, great northern, and other market classes of commercial dry bean cultivars in the United States, where, for example, the *Ur-11* and *Ur-3* genes formed the basis of breeding efforts to pyramid major rust resistant genes (MacQueen et al. 2020; Urrea et al. 2021). The objective of this study was to describe the fine-mapping of *Ur-11* and the development and validation of a DNA marker tightly linked to *Ur-11*.

## MATERIALS AND METHODS

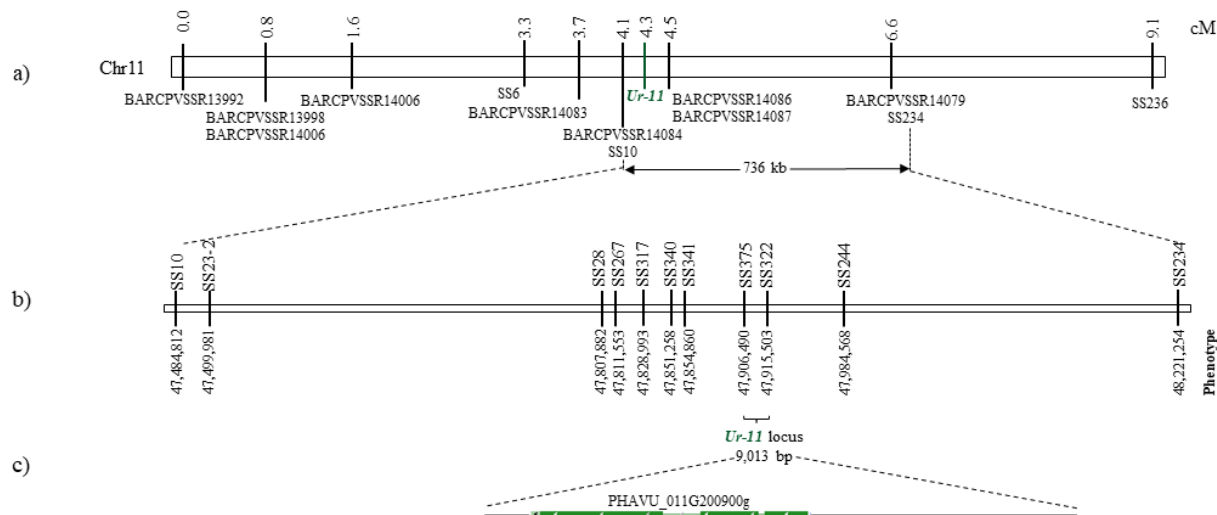
The analysis of the inheritance of resistance of *Ur-11* was performed by inoculating 124 F<sub>2</sub> plants derived from the Pinto 114 (susceptible) and PI 181996 (resistant) cross with races 15-1 (41), 15-3 (47), 31-1 (53) and 31-22 (67) of *U. appendiculatus*. Three susceptible bulks were prepared and genotyped with the BARCBEAN6K\_3 BeadChip. The genomic region containing *Ur-11* was targeted for development of specific SSR and KASP markers to be used to advance the mapping of *Ur-11*. The fine mapping was performed by inoculating 1,639 F<sub>3</sub> plants, derived from 162 F<sub>2:3</sub> families, with race 31-1 (53) and genotyped with the SS10 and SS234 KASP markers flanking the *Ur-11* gene. F<sub>3</sub> plants showing recombination between the flanking markers were selected for fine mapping. The region between SS10 and SS234 was screened for the presence of new SNPs to develop KASPs markers. SNPs were selected based on the whole genome re-sequencing of 42 common bean cultivars with and without *Ur-11*. The KASP markers were tested on the recombinant F<sub>3</sub> plants. Then, SS322, one of the closest markers to *Ur-11*, was validated on a panel of 205 cultivars from different gene pools, market classes, and with different rust resistant genes.

## RESULTS AND DISCUSSION

Using bulk segregant analysis and SNP genotyping, the single and dominant *Ur-11* gene was first positioned in a 2.3 Mb genomic region on chromosome Pv11. Subsequent genetic mapping of *Ur-11*, using a combination of KASP and SSR markers, positioned *Ur-11* in a smaller 482 kb genomic region between KASP SS10 and SSR BARCPVSSR14086 markers (Fig. 1a). To advance the fine mapping of *Ur-11*, 1,639 F<sub>3</sub> plants were phenotyped with race 31-1 (53) and genotyped with the SS10 and SS234 KASP markers flanking *Ur-11*. We identified 83 F<sub>3</sub> recombinant plants. These results indicated that the *Ur-11* gene was positioned in a smaller genomic region (9.01 kb) flanked



by KASP markers SS375 (47,906,490 bp) and SS322 (47,915,503 bp) (V1.0) (Fig. 1b). This region included a candidate gene encoding a nucleotide-binding site and leucine rich-repeat domain with a pathogen resistance function (Fig 1c). To validate the KASP SS322 marker, a panel of 205 diverse common bean cultivars was phenotyped with races 22-6 (49), 31-1, 31-22, and 22-52 of *U. appendiculatus* and genotyped with the SS322 marker. This panel included Andean and Middle American cultivars and snap and dry beans from different market classes. Some of the cultivars, had the *Ur-11* gene alone, while other cultivars had *Ur-11* in combination with other rust resistant genes. The presence or absence of SS322 was accurately detected in 200 cultivars. However, the presence of SS322 was not accurately detected in five cultivars: Stampede, PT-11-13-1, USDA-Rattler, NE2-17-37, and NE4-17-10. In all five cultivars, the *Ur-3* and *Ur-11* genes were segregating; that is, some plants had *Ur-3* alone, and others had *Ur-3+Ur-11*. SS322 was observed in plants of Stampede, PT-11-13-1, and USDA-Rattler with *Ur-3* alone and in plants combining *Ur-3* and *Ur-11*. Conversely, SS322 was not observed in plants of NE2-17-37 and NE4-17-10 that combined *Ur-11* and *Ur-3*. However, the inability of SS322 to identify *Ur-11* in plants combining *Ur-11* and *Ur-3* was not widespread. For instance, SS322 accurately (100%) detected the presence of *Ur-11* in an F<sub>2</sub> population from the BelMiNeb-RR-2 (*Ur-11*) × Aurora (*Ur-3*) cross. At present, we are studying why SS322 is effective in some combinations of *Ur-11/Ur-3* but not in others.



**Figure 1.** Fine mapping *Ur-11* rust resistance gene in PI 181996. a) Genetic map of common bean Pv11 containing *Ur-11* and the linked SSRs and SNPs markers used to genotype the F<sub>2</sub> population from the Pinto 114 × PI 181996 cross; b) Flanking SNP markers SS10 and SS234 and nine KASP markers used to fine map the *Ur-11* locus to a 9.01 kb genomic region using 83 recombinants F<sub>3</sub> plants; c) The *Ur-11* candidate region included a gene in the reference genome of G19833 encoding a nucleotide-binding site and leucine rich-repeat domain with pathogen resistance functions. The marker position is based on version 1.0 of the RefGen G19833.

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# PRELIMINARY SURVEY OF SOME DISEASES ON COMMON BEAN AND IDENTIFICATION OF ASSOCIATED PATHOGENS

Adomako, J.<sup>1</sup>, Yeboah, S.<sup>1</sup>, Oteng-Darko, P.<sup>1</sup>, Asibuo, J.<sup>1</sup>, Acheampong, P.P.<sup>1</sup>,  
and Butare, L.<sup>2</sup>

<sup>1</sup>CSIR-Crops Research Institute, P. O. Box 3785, Kumasi-Ghana

<sup>2</sup>International Center for Tropical Agriculture-Nairobi, Kenya

## INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is a newly introduced crop in Ghana with the potential for improving nutritional security of the population, most especially the resource poor. Field trials show it grows well in both the transition and forest zones of the country and that it has high adoption potential. Despite its significance, the crop is prone to several diseases. To develop sustainable disease management strategies for farmers, there was the need to identify and document some common diseases attacking the crop and to identify the associated pathogens.

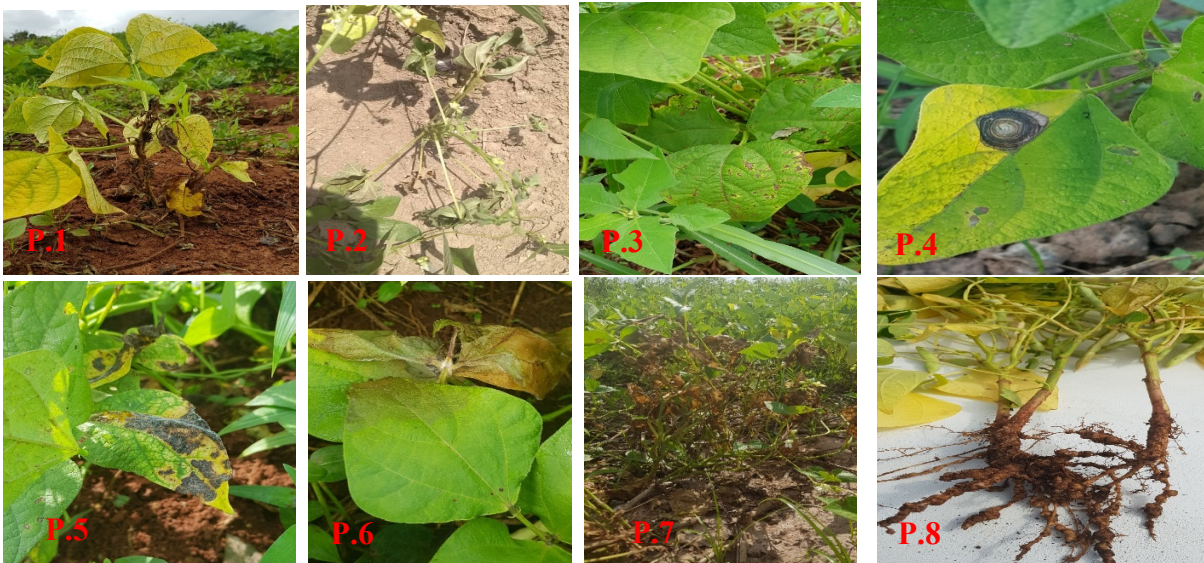
## MATERIALS AND METHODS

Surveys were carried out during the cropping season of 2019 in common bean research fields across the transition and forest agro-ecological zone of Ghana. In each field, 20 plants in the two central rows were selected systematically and assessed for disease symptoms such as leaf spots, leaf blight, lesions/spots on pods, and leaf chlorosis. Selected plants were also uprooted and stems and roots assessed for signs of fungal and plant parasitic nematode infection such as mycelia, presence of cankers, and root rots. Standard laboratory protocols were followed during fungal and nematode pathogen isolation and identification.

## RESULTS AND DISCUSSION

Surveys across the fields revealed leaf chlorosis (P.1), wilting (P.2), leaf spots (P.3), spots with concentric rings (P.4), leaf lesions (P.5), leaves appearing as though they had been scalded by hot water (a common symptom of web blight, P.6) and common bean blight (P.7) with total defoliation of the bean plant under severe conditions as the most prevalent above ground symptom. In addition to the above ground symptoms, root gall (P.8), a common symptom of root knot nematode infestation, was also identified on roots of plants.

Several fungal species: *Colletotrichum* spp., *Cercospora* spp., *Curvularia* spp., *Phaseoriopsis* spp., *Rhizoctonia* spp. and *Phoma* spp. were found associated with the foliar symptoms. *Phoma* spp. and *Rhizoctonia* spp. were isolated from leaf spots with concentric rings and web blight disease, respectively, whilst *Fusarium* spp. and *Sclerotium* spp. were isolated from roots and stems of plants showing wilting and root rot symptoms. Root knot nematode (*Meloidogyne* spp.) was recovered from roots and the rhizosphere soil of plants showing symptoms of root gall. The pathogens, *Colletotrichum* spp. and *Phaseoriopsis* spp., are known to cause Anthracnose and Angular Leaf Spot diseases, respectively. These diseases have been reported as major constraints to bean production, reducing productivity (ECABREN, 2003). Phyto-nematode infestation reduces nodule formation (Wood et al., 2018) and hampers the ability of plants to efficiently absorb nutrients and water, leading to symptoms such as wilting and leaf chlorosis in affected plants.



Pathogenicity studies will be carried out to confirm each of the isolated pathogens as a disease-causing organism in common bean.

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# GENOME-WIDE ASSOCIATION STUDY FOR PHENOLIC COMPOUNDS OF COMMON BEAN SEEDS

A. Campa, R. Rodríguez, M. Jurado, C. García-Fernández, B. Suárez, J.J. Ferreira

Agri-Food Research and Development Regional Service (SERIDA), Asturias, Spain

## INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) contains flavonoids (flavonols, anthocyanins, and condensed tannins) in their seed coats whose concentration and profile varies widely depending on genotype. The objective of this work was to conduct a genome-wide association study (GWAS) using the Spanish Diversity Panel (SDP) to identify genomic regions associated with the synthesis of seed coat flavonols and anthocyanins.

## MATERIALS AND METHODS

SDP is a panel considered representative of the Spanish diversity for this species (Campa et al. 2018). In all, 274 SDP lines were grown in a greenhouse (2018) using a randomized complete block design with one replicate (10 plants) per line. Dry seeds were harvested and maintained under vacuum at -20°C until the chemical analysis (Rodríguez et al. 2020). The extractable phenolic profile of 4 anthocyanins (Cya\_G, Del\_G, Pel\_G, Pet\_G) and 7 flavonols (Kae, Kae\_G, Kae\_GAcII, Myr\_G, Qu\_G, Que\_Rut, Que\_GAc) were analyzed in each line (Table 1).

The SDP was genotyped by GBS and aligned to the *Phaseolus vulgaris* v2.1 reference genome. After filtering (missing values < 10%; MAF > 0.05) 11,763 SNPs were considered for GWAS, conducted in the R project (mrMLM package) using the FASTmrEMMA model. A critical threshold of significance  $LOD \geq 5.4$  was considered after Bonferroni correction. A 350-Kb window centered for each associated SNP was considered for candidate gene exploration. When two windows overlapped, they were considered as a unique quantitative trait loci (QTL). The QTL was named using the prefix Phe (phenolic), chromosome number, and the start physical position in Mbp. Candidate genes underlying each QTL were considered based on: (i) search by keywords of molecules involved in the phenolic pathway in Phytozome or Kyoto Encyclopedia of Genes and Genomes (KEGG); (ii) genes previously described in common bean (Reinprecht et al. 2013); (iii) BLASTp with genes described in *Arabidopsis thaliana* (Kubasek et al. 1992; Baxter et al. 2005).

## RESULTS AND DISCUSSION

Table 1 shows the observed variation for each metabolite. Kae\_G showed the highest concentrations (from 0 to 2042.4 µg/g) while Que\_Rut showed the lowest (from 0 to 18.8 µg/g). A total of 10 SNPs were significantly associated with one or several metabolites (except for Pet\_G and Que\_Rut) and they were grouped in 8 QTL (Table 2). Twenty-one candidate genes were identified for all QTL except for Phe\_01(1.3), Phe\_02(30.8), and Phe\_10(10.3).

Chromosome Pv08 seems to be highly involved in the flavonoid pathway, including the QTL Phe\_08(2.7) associated with the 6 metabolites Que\_G, Kae\_G, Kae\_GAcII, Que\_GAc, Cya\_G, and Pel\_G. For this QTL, 9 candidate genes were identified, including a cluster of 4 genes (*Phvul.008G038200*, *Phvul.008G038400*, *Phvul.008G038500*, *Phvul.008G038600*) that encode a MYB113 transcription factor, a regulator of anthocyanin biosynthesis, and a cluster of 2 genes (*Phvul.008G042350*, *Phvul.008G042400*) that encode flavone-malonyltransferases. This chromosome region, spreading from 2.7 to 4.0 Mbp of chromosome Pv08, probably corresponds

to the complex *C* locus involved in the seed coat color. In fact, the candidate gene *Phvul.008G038400* is responsible for the black seed coat color (García-Fernández et al. 2021). The 21 candidate genes identified and their implication in the genetic control of the flavonoid biosynthesis pathway will be studied in more detail.

**Table 1.** Observed variation (in µg/g) for 4 anthocyanins and 7 flavonols in the 274 bean lines included in the SDP. Mean, standard deviation (SD), minimum (Min), and maximum (Max) values

Phenolic Compound (abbreviation)	Mean	SD	Min-Max
cyanindin3-O-glucoside (Cya_G)	11.7	35.5	0 - 334.3
delphinidin3-O-glucoside (Del_G)	42.2	127.1	0 - 871.3
pelargonidin3-O-glucoside (Pel_G)	10.5	27.5	0 - 227.3
petunidin3-O-glucoside (Pet_G)	7.8	32.9	0 - 258.3
Kaempferol (Kae)	4.5	14.6	0 - 158.5
kaempferol3-O-glucoside (Kae_G)	178.4	376.1	0 - 2042.4
kaempferol3-O-acetylglucosideII (Kae_GAcII)	46.6	82.8	0 - 454.7
myricetin3-O-glucoside (Myr_G)	13.0	45.6	0 - 300.3
quercetin3-Oglucoside (Que_G)	16.6	30.7	0 - 165.2
quercetin3-O-rutinoside (Que_Rut)	0.7	1.7	0 - 18.8
quercetin3-Oacetylglucoside (Que_GAc)	4.8	11.5	0 - 113.6

**Table 2.** QTL identified in the 274 SDP lines. Na/Nc, number of annotated/candidate genes

QTL	SNP	Trait/s	Na/Nc	Candidate genes
Phe_01(1.3)	SNP01_1719386	Que_GAc	69/0	-
Phe_02(30.8)	SNP02_31169826	Del_G	65/0	-
Phe_04(1.2)	SNP04_1611602	Del_G, Myr_G	69/1	<i>Phvul.004G012500</i>
Phe_08(2.7)	SNP08_3066515	Que_G, Kae_G, Kae_GAcII	139/9	<i>Phvul.008G038200, Phvul.008G038400, Phvul.008G038500, Phvul.008G038600, Phvul.008G042350, Phvul.008G042400, Phvul.008G034000, Phvul.008G035800, Phvul.008G040232</i>
	SNP08_3181860	Que_Gac		
	SNP08_3694747	Cya_G, Pel_G		
Phe_08(62.5)	SNP08_62934084	Pel_G	70/5	<i>Phvul.008G287200, Phvul.008G287300, Phvul.008G289200, Phvul.008G289400, Phvul.008G289500</i>
Phe_09(35.7)	SNP09_36051750	Que_GAc	57/2	<i>Phvul.009G239000, Phvul.009G238400</i>
Phe_10(10.3)	SNP10_10709290	kae	9/0	-
Phe_11(51.3)	SNP11_51718220	kae	63/4	<i>Phvul.011G200700, Phvul.011G201700</i>
				<i>Phvul.011G202000, Phvul.011G202500</i>

## ACKNOWLEDGMENTS

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# A CORE SET OF BEAN GENOTYPES ESTABLISHED FROM PHENOTYPING A SNAP BEAN PANEL

C. García-Fernández<sup>1</sup>, M. Jurado<sup>1</sup>, A. Campa<sup>1</sup>, C. Brezeanu<sup>2</sup>, V. Geffroy<sup>3</sup>, E. Bitocchi<sup>4</sup>, R. Papa<sup>4</sup>, J. J. Ferreira<sup>1</sup>

<sup>1</sup>Agri-Food and Development Regional Service (SERIDA), Asturias, Spain; <sup>2</sup>Stațiunea de Cercetare Dezvoltare pentru Legumicultură, Bacău, Romania; <sup>3</sup>Université Paris-Saclay, CNRS, INRAE; <sup>4</sup>Università Politecnica delle Marche, Ancona, Italy

## INTRODUCTION

Snap beans (green beans, French beans) are a group of common bean cultivars (*Phaseolus vulgaris* L.) whose immature pods can be consumed as a green vegetables. The main objective of this work was to investigate the phenotypic diversity in pod traits from a large panel of snap beans (SBP) and establish a core set (Core-SBP) with maximum phenotypic diversity and minimum redundancy.

## MATERIALS AND METHODS

**Plant Material.** A total of 311 snap bean accession collected from European gene banks, working collections, and seed companies were included in the SBP. Among these accessions were included genotypes classified as landraces, old and elite cultivars, as well as 39 snap bean lines from the Spanish Diversity Panel<sup>[1]</sup>. One homozygous line per accession was obtained by self-pollination in a greenhouse of one plant derived from each accession.

**Phenotyping.** The SBP was evaluated at Villaviciosa, Spain (43°29'01"N, 5°26'11"W; elevation 6.5 m) in a greenhouse (2018) and field (2020) using a randomized design with a plot per line, and 10 plants/plot. Phenotypic characterization was based on 14 quantitative and 2 qualitative traits from 10 pods per line in each trial. Twelve quantitative traits corresponding to morphometric measurements (see Figure 1) were digitally phenotyped using Tomato Analyzer software v3<sup>[2]</sup>. The rest of the quantitative (number of seeds per pod, and 25 seeds weight) and qualitative (pod color, and main seed coat color) traits were recorded manually.

**Statistical analysis:** A HCPC (Hierarchical Clustering on Principal Components) analysis was carried out to identify the main clusters from the quantitative pod traits. Statistical analyses were carried out using the mean phenotypic data and performed with the packages FactoMineR and FactoExtra in the R platform<sup>[3,4]</sup>.

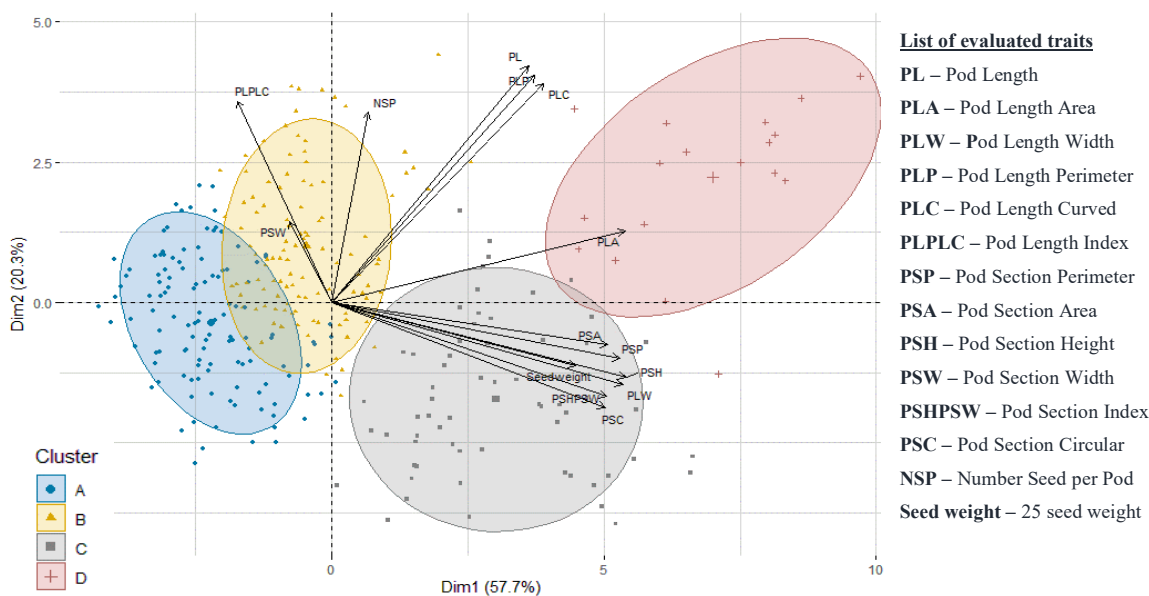
**Core-SBP establishment:** A hierarchical method was followed to select a subset of lines that represents most of the phenotypic diversity gathered in the SBP<sup>[5]</sup>. The lines were grouped first according to the results provided by HCPC and then classified according to the pod color, and main seed coat color into each group.

## RESULTS AND DISCUSSION

The SBP lines showed a wide phenotypic variation for all morphological pod traits. For instance, pod length (PL) and the number of seeds per pod (NSP) ranged from 7.3 cm (observed in SBP382) to 25.86 cm (SBP299), and from 3.7 seeds (SBP280) to 8.75 seeds (SBP012), respectively. Pod color phenotype also showed a wide diversity with green (212 lines), yellow (80), purple (7), green mottled (11), and yellow mottled (1) pod colors (see <https://zenodo.org/record/5557139>). Additionally, more than half the lines had a seed coat color (170) including cream (22), canella (15), brown and dark brown (66), red (4), purple (10), and black (53).

HCPC analysis using the 14 quantitative pod traits revealed two main dimensions explaining 78 % of the variation, and four main clusters (see Figure 1) with significant differences among them for shape and size. Considering these four clusters provided by HCPC analysis, the five pod colors, and the nine main seed coat colors, a total of 54 different phenotypic classes were identified in the SBP. Cluster C was the most diverse with 17 classes followed by cluster A (15 classes), cluster B (13), and cluster D (9). The largest class has short green pods and white seed (73 lines included in cluster A) followed by the class with medium-length green pods and white seeds (23 lines included in cluster B) and a class with medium-length green pods and black seeds (20 lines included in cluster B). Finally, the phenotypic diversity of the SBP (N=311) was conserved in the Core-SBP (N=54) from the random selection of a single line per phenotypic class identified. The Core SBP established is a first attempt to classify snap bean cultivars based on morphological pod traits and constitutes a valuable source of traits for future breeding programs and genetic analysis.

**Figure 1.** Biplot showing the distribution of the 311 snap bean lines considering the two main estimated dimensions revealed by Hierarchical clustering on Principal Component Analysis (HCPC). Ellipses representing the clusters were drawn considering a confidence interval > 0.8.



## ACKNOWLEDGMENTS

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# WHITE BEAN CULTIVAR MIXTURES: MIXING EFFECTS ON YIELD

Yarmilla Reinprecht, Lyndsay Schram, Thomas H. Smith, and K. Peter Pauls

University of Guelph, Department of Plant Agriculture, Guelph, ON N1G 2W1, Canada

## INTRODUCTION

Conventional bean breeding is organized to deliver high yielding, genetically pure cultivars in various market classes. However, crossing of elite-by-elite cultivars has led to the reduction of genetic diversity, especially in large-seeded beans. We have been exploring the feasibility of increasing genetic diversity in cropping systems by using mixtures of common bean cultivars instead of monocultures. Numerous studies have demonstrated that cultivar mixture cropping has benefits over monoculture cropping in controlling disease, increasing water use efficiency and increasing yield stability.

## MATERIALS AND METHODS

Six diverse white bean cultivars (Mist, Rexeter, Bolt, AAC Shock, Nautica, and AAC Argosy - recommended by bean growers/processors) were selected for this study. The genotypes were evaluated in pure stands and as completely random binary mixtures (1:1 seed number) at two University of Guelph research stations (Elora and Woodstock, ON) as RCBD trials (8-row plots and 4 replications) over three years (2019-2021). In all mixtures, both genotypic components were harvested and analyzed together. Plot-based crop data were collected for the conventional above ground traits. Crop data were analyzed using the generalized linear mixed models (Glimmix) procedure in SAS (Statistical Analysis System) v.9.4 software (SAS Institute, 2013). The Relative Yield of the Mixture (RYM) index was used to evaluate mixing efficiency of biblends with the formula:  $RYM = \text{Yield}_{\text{mixture}} / [(\text{Yield}_{\text{pure 1}} + \text{Yield}_{\text{pure 2}}) / 2]$  (Trenbath, 1974). Diallel analysis was used to determine general mixing abilities (GMA) of cultivars in pure stands and specific mixing abilities (SMA) of their mixtures in PTools (IRRI, 2014).

## RESULTS AND DISCUSSION

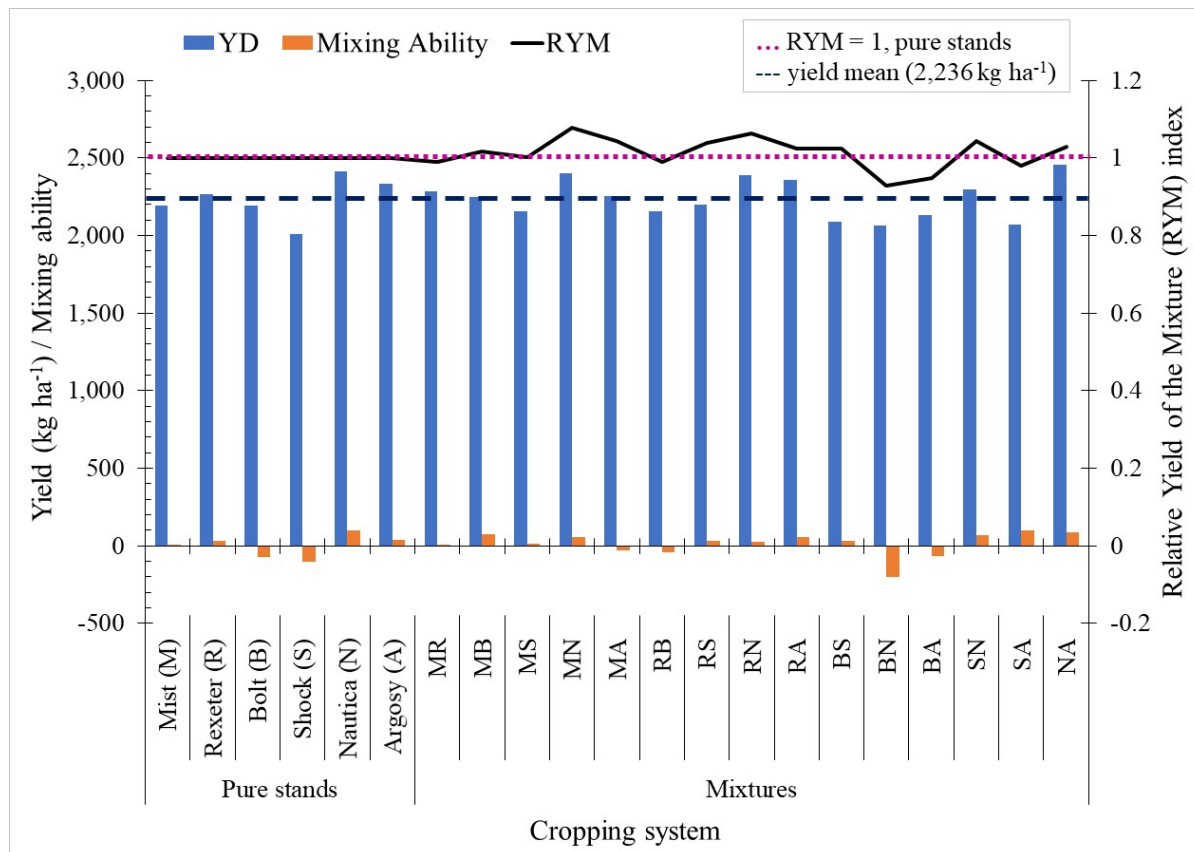
Significant differences were identified among six white bean cultivars and their binary mixtures for yield and yield-related traits. Yields from some mixtures were similar to, or better than, the average of the two cultivars grown as monocrops and they had  $RYM > 1$ . The mixture of Nautica/Argosy (NA) was 3% higher yielding than the best pure stand component (Nautica). Higher yields and greater RYMs identified in some bean mixtures may be attributed to a more efficient use of limited plant resources (light, water, and nutrients) or greater resiliency to environmental stress. The results from this study showed that the identification of the best mixtures needs to be carried out using several approaches (trait-, index- and/or diallel-based selection). The best performing mixtures were Mist/ Nautica (MN), Rexeter/ Nautica (RN), Rexeter/ Argosy (RA) and Nautica/Argosy (NA) - they had  $RYM > 1$ , positive specific mixing abilities (SMA) and high and stable yields (YD, **Figure 1**) averaged over six Ontario environments (location/year).

## Implications

The study evaluated a set of 15 binary mixtures formed from six white bean cultivars (selection recommended by bean growers and processors) in two Ontario locations over three years. The superior performances of some compatible mixtures indicated that there is a possibility (or even an advantage) to establish bean crops with mixtures of cultivars, instead of a single cultivar monoculture. Growing crops from mixtures of white navy beans, or mixtures within the same



market class of other market classes (such as pinto or kidney beans) might find immediate acceptance by bean producers. By planting superior performing mixtures of one market type, producers may benefit from crops that have high yield and greater genetic variability to allow them to respond to disease or other environmental challenges with resiliency, and uniform appearance and do not require sorting into separate market classes.



**Figure 1.** Selection of the best performing bean cultivar mixtures based on the yield, RYM index and mixing abilities.

### ACKNOWLEDGEMENTS

This project is a part of the Food from Thought research program at the University of Guelph. The research was undertaken in part thanks to generous funding from the Canada First Research Excellence Fund, the Ontario Bean Growers, Agriculture and Agri-Food Canada, the Ontario Ministry for Food and Rural Affairs and the Ontario Ministry for Research and Innovation.

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## ANDEAN COMMON BEAN GERMPLASM FROM BRAZIL: A MISSED OPPORTUNITY?

Stephen Beebe

CIAT Bean Program, Cali, Colombia

Concepts of Andean bean diversity were articulated by Singh et al. (1992) whereby three races were defined: Nueva Granada (referring to the name of the colonial territory of the northern Andes), Peru, and Chile. The governing principles for the definition of races were growth habit and adaptation range, which were contrasting for the three suggested races. However, subsequent analysis of DNA by AFLP (Beebe et al., 2001) or by microsatellites (Kwak and Gepts, 2009) demonstrated that the three races were very closely related, and genetic differences that could be detected among races were at best modest. The distinguishing factors among races appeared to have resulted from human intervention and selection, likely diverging from a common origin.

A prior analysis of a core collection of wild *Phaseolus vulgaris* had revealed at least four major groups, with sub-groups within groups. In the Andean accessions, a group suggested to be ancestral in northern Peru and Ecuador was recognized as distinct (Tohme et al., 1996) and was subsequently renamed as a new species, *P. debouckii* (Rendon, et al., 2017). Another group occurred in Colombia and the northern Andes, while wild beans from Peru, Bolivia and Argentina represented subgroups within a broader Andean group (Tohme et al., 1996).

When a sub-set of Andean wild beans representing the several groups were included in an AFLP analysis of cultivated Andean beans, to our surprise most wild types that overlapped with cultivated bean were derived from Bolivia (Beebe et al., 2001). Little attention had been paid to Bolivia as a center of diversity of common bean, likely due to the low importance of bean in modern agricultural statistics of that country. The very names of the three Andean races (Nueva Granada, Peru and Chile) demonstrate the geographic focus of attention in the 1990's when interest was high in understanding genetic diversity. However, the prospect of an early domestication event in Bolivia – indeed, what could have been the primary domestication event of the Andean gene pool! – invites ample reflection.

Such an event would imply that cultivated bean expanded to the west, north and south through the Andes, to form those three races. Experience with Bolivian wild beans suggests that this was feasible, since these had been noted to have wide adaptation in the sense of producing seed more readily than other wild beans on the CIAT research station in Cali, Colombia (Orlando Toro, pers. com.).

Furthermore, a domestication event in Bolivia has further implications, given the proximity of the Bolivian inter-Andean valleys to Brazil. Normally Brazil is associated with common bean of the Mesoamerican gene pool, given its role as the bean producer of greatest volume in the entire world, largely in cream-striped (carioca) or small black market classes. However, a range of Andean grain types are native to Brazil, and these are believed to predate the colonial era. Grain colors include yellows, cream striped (cranberry), pink striped, red, gray speckled, and black. If the hypothesis about the expansion of cultivated bean from Bolivia is correct, this would imply that not only domestication but much of the formation of the modern cultivar phenotype occurred in Bolivia, since phenotypically the Brazilian germplasm is similar to that of race Nueva Granada in seed size and shape, and in plant growth habit, and selection of this phenotype would have occurred prior to dissemination in different directions.

Although similar to other Andean bush beans in general phenotype, over several years of observation Brazilian Andean types have been noted to present traits that are uncommon in Andean bush beans: insensitivity to photoperiod; a modest adaptation to low phosphorus soils; type II growth habit. An observation on productivity of a handful of Andean accessions from Brazil under high temperatures led us to evaluate a larger collection of 156 accessions from the CIAT gene bank. These were evaluated under high temperatures on the Nataima research station of the Agrosavia national research institution of Colombia, (365 masl, 28°C average temperature, and 22°C night temperature). More than 20 accessions were selected for further study, with productivity comparable to bred lines introgressed with genes from *P. acutifolius* in early efforts at breeding for heat tolerance. Preliminary observations on tolerance of the Brazilian accessions are being confirmed in subsequent evaluations.

Finding heat tolerance in Andean germplasm at first seemed counter-intuitive, given our concepts of Andean germplasm being adapted to mid-to-high altitudes. However, considering this germplasm as having evolved in proximity to the inter-Andean valleys of Bolivia puts these in a different light. These valleys descend gradually from more than 2000 masl to the lowlands, allowing a gradual adaptation to warmer climates.

These observations suggest that Andean accessions from Brazil may represent a small but unusual and significant segment of the Andean gene pool that warrants more attention. We have no way of knowing over what period of time these accessions might have been subjected to selection pressures of higher temperature, acid soil, or the associated biotic factors, but it is feasible that this could have been many centuries. It is also of interest that this same region gave rise to cultivated peanuts, *Arachis hypogaea*, when Native American plant domesticators recognized a rare event of polyploidization. Pineapple and cassava might have been domesticated on the southern rim of the Amazon basin. Was this a region of active plant domestication that also contributed to cultivated common bean?

This experience also illustrates the possible pitfalls in permitting current production patterns to influence our understanding of the evolution of genetic diversity, either in underestimating the historical role of Bolivia in bean domestication, or neglecting the possible value of native Brazilian germplasm of Andean origin.

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# GENE EXPRESSION ANALYSIS IN CALIFORNIA DARK RED KIDNEY COMMON BEAN CULTIVAR DURING INCOMPATIBLE INTERACTION WITH *Colletotrichum lindemuthianum*

Lovatto M<sup>1</sup>, Gonçalves-Vidigal MC<sup>1</sup>, Calvi A<sup>1</sup>, Vaz-Bisneta M<sup>1</sup>, Vidigal Filho PS<sup>1</sup>

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

**INTRODUCTION:** Anthracnose is a severe disease in common beans (*Phaseolus vulgaris* L.); resulting in up to 100 percent yield losses (Pastor-Corrales et al. 1994). Cultivars resistant to *C. lindemuthianum*, the pathogen that causes anthracnose, offer a cost-effective, easy-to-use, and environmentally friendly management strategy. The common bean California Dark Red Kidney is resistant to races 2, 9, 39, 55, 64, 65, 73, 89, 1545, 2047, and 3481 of *C. lindemuthianum*. The resistance locus *CoPv01<sup>CDRK</sup>* was fine mapped to a 33 Kb interval on chromosome Pv01, harboring five candidate genes (Gonçalves-Vidigal et al., 2020). In the present study, the relative expression of the candidate genes close to the *CoPv01<sup>CDRK</sup>* locus and other resistance genes was evaluated during an incompatible interaction between California Dark Red Kidney common bean cultivar with race 73 of *C. lindemuthianum*.

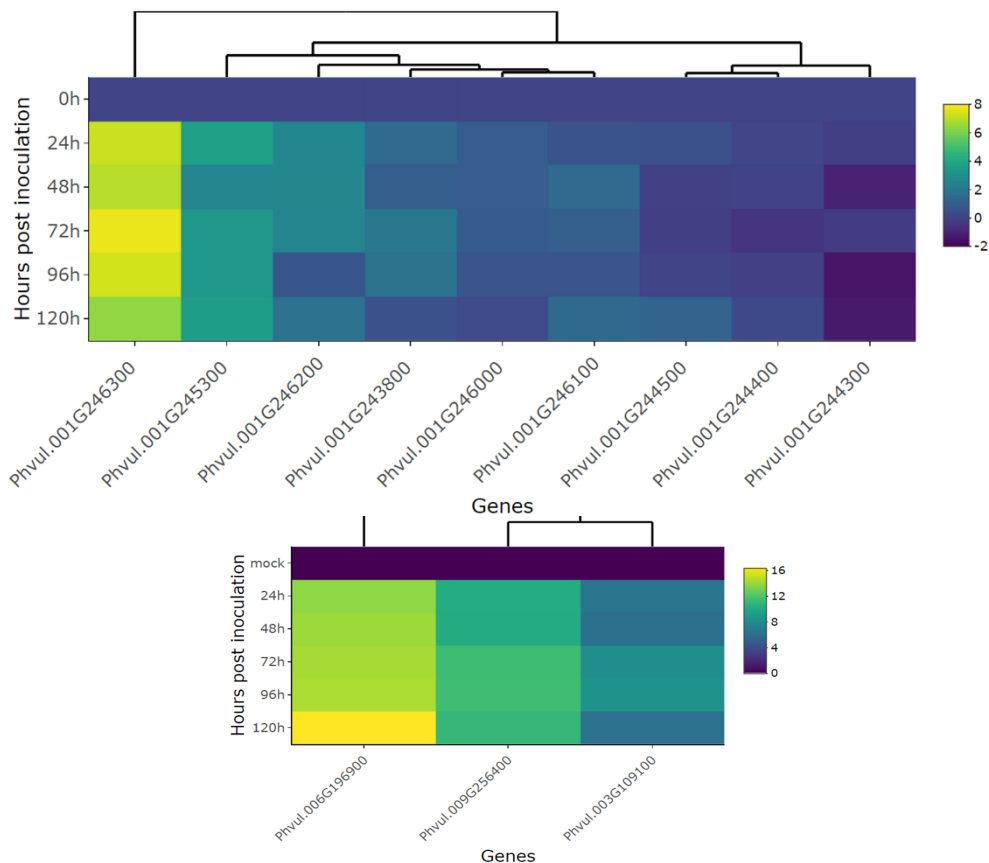
**MATERIALS AND METHODS:** The California Dark Red Kidney (CDRK) cultivar and race 73 of *C. lindemuthianum* were used in this study. The plants were inoculated with a spore suspension containing  $2.0 \times 10^6$  spores mL<sup>-1</sup> of race 73, at the v<sub>3</sub> development stage. Leaf samples of the first trifoliolate were collected at 0, 24, 48-, 72-, 96-, and 120-hours post inoculation (hpi) from three biological replicates. The total RNA was extracted, purified and the cDNA was synthesized. The relative expression of the candidate genes *Phvul.001G246000*, *Phvul.001G246100*, *Phvul.001G246200*, *Phvul.001G246300*, and *Phvul.001G245300* proposed by Gonçalves-Vidigal et al. (2020) for the *CoPv01<sup>CDRK</sup>* locus were obtained. The candidate genes *Phvul.001G244300*, *Phvul.001G244400*, and *Phvul.001G244500* of the *Co-AC* locus (Gilio et al. 2020), *Phvul.001G243800* of the *Co-I<sup>2</sup>* locus (Zuiderveen et al. 2016) and the known resistance genes *Phvul.003G109100* (PR1a), *Phvul.006G196900* (PR1b) and *Phvul.009G256400* (PR2) were also evaluated. Quantitative RT-PCR was performed with StepOnePlus™ Real-Time PCR Systems. Mean cycle threshold (CT) values were calculated for each gene at each data point. Gene expression levels were normalized based on the expression of *Phvul.001G133200* (IDE) and *Phvul.008G011000* (ACT). Relative expression was carried out by the 2<sup>- $\Delta\Delta$ CT</sup> method (Livak and Schmittgen 2001), where:  $\Delta\Delta$ CT = [(CT gene of interest – CT Reference gene) time × - (CT gene of interest - CT Reference gene) time 0)]. The calibrator condition was the mock. R software was used to perform the data analysis, and a heatmap was built using the heatmaply package.

**RESULTS:** Among the candidate genes close to the *CoPv01<sup>CDRK</sup>* locus we observed that the *Phvul.001G246300* gene was the most responsive to race 73 of *C. lindemuthianum*. It was highly expressed in the CDRK cultivar at 24, 72, and hpi suggesting its potential in the resistance response. This gene encodes an abscisic acid (ABA) receptor PYL5 protein that plays a central role in crosstalk between ABA and jasmonic acid (JA) responses.

Previous studies conducted by Zuiderveen et al. (2016) revealed that resistance to race 73 of *C. lindemuthianum* can be attributed to the *Co-I* locus, associated with the marker ss715645251, located within the exon of *Phvul.001G243800*. Through expression analysis of candidate genes of the *Co-I<sup>2</sup>* resistance locus against *C. lindemuthianum* race 73 the *Phvul.001G243800* gene was

identified with high levels, being the most responsive gene to the pathogen (Mahiya-Farooq et al., 2019).

The present study observed highly expressed candidate gene *Phvul.001G246300*, known as a defense gene, for *CoPv01<sup>CDRK</sup>* locus in the California Dark Red Kidney cultivar. Furthermore, the *Phvul.001G243800* (*Co-1 locus*) showed low expression in the CDRK cultivar.



**Fig. 1** Heatmap of relative expression of the candidate genes to the *CoPv01<sup>CDRK</sup>* locus (A) and other resistance genes (B) 0, 24, 48-, 72-, 96-, and 120-hours post inoculation in the California Dark Red Kidney cultivar inoculated with race 73 of *C. lindemuthianum*.

**CONCLUSIONS:** Based on different resistance spectra, the physical positions of the molecular markers used for mapping anthracnose resistance genes, and relative expression of candidate genes for each *locus*, we conclude that *CoPv01<sup>CDRK</sup>* is positioned downstream of the *Co-1 locus*. Likewise, through expression analysis, we identified the candidate gene *Phvul.001G246300* for *CoPv01<sup>CDRK</sup>* as highly responsive to the race 73 of *C. lindemuthianum* in the resistant common bean cultivar California Dark Red Kidney.

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# COMPARING DESTRUCTIVE AND NON-DESTRUCTIVE METHODS FOR *Sclerotinia sclerotiorum* INOCULATION IN COMMON BEAN

Alex Naves Ferreira, Fernanda Aparecida Castro Pereira, Rafael Novais de Miranda, Gleice Aparecida da Silva Lima, Elaine Aparecida de Souza\*

Universidade Federal de Lavras, Departamento de Biologia, Lavras, MG, Brazil

\*Corresponding author: easouza@ufla.br

## INTRODUCTION

White mold (WM), caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, is one of most important diseases affecting the common bean crop (*Phaseolus vulgaris* L.). The choice of inoculation method for the assessment of genotypes is a great challenge in plant breeding for resistance to *S. sclerotiorum* (Ferreira et al. 2019). Furthermore, optimization of the original methods have been proposed (Arkwazee and Myers 2017). The comparison between destructive and non-destructive methods of inoculation of *S. sclerotiorum* in common bean has been carried out and the implications are discussed in this work.

## MATERIALS AND METHODS

Reaction of common bean lines (VC17, A195, Beryl, Cornell 605 and IPR Corujinha) and progenies from a recurrent selection program for WM resistance of UFLA, Brazil (CXII-53/10, 56/8, 64/9, 59/8 e 64/14) was assessed using two isolates of *Sclerotinia sclerotiorum* (UFLA 65; RP:UFLA 145) that were inoculated using different methods (I and II). The isolates were grown on Petri dishes containing BDA medium. The experiment was carried out in a randomized complete blocks design with three replicates and three plants per plot. **Method I:** Straw test proposed by Petzoldt and Dickson (1996), that consists of cutting the main stem of the plant above the third node and inoculating it with a straw containing the fungus mycelium. **Method II:** that consists of inoculating a straw containing the fungus mycelium in the petiole of the leaf inserted in the third node of the plant. Inoculation of plants was carried out 28-35 days after sowing in greenhouse with temperatures of <25°C and a humidity of 80%. Severity of WM in plants was assessed seven days after inoculation using a diagrammatic scale with scores 1 to 9 (Singh et al. 2014). The data were submitted to analysis of variance and averages were clustered using the Scott-Knott test. Moreover, Pearson correlations were obtained between methods and isolates.

## RESULTS AND DISCUSSION

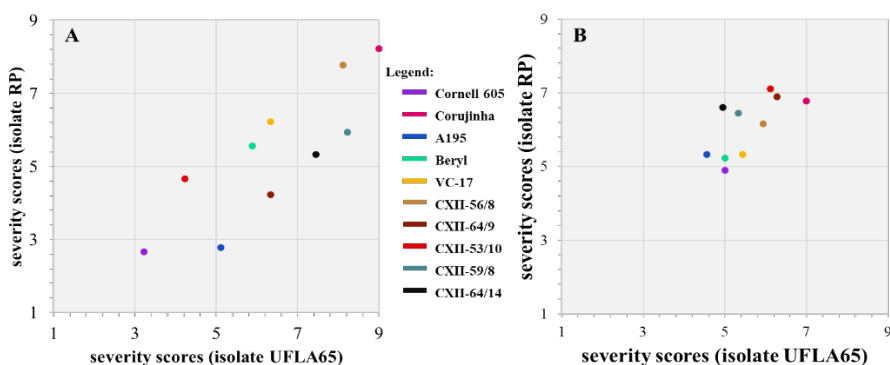
Correlation between the severity of WM scores of two methods was not observed (Pearson correlation coefficient = 0.23,  $P > 0.05$ ). Therefore, there were different physiological responses between the non-destructive inoculation with continued metabolism, growth and development (Method II) and when its growth was stopped (Method I). We found correlations between the isolates in each method (Figure 1). According to Table 1 we observed that mean scores of WM severity of genotypes were different for each method except for the Corujinha line that was classified as susceptible in all treatments. The classification was according to Paula Jr. et al. (2012) (scores 1 to 3: resistant; scores 3.1 to 6: moderately resistant; scores >6.1: susceptible). Method I allowed for better discrimination between the genotypic reaction to *S. sclerotiorum* according to the criteria of classification of Paula Jr. et al. (2012).

**Table 1.** Reaction of common bean genotypes to the two isolates using the two inoculation methods.

Genotypes	Method I		Method II	
	Straw test		Straw test modified	
	RP	UFLA 65	RP	UFLA 65
CXII-53/10	4.2 C	4.6 C	6.1 A	7.1 A
VC-17	6.3 B	6.2 B	5.4 B	5.3 B
CXII-56/8	8.1 A	7.7 A	5.9 A	6.1 A
CXII-64/9	6.3 B	4.2 C*	6.2 A	6.8 A
CXII-59/8	8.2 A	5.9 B*	5.3 B	6.4 A
CXII-64/14	7.4 A	5.3 B*	4.9 B	6.6 A
A195	5.1 B	2.7 D*	4.5 B	5.3 B
Beryl	5.8 B	5.5 B	5.0 B	5.2 B
Cornell 605	3.2 C	2.6 D	5.0 B	4.8 B
IPR Corujinha	9.0 A	8.2 A	7.0 B	6.7 A

Means followed by the same letter belong the same group ( $P < 0.05$ ) according to Scott-Knott test. \*Significative differences between isolates by F test of ANOVA ( $P < 0.05$ ).

Cornell 605 e A195 lines were classified as resistant to isolate UFLA65 and the others were moderately resistant and susceptible for both isolates. The choice of inoculation method depends on the objective of the breeding program. If the objective is to identify genotypes resistant to WM or identify the most aggressive isolate, Method I would be the most useful. Method II is useful when the breeder intends to carry out hybridizations in inoculated plants, that is, the hybridization occurs in the same cycle of selection, which allows for a reduction in the time required to conduct a breeding program. Therefore, genetic gain can be increased per time unit.



**Figure 1.** Severity scores of white mold and dispersion of common bean genotypes inoculated according to methods I (A) and II (B) with isolates RP and UFLA 65 of *Sclerotinia sclerotiorum*.

**ACKNOWLEDGEMENTS:** CNPq, CAPES and FAPEMIG (Brazil).

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## RESPONSE OF ELITE CARIOCA CULTIVARS OF COMMON BEAN RECOMMENDED IN BRAZIL TO ANTHRACNOSE AND ANGULAR LEAF SPOT

Jussara Mencialha<sup>1\*</sup>, Mariana Andrade Dias<sup>2</sup>, Elaine Aparecida de Souza<sup>2</sup>,  
Pedro Crescêncio Souza Carneiro<sup>1</sup>, Vinícius Quintão Carneiro<sup>2</sup>,  
José Eustáquio de Souza Carneiro<sup>1</sup>

<sup>1</sup>Departamento de Agronomia, Universidade Federal de Viçosa (UFV), 36570-900, Viçosa, Minas Gerais, Brasil; <sup>2</sup>Departamento de Biologia, Universidade Federal de Lavras (UFLA), 37200-900, Lavras, Minas Gerais, Brasil;

\*Corresponding author: jussara.mencialha@ufv.br

**INTRODUCTION:** Anthracnose (*Colletotrichum lindemuthianum*) and angular leaf spot (*Pseudocercospora griseola*) are among the most important diseases of common bean, causing considerable economic losses and increasing production costs. The use of resistant cultivars is the most effective and economically viable strategy to control these diseases. Brazilian breeding programs have made efforts in the development of cultivars resistant to the main races of *C. lindemuthianum* and *P. griseola*. The reaction of cultivars to these pathogens is assessed by the disease response in experiments with artificial inoculation of the pathogen in seedlings at stage V2. Thus, the objective of study was to assess the reaction of elite carioca cultivars of common bean to races 65, 73, 81 and 89 of *C. lindemuthianum* and to races 63-23 and 63-63 of *P. griseola*.

**MATERIAL AND METHODS:** Eleven elite carioca cultivars of common bean were evaluated for reaction to races 65, 73, 81 and 89 of *C. lindemuthianum* and to races 63-23 and 63-63 of *P. griseola*. The cultivar BRS Esplendor was used as resistant control to *C. lindemuthianum* and the line MAIII-16159 as resistant control to *P. griseola*. The experiments were carried out in a greenhouse, in a randomized block design with three replications and nine plants per plot. Seedlings with fully expanded primary leaves were sprayed with a solution at a concentration of  $1.2 \times 10^6$  conidia mL<sup>-1</sup> when inoculated with *C. lindemuthianum* races and  $2.0 \times 10^4$  conidia mL<sup>-1</sup> when inoculated with *P. griseola* races. The seedlings inoculated with *C. lindemuthianum* races were incubated in a moisture chamber for 48 h ( $20 \pm 1$  °C and >95% relative humidity) under a 12-h photoperiod. Subsequently, the seedlings were transferred to a greenhouse and assessed for the anthracnose response according to the 1 to 9 scale, 12 days after inoculation, as described by van Schoonhoven and Pastor-Corrales (1987). The experiments to assess the angular leaf spot response were assessed eighteen days after inoculation according to the 1 to 9 scale proposed by Librelon et al. (2015). The severity data for each race were submitted to individual analysis of variance and the severity mean scores of cultivars were compared to the resistant control using the Dunnett's test (1955). Data analyzes were performed using the GENES software (Cruz, 2013).

**RESULTS AND DISCUSSION:** The cultivars differed in their reaction to races 65, 73, 81 and 89 of *C. lindemuthianum* and to races 63-23 and 63-63 of *P. griseola*. The cultivars BRSMG Amuleto, BRSMG Madrepérola and BRS FC104 were resistant to all races of *C. lindemuthianum* and *P. griseola* assessed are suitable sources of resistance to these pathogens for use in breeding programs (Table 1). The cultivar IAC Formoso was resistant to the four races of *C. lindemuthianum*, but was susceptible to angular leaf spot. The cultivars BRSMG Zape and BRS Estilo were resistant to two of the four races of *C. lindemuthianum* and to the two races of *P. griseola*. The cultivars TAA Dama, BRS FC406 and BRSMG Uai were resistant to only two races of *C. lindemuthianum*, while the cultivars ANFc 9 and Pérola were susceptible to all races of *C.*



*lindemuthianum* and resistant to only one race of *P. griseola*. The BRSMG Amuleto and BRSMG Madrepérola cultivars stand out, which, in addition to being resistant to anthracnose and angular leaf spot, have high commercial grain quality. The cultivar BRS FC104, in addition to having a wide spectrum of resistance to anthracnose and angular leaf spot, shows early maturity.

**Table 1.** Anthracnose and angular leaf spot mean scores of elite carioca cultivars of common bean recommended in Brazil.

Cultivars	Anthracnose severity mean scores				Angular leaf spot severity mean scores	
	Races of <i>C. lindemuthianum</i>				Races of <i>P. griseola</i>	
	65	73	81	89	63-23	63-63
BRSMG Amuleto	1.71 a <sup>1</sup>	1.66 a	1.56 a	2.16 a	2.44 b <sup>1</sup>	2.13 b
BRSMG Zape	1.83 a	4.52	1.65 a	9.00	2.19 b	1.85 b
IAC Formoso	2.20 a	1.81 a	1.10 a	1.21 a	5.05	3.96
BRSMG Madrepérola	2.29 a	2.24 a	1.88 a	1.95 a	1.89 b	1.59 b
BRS FC104	2.32 a	1.61 a	1.72 a	2.07 a	1.71 b	2.12 b
BRS Estilo	5.27	3.32 a	6.30	2.40 a	1.98 b	2.10 b
TAA Dama	5.83	1.27 a	1.52 a	9.00	5.69	3.70
ANFc 9	6.99	7.20	5.85	9.00	5.04	3.36 b
BRS FC406	8.37	1.78 a	2.00	2.00 a	5.12	3.95
BRSMG Uai	8.92	2.29 a	4.68	2.05 a	6.00	5.66
Pérola	9.02	8.08	9.00	9.00	3.34 b	3.50
BRS Esplendor <sup>2</sup>	1.76 a	1.11 a	1.04 a	1.87 a		
MAIII-16159 <sup>3</sup>					2.14 b	2.19 b

<sup>1</sup>Mean scores followed by the same letter in a column do not differ significantly at the 0.05 probability level with the control using Dunnett's test; <sup>2</sup>Resistant control to races 65, 73, 81 e 89 of *C. lindemuthianum*; <sup>3</sup>Resistant control to races 63-23 e 63-63 of *P. griseola*.

**ACKNOWLEDGEMENTS:** CNPq, CAPES e FAPEMIG for the financial support.

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# NEW SOURCES OF WHITE MOLD RESISTANCE DERIVED FROM WIDE CROSSES IN COMMON BEAN AND EVALUATED IN THE GREENHOUSE AND FIELD USING MULTI-SITE SCREENING NURSERIES

Rebecca Higgins<sup>1</sup>, Evan Wright<sup>2</sup>, Halima Awale<sup>2</sup>, Valerio Hoyos-Villegas<sup>3</sup>, Phillip Miklas<sup>4</sup>, James Myers<sup>5</sup>, Juan Osorno<sup>6</sup>, Carlos Urrea<sup>7</sup>, Michael Wunsch<sup>8</sup>, Sydney Everhart<sup>9</sup>, Francisco E. Gomez<sup>2</sup>

<sup>1</sup>University of Nebraska, Lincoln, NE; <sup>2</sup>Michigan State University, East Lansing, MI; <sup>3</sup>McGill University, Montreal, Quebec, Canada; <sup>4</sup>USDA-ARS, Prosser, WA; <sup>5</sup>Oregon State University, Corvallis, OR; <sup>6</sup>North Dakota State University, Fargo, ND; <sup>7</sup>University of Nebraska, Scottsbluff, NE; <sup>8</sup>North Dakota State University, Carrington, ND, University of Connecticut, Storrs, CT

**INTRODUCTION:** In 2021, field and/or greenhouse evaluations were used to screen 27 candidate dry bean lines in greenhouse trials and 9 candidate lines in field trials as putative sources of white mold disease tolerance in adapted backgrounds. Evaluations were conducted at multiple sites located in six states (CO, MI, NE, ND, OR, WA) and in one province in Canada (Quebec). Collectively, these locations represent the major bean production areas of the North American continent. Multi-site testing is essential for robust evaluation under different environmental conditions and with white mold pathogen populations that previous research has shown are significantly different in both genetic variation and aggressiveness.

**MATERIALS AND METHODS:** Greenhouse evaluations were conducted using a straw test that consistently identifies sources of physiological resistance in adapted and unadapted bean germplasm and requires only a small number of seeds to confirm resistance. Twenty-seven bean lines were evaluated, plus G122 (with partial resistance), Bunsu (mostly field avoidance) and Beryl (susceptible) that were included as the control lines. Greenhouse evaluations were conducted at all locations except ND.

Field tests were conducted in all locations except CO, which is too dry to produce consistent disease pressure. Nine bean lines were evaluated in addition to the three controls described above. Unfortunately, data was collected from only four of the locations due to multiple causes, most commonly a lack of disease pressure as a result of hot, dry weather during the summer growing season. One location was removed due to a common bacterial blight outbreak and no data was collected from this site. As in years past, this illustrates the necessity of multiple sites for generating data considering weather or other natural complications in field trials.

**RESULTS AND DISCUSSION:** Results of the greenhouse trials identified six promising candidate lines ('P3-032-2', 'M25-13-1', 'USG-WM-3-1', 'P3-047-1', 'ND122454', 'USPT-WM-12-1') that performed better than the tolerant / resistant 'G122' line. These materials represent useful sources of resistance with potential for improving pinto bean and the other Durango market classes and will be submitted for further testing in 2022. Furthermore, greenhouse results demonstrated that an isolate used in MI was more aggressive than those at other sites resulting in higher disease severity. Results of field trials were inconclusive. Sites were plagued by unprecedented damage to plots from common bacterial blight (WA) and insufficient disease pressure (CAN, OR, ND). However, results from just two sites (NE and MI) were able to be analyzed but lacked the statistical power to differentiate between the tolerant/resistant check 'G122' from the partially resistant line 'Bunsu'. Yet numerically, one line was identified ('N19246') to be more resistant than 'Bunsu', but not 'G122'.

**Table 1.** Greenhouse test mean disease ratings\* in 2021 with control lined highlighted in bold.

Line	NE	WA	OR	MI	Mean	Grouping**
P3-032-2	3.90	3.00	3.80	5.80	4.10	K
M25-13-1	3.20	4.40	4.00	6.20	4.50	JK
USGN-WM-3-1	4.50	2.50	4.20	6.70	4.50	JK
P3-047-1	4.80	3.00	3.70	6.50	4.50	IJK
ND122454	4.30	3.80	4.00	6.20	4.60	HIJK
USPT-WM-12-1	5.40	2.40	3.70	7.50	4.70	GHIJK
<b>G122</b>	<b>5.00</b>	<b>4.60</b>	<b>4.10</b>	<b>6.20</b>	<b>5.00</b>	<b>FGHIJK</b>
Z0726-9-50-1	5.80	3.30	4.00	8.00	5.30	FGHIJK
P2-087-1	4.90	4.60	4.60	7.40	5.40	EFGHIJK
WMM-820-1	5.40	4.60	4.30	7.20	5.40	EFGHIJK
P2-124-1	5.70	3.80	4.80	7.80	5.50	EFGHIJ
M25-18-1	4.80	5.30	3.70	8.70	5.60	EFGHIJ
WMM-219-1	5.20	4.50	4.10	8.70	5.60	EFGHIJ
SR16-1	4.70	5.50	4.20	8.70	5.80	EFGHI
Z0726-9-55-1	5.80	5.50	3.70	8.10	5.80	EFGHI
SR9-5	5.70	5.00	3.80	8.60	5.80	EFGHI
PS18-014-1-1	6.40	3.20	4.90	8.80	5.80	EFGH
USPT-WM-1-2	6.70	5.20	4.20	7.90	6.00	DEFG
WM-16079-1	6.50	5.30	3.90	8.90	6.20	CDEF
<b>Bunsi</b>	<b>7.50</b>	<b>5.90</b>	<b>4.60</b>	<b>6.70</b>	<b>6.20</b>	<b>CDEF</b>
PT11-13-31-1	5.80	4.40	6.20	8.50	6.20	CDEF
S18904	7.50	4.70	5.20	9.00	6.60	BCDE
SR16-2	7.10	7.40	5.60	9.00	7.30	ABCD
ND132162	7.80	8.60	4.80	8.00	7.30	ABC
ND172568	7.20	6.70	6.60	9.00	7.30	ABC
<b>Beryl</b>	<b>7.50</b>	<b>8.10</b>	<b>5.10</b>	<b>9.00</b>	<b>7.40</b>	<b>ABC</b>
ND121315	8.30	8.20	4.60	9.00	7.50	AB
NE1-17-36	8.80	7.80	5.10	8.80	7.60	AB
N19246	7.70	9.00	5.60	8.60	7.70	AB
B19309	8.30	8.80	6.60	9.00	8.20	A

\* Petzoldt & Dickson scale: 1-3 = resistant, 4-6 = intermediate, 7-9 = susceptible

\*\*Levels not connected by the same letter are significantly different at  $\alpha = 0.5$ .

**Table 2.** Field test mean disease ratings\* 2021 with control lines highlighted in bold.

Line	NE	MI	Mean	Grouping**
<b>G122</b>	<b>3.00</b>	<b>3.00</b>	<b>1.90</b>	<b>C</b>
N19246	2.00	3.33	2.05	C
<b>Bunsi</b>	<b>3.00</b>	<b>5.00</b>	<b>2.28</b>	<b>BC</b>
ND132162	3.00	5.33	2.48	BC
ND172568	2.00	7.00	3.04	BC
SR9-5	3.00	5.00	2.50	BC
SR16-1	4.00	5.67	2.95	BC
B19309	3.00	5.33	2.60	BC
S18904	2.00	6.67	2.69	BC
NE1-17-36	3.00	5.67	2.51	BC
SR16-2	3.00	8.00	3.13	AB
<b>Beryl</b>	<b>7.00</b>	<b>8.67</b>	<b>4.29</b>	<b>A</b>

\*CIAT scale- 1-9; 1= no disease, 9 = dead plant.

\*\*Levels not connected by the same letter are significantly different at  $\alpha = 0.5$ .

## IDENTIFICATION OF RECOMBINANT RUST-RESISTANT BLACK BEAN LINES UNDER FIELD CONDITIONS

Ibarra-Pérez FJ<sup>1</sup>, Tosquy-Valle OH<sup>1</sup>, Esqueda-Esquivel VA<sup>1</sup> and Rodríguez-Rodríguez, JR

<sup>1</sup>INIFAP, Campo Experimental Cotaxtla, Veracruz, Mexico; <sup>2</sup>INIFAP, Campo Experimental Ixtacuaco, Veracruz, Mexico

**INTRODUCTION:** Bean rust [*Uromyces appendiculatus* var. *appendiculatus* (Pers.) Unger] is one of the fungal diseases that most affects the bean crop in tropical southeastern Mexico, including the state of Veracruz. This situation is critical because the vast majority of farmers use local bean landraces and some improved cultivars that are susceptible to this disease (López *et al.*, 2012). Rust chemical control increases the cost of production; therefore, the development of cultivars resistant to this disease is considered the most appropriate strategy to counteract this problem (Esqueda *et al.*, 2020). The objective of this research was to determine the level of resistance to rust in a group of recombinant black bean lines evaluated under farmers' field conditions and to identify those that show higher yields than the control bean cultivars.

**MATERIALS AND METHODS:** A field experiment was conducted in three environments of the Orizaba region in the central mountainous zone of Veracruz, Mexico. Two field trials were carried out during the fall-winter (October-January) of 2019 with soil residual moisture, and one trial in the winter-spring (February-May) of 2020, under field irrigation. The experiment included eleven advanced recombinant black bean lines, which were previously assessed for their response to artificial inoculation under greenhouse conditions, using different mono-pustule rust isolates collected in commercial bean crops across tropical southern Mexico (Garrido *et al.*, 2020). Negro Jamapa, Negro Medellín and Verdín cultivars were used as rust susceptible controls. The experimental design used was a RCBD with three replications. The reaction of the genotypes to the natural incidence of bean rust was determined using the CIAT rust scale of 1 to 9 (Schoonhoven and Pastor-Corrales, 1987), as well as seed yield (kg ha<sup>-1</sup>) at 14% humidity. The disease incidence and seed yield data were analysed individually (ANOVA) and jointly; for the separation of means, the Least Significant Difference (LSD,  $\alpha = 0.05$ ) was applied. Correlation analyses were also carried out between the rust incidence values and the yield of the genotypes at each evaluation site.

**RESULTS AND DISCUSSION:** The bean rust significantly reduced bean yield only in the winter-spring irrigated environment 2020 ( $r = -0.61^*$ ), due to a significantly higher and earlier incidence of this disease (López *et al.*, 2006). In contrast, no correlation was found in both fall-winter 2019 environments, where beans grew under residual moisture ( $r = -0.29$  ns and  $r = -0.20$  ns). Nine recombinant bean lines had average rust incidence scores between 1.78 and 3.33, indicating that they were resistant to rust under field conditions (Schoonhoven and Pastor-Corrales, 1987). These bean lines had previously shown a hypersensitivity reaction to rust when assessed under greenhouse conditions, indicating that these lines were highly resistant and showed only small chlorotic spots without sporulation (Garrido *et al.*, 2020). In turn, the three control cultivars presented the greatest damage from this disease (Table 1). From the group of the outstanding bean lines with low reaction to rust, Jamapa Plus/XRAV-187-3-4-4 stands out because it was the most productive across the test environments. This bean line obtained a statistically similar average seed yield of that obtained by two other bean lines, Jamapa Plus/XRAV-187-3-4-1 and Jamapa Plus/XRAV-187-3-1-2, and that of check cultivar Verdín, and superior to that of the rest of the genotypes (Table 1). The other check cultivars, Negro Jamapa and Negro Medellín, had the lowest

average seed yield, mainly because they presented significant rust damage in two of the three evaluation environments (data not shown).

**Table 1.** Average rust incidence scores and seed yield of 14 black bean genotypes assessed under field conditions in three environments of Veracruz, Mexico in years 2019 and 2020.

Genotype	Rust incidence (scores 1 to 9)	Seed yield (kg ha <sup>-1</sup> )
Negro Papaloapan/SEN 46-2-6	3.00 def	1841.78 abcd
Negro Papaloapan/SEN 46-3-2	4.00 bcd	1793.33 bcd
Negro Papaloapan/SEN 46-7-7	3.00 def	1714.44 bcde
Negro Papaloapan/SEN 46-7-10	3.22 cde	1695.11 bcde
Negro Papaloapan/SEN 46-7-12	3.00 def	1745.00 bcd
Negro Citlali/XRAV-187-3-1-5	1.78 f	1584.44 de
Negro Citlali/XRAV-187-3-1-6	1.78 f	1716.56 bcde
Negro Citlali/XRAV-187-3-1-8	3.78 cd	1614.67 cde
Jamapa Plus/XRAV-187-3-1-2	2.78 def	1962.33 abc
Jamapa Plus/XRAV-187-3-4-1	2.00 ef	2043.89 ab
Jamapa Plus/XRAV-187-3-4-4	3.33 cd	2183.44 a
Negro Medellín (RC)	5.33 a	1518.56 de
Negro Jamapa (RC)	4.44 abc	1350.00 e
Verdín (RC)	5.22 ab	1847.44 abcd
Location average	3.33	1757.93
ANOVA	**	**
LSD (0.05)	1.25	375.78

RC = Regional check cultivar. \*\* =  $P \leq 0.01$ . Means with the same letters in each column are not statistically different according to the Least Significant Difference (LSD, 0.05).

**CONCLUSIONS:** Under the environmental conditions of the central mountainous zone of Veracruz, Mexico, Jamapa Plus/XRAV-187-3-4-4, Jamapa Plus/XRAV-187-3-4-1 and Jamapa Plus/XRAV-187-3-1-2 recombinant bean lines showed resistance to rust under field conditions and had significantly higher grain yield than check cultivars Negro Jamapa and Negro Medellín. It is important to mention that cultivar Verdín also produced a seed yield statistically similar to the most productive lines, despite being significantly affected by rust.

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## REACTION OF DRY BEAN GENOTYPES TO THE INCIDENCE OF BEAN GOLDEN YELLOW MOSAIC VIRUS IN CHIAPAS, MEXICO

Tosquy-Valle OH<sup>1</sup>, Ibarra-Pérez FJ<sup>1\*</sup>, Villar-Sánchez B<sup>2</sup>, Anaya-López JL<sup>3</sup> and Rodríguez-Rodríguez JR<sup>4</sup>

<sup>1</sup>INIFAP, Campo Experimental Cotaxtla, Veracruz, Mexico; <sup>2</sup>INIFAP, Campo Experimental Centro de Chiapas, Chiapas, Mexico; <sup>3</sup>INIFAP, Campo Experimental Bajío, Guanajuato, Mexico; <sup>4</sup>INIFAP, Campo Experimental Ixtacuaco, Veracruz, Mexico

**INTRODUCTION:** In the tropical areas of Chiapas, Mexico, bean production is affected by various biotic and abiotic factors, among them, one stands out: the incidence of bean golden yellow mosaic virus (BGYMV), whose vector is the whitefly [*Bemisia tabaci* (Gennadius)] (Cuellar and Morales, 2006). In this area terminal drought, particularly during the fall-winter cropping cycle where dry beans grow commonly under residual moisture, is combined with acid soils of low fertility (Villar *et al.*, 2003). The objective of this research was to identify bean genotypes resistant to BGYMV with higher productivity than the Negro Jamapa variety, commonly planted in Chiapas.

**MATERIALS AND METHODS:** During the 2019-20 fall-winter growing cycle, a field experiment was conducted under conditions of residual moisture and strongly acidic soil (pH ranging from 4.26 to 5.74) across three locations in central Chiapas, Mexico. Eleven advanced black bean breeding lines derived from three different crosses (Papaloapan/SEN 46, Negro Citlali/XRAV-187-3, and Jamapa Plus/XRAV-187-3) were field evaluated; three improved bean cultivars were used as checks, including Negro Jamapa, one of the most planted in Chiapas. An RCBD experimental design was used with three replications. The incidence of BGYMV in stage R8 was determined using the CIAT scale from 1 to 9 (Schoonhoven and Pastor-Corrales, 1987), and seed yield (kg ha<sup>-1</sup>) at 14% humidity was determined. Data from the three localities were analyzed individually and jointly, but for lack of space, only the results of the combined analyses of variance are presented. For the separation of means, the LSD test ( $\alpha = 0.05$ ) was applied. Correlation analyses between the incidence of BGYMV and seed yield of genotypes were also performed.

**RESULTS AND DISCUSSION:** BGYMV significantly reduced the seed yield of bean genotypes grown in the three evaluation sites (Villa Corzo,  $r = -0.562$  \*; CECECH,  $r = -0.757$  \*\* and El Gavilán,  $r = -0.552$  \*). This can be because the whitefly and the symptoms appeared as early as the V4 bean plant stage, which is when this disease can cause greater damage (López *et al.*, 2002). The Jamapa Plus/XRAV-187-3-4-1 breeding line showed the highest susceptible reaction to BGYMV, with an average incidence rating of 6.0, significantly higher than the rest of the genotypes. In contrast, four genotypes: Negro Citlali/ XRAV-187-3-1-6, Papaloapan/SEN 46-7-7, Jamapa Plus/XRAV-187-3-4-4, and the Verdín cultivar showed the highest resistance to BGYMV, with reaction scores between 1.67 and 3.22, statistically lower than that of Negro Jamapa. The excellent plant reaction to BGYMV of the line Negro Citlali/XRAV-187-3-1-6 and Verdín is largely because this cultivar has the co-dominant molecular marker SR2, linked to the *bgm-1* gene, which confers genetic resistance to this viral disease (Anaya-López *et al.*, 2018). The resistance reaction of Negro Papaloapan/SEN 46-7-7 and Jamapa Plus/XRAV-187-3-4-4, may be because these breeding lines could carry other resistance genes or have other mechanisms that contributed to a more stable resistance response across environments. The latter could be most likely since

their parents (Negro Papaloapan and XRAV-187-3) in addition to having the *bgm-1* gene; also carry a QTL conferring a high level of resistance to BGYMV (Anaya-López *et al.*, 2018). It should be noted that these four genotypes also obtained outstanding average seed yields (greater than 934 kg ha<sup>-1</sup>) and higher than that of Negro Jamapa (Table 1).

**Table 1.** Average values of BGYMV incidence and average seed yield of 14 black bean genotypes field evaluated in three locations in Chiapas, Mexico, during the fall-winter 2019-20 crop cycle.

Genotype	BGYMV incidence (scale 1 to 9)	Seed yield (kg ha <sup>-1</sup> )
Negro Papaloapan/SEN 46-2-6	4.00 bcde	780.11 def
Negro Papaloapan/SEN 46-3-2	4.33 bcd	877.22 cdef
Negro Papaloapan/SEN 46-7-7	2.11 gh	1070.00 ab
Negro Papaloapan/SEN 46-7-10	3.33 cdef	924.89 abcde
Negro Papaloapan/SEN 46-7-12	3.56 bcdef	887.89 bcdef
Negro Citlali/XRAV-187-3-1-5	3.00 efg	864.89 cdef
Negro Citlali/XRAV-187-3-1-6	1.67 h	957.22 abcd
Negro Citlali/XRAV-187-3-1-8	3.44 cdef	924.00 abcde
Jamapa Plus/XRAV-187-3-1-2	3.89 bcde	989.56 abc
Jamapa Plus/XRAV-187-3-4-1	6.00 a	762.89 ef
Jamapa Plus/XRAV-187-3-4-4	3.22 defg	1089.11 a
Negro Medellín	4.67 b	723.89 f
Negro Jamapa	4.44 bc	738.11 f
Verdín	2.56 fgh	934.67 abcde
Location average	3.59	894.60
ANOVA	**	**
LSD (0.05)	1.134	185.40

BGYMV = Bean golden yellow mosaic virus. \*\* =  $P \leq 0.01$ . Means with the same letters in each column are not statistically different according to the Least Significant Difference (LSD, 0.05).

**CONCLUSIONS:** Under the conditions of residual moisture and acid soils of Chiapas, the lines Jamapa Plus/XRAV-187-3-4-4, Negro Papaloapan/SEN 46-7-7, and Negro Citlali/XRAV-187-3-1-6, and the Verdín cultivar were resistant to BGYMV and had significantly higher average seed yield than the check cultivar Negro Jamapa.

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# BEAN RUST IN SRI LANKA: IDENTIFYING THE PATHOGEN'S FIRST RACES AND GENES FOR DEVELOPING RUST-RESISTANT SNAP BEANS

H.M.P.S. Kumari<sup>1</sup>, Marcial A. Pastor-Corrales\*<sup>2</sup>, R.G.A.S. Rajapaksha<sup>1</sup>, P.C.G. Bandaranayake<sup>3</sup>, Cholani Weebadde<sup>4</sup>

<sup>1</sup>Horticultural Crops Research and Development Institute, Gannoruwa, Sri Lanka; <sup>2</sup>USDA-ARS, Soybean Genomics and Improvement Lab., USDA-ARS, BARC-West, Beltsville, MD, 20705; <sup>3</sup>Agricultural Biotechnology Centre, University of Peradeniya, Sri Lanka; <sup>4</sup>Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI

## INTRODUCTION

Snap beans are widely cultivated in the Badulla district of the Uva province and in the Matale, Kandi, and Nuwara Eliya districts of the Central province of Sri Lanka. In these districts, the bean rust disease, caused by the fungus *Uromyces appendiculatus*, produces yield and quality losses (Abeyasinghe 2009). *U. appendiculatus* recurrently produces new virulent strains that often infect common bean varieties that previously were resistant. Hundreds of different virulent strains of *U. appendiculatus* have been characterized and reported as races. The development of rust resistant common bean cultivars requires a detailed understanding of the virulence spectrum of *U. appendiculatus* and the availability of rust resistance genes that can be used to develop cultivars with effective resistance. Both topics are unknown in Sri Lanka. The first objective of this study was to characterize the virulence of the pathogen. The second objective aimed to identify rust resistance genes to develop snap beans with effective and durable rust resistance in Sri Lanka.

## MATERIALS AND METHODS

During the 2017 and 2019 growing seasons, approximate 500 leaves of snap bean varieties with visible symptoms of the rust disease were collected from 57 locations in four snap bean-producing districts of the Central and Uva provinces of Sri Lanka: 25 locations in Badulla, the largest snap bean producing district in Sri Lanka, 12 locations in Kandy, and 10 locations each in the Matale and Nuwara Eliya districts. We obtained 57 single pustule isolates to identify the races of *U. appendiculatus* in Sri Lanka. To that end, each of these isolates were individually inoculated on the internationally accepted set of 12 differentials cultivars (Steadman et al., 2003). Plants of each differential cultivar were evaluated for their reactions to each of the isolates. The virulence phenotype of each isolate of *U. appendiculatus* and the name of the races identified were obtained using a standard scale and the binary nomenclature described by Steadman et al. (2003).

## RESULTS AND DISCUSSION

From a total of 57 single pustule isolates of *U. appendiculatus* collected in four snap bean producing districts of Sri Lanka, four different races were identified. These races were: 23-5, 31-1, 31-11, and 63-21 (Table 1). Race 63-21 exhibited the broadest virulence spectrum; it infected nine (75%) of the 12 differential cultivars, six Andean and three Middle American. This was followed by race 31-11 that infected eight (66.6%) of the 12 differential cultivars, five Andean and three Middle American. Races 23-5 and 31-1 exhibited a narrower virulence spectrum; both infected six (50%) of the 12 differential cultivars; albeit not the same cultivars. Race 23-5 infected four Andean and two Middle American differential cultivars, while race 31-1 infected five Andean and one Middle American differential cultivar (Table 1). From the 24 interactions between the six Andean differential cultivars with the four races of *U. appendiculatus* in Sri Lanka, 20 (83.3%) were susceptible, while only four (16.7%) were resistant. Inversely, from the 24 interactions between the six Middle American



differential cultivars with the same four races of *U. appendiculatus*, nine (37.5%) were susceptible and 15 (62.5%) were resistant. Thus, the Andean differential cultivars were significantly more susceptible (83.3%) than the Middle American cultivars. That is, all four races of *U. appendiculatus* identified in Sri Lanka, were significantly more virulent on the Andean (83.3%) than on the Middle American (37.5%) differential cultivars. Specifically, the Middle American differential cultivars were significantly more resistant (62.5%) than the Andean (16.7%) differential cultivars to the four races. PI 181996, a Middle American differential cultivar having the *Ur-11* gene, conferred resistance to all four races. Moreover, three other Middle American cultivars were each resistant to three races. Conversely, all but one of the six Andean differential cultivars were susceptible to all four races. PI 1260418 was the only Andean differential cultivar with resistance to three races. These results suggested that the *Ur-11*, *Ur-3*, *Ur-3+*, and *Ur-5* genes of Middle American origin, could be combined with the resistance in the Andean PI 260418 to develop snap bean varieties with broad resistance to all races of *U. appendiculatus* in Sri Lanka.

**Table 1.** Races of *Uromyces appendiculatus*, the bean rust pathogen, occurring on snap bean in Sri Lanka identified using the international set of 12 differential cultivars, six Andean and six Middle American, and the binary system to name races of the pathogen

Bean Differential Cultivars	Rust Resistant Genes	Gene Pool	Binary Value of susceptible cultivars	Resistant (R) or susceptible (S) reactions of differential cultivars to the 57 isolates of <i>U. appendiculatus</i>			
<b>Andean differential cultivars</b>							
Early Gallatin	<i>Ur-4</i>	A/MA	1	S	S	S	S
Redlands Pioneer	<i>Ur-13</i>	A	2	S	S	S	S
Montcalm	<i>Ur-?</i>	A	4	S	S	S	S
P.C. 50	<i>Ur-9. Ur-12</i>	A	8	R	S	S	S
G.G.W.	<i>Ur-6</i>	A/MA	16	S	S	S	S
PI 260418	<i>Ur-?</i>	A	32	R	R	R	S
<b>Middle American differential cultivars</b>							
G. N. 1140	<i>Ur-7</i>	MA	1	S	S	S	S
Aurora	<i>Ur-3</i>	MA	2	R	R	S	R
Mexico 309	<i>Ur-5</i>	MA	4	S	R	R	S
Mexico 235	<i>Ur-3+</i>	MA	8	R	R	S	R
CNC	<i>Ur-?</i>	MA	16	R	R	R	S
PI 181996	<i>Ur-11</i>	MA	32	R	R	R	R
Race designation				<b>23-5</b>	<b>31-1</b>	<b>31-11</b>	<b>63-21</b>
The virulence phenotype (R or S) of each of isolate of <i>U. appendiculatus</i> and the name of the races identified were obtained using a standard scale and the binary nomenclature described by Steadman et al., 2003							

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# VALIDATION AND DEPLOYMENT OF RESISTANCE-LINKED SCAR MOLECULAR MARKERS FOR MARKER-ASSISTED IMPROVEMENT OF MULTIPLE DISEASE RESISTANCE IN BIOFORTIFIED COMMON BEAN

Stanley T. Nkalubo<sup>1</sup>, Annet Namayanja<sup>1</sup>, Annet Namusoke<sup>1</sup>, Jane Mukabaranga<sup>1</sup>, Nampijja Shakirah<sup>1</sup> and Paul Gepts<sup>2</sup>

<sup>1</sup>National Crops Resources Research Institute (NaCRRI), Namulonge, Kampala, Uganda.

<sup>2</sup>Department of Plant Sciences, University of California, Davis, CA 95616-8780, United States

**INTRODUCTION:** Development and utilization of biofortified cultivars is the most effective, sustainable, and potentially long-lasting strategy for reducing micronutrient deficiencies in Africa because it ensures wide availability, regular access, and is low cost. Consequently, through the global biofortification program initiated in July 2003 by the Harvestplus Challenge Program, many breeding programs in Africa have been able to release biofortified (72-83ppm iron and 35-40ppm zinc) bean varieties. Unfortunately, in Uganda, the newly released biofortified common bean varieties NAROBAN 3 (MORE 88002) and NAROBAN 4C (MAC 44) have shown susceptibility to anthracnose and *Pythium* root rot disease in farmers' fields. This drastically limits wide use and nutritional benefits associated with these varieties. Genetic resistance is considered as the most effective control strategy against these diseases.

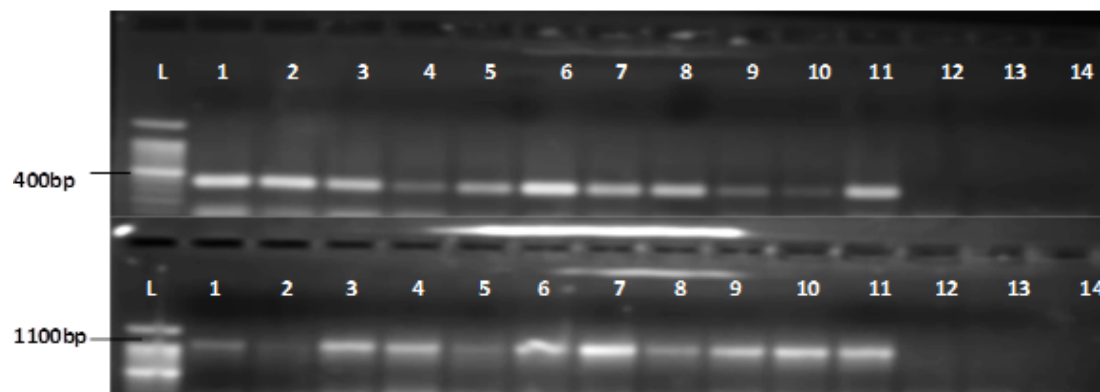
Considering that diseases generally appear in farmers' fields at the same time, there is a need to introgress multiple disease resistances in the background of varieties as the best control measure. The backcross breeding method has been largely used to transfer disease resistance genes from various resistance sources to elite but susceptible genotypes. To accelerate the process of backcrossing, molecular marker-assisted selection (MAS) is being used. Therefore, the present study seeks to validate the utility of selected SCAR markers linked to resistance genes for anthracnose disease and *Pythium* root rot for their ability to show polymorphism to introgress multiple disease resistances into biofortified bean varieties, NAROBAN 3 and NAROBAN 4C.

**MATERIALS AND METHODS:** Three dominant markers, namely SAB3 (Vallejo and Kelly, 2001) and SH18 (Awale and Kelly, 2001) linked to the *Co-5* and *Co-4*<sup>2</sup> anthracnose resistance genes, respectively, and the PYAA19<sub>800</sub> (Mahuku *et al.*, 2007) linked to the *Pythium* root rot resistance gene were studied for their ability to show polymorphism among resistant and susceptible parental genotypes. The genotypes consisted of: i) 10 NABE14/G2333 and 5 NABE12C/ RWR 719 /G 2333/ Mexico 54 backcross-derived lines generated in our previous breeding project funded by the Kirkhouse Trust ABC program, ii) two recurrent parents (NAROBAN 3 and NAROBAN 4C), and iii) G2333, RWR719, NABE14 and NABE12C as controls. DNA was extracted from young trifoliolate leaves of each of the selected lines and analysed for presence/absence of positive bands associated with the markers.

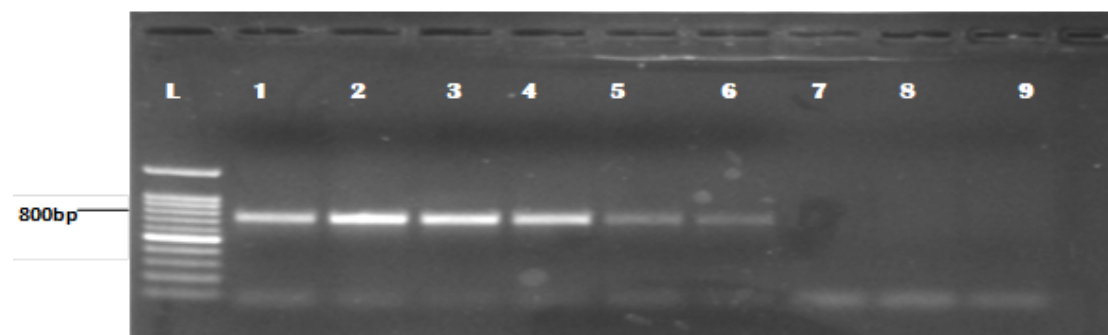
**RESULTS AND DISCUSSION:** The DNA amplification products showed that the SH18 and SAB3 amplified the expected band (sizes of 1,100 and 400 bp, respectively) in the NABE14/G2333 backcross derived lines (B 93, B 133, B 157, B 160, B189, B 264, 24-1, 6-2, 35-3 and 35-5) and the genotype (G2333) (Figure 1); these bands were absent in the two recurrent parents (NAROBAN 3 and 4C) and the negative control NABE14.

While the *Pythium* root rot resistance-linked marker, PYAA 19, amplified the expected band size (800 bp) in the NABE12C/RWR 719/G 2333/Mexico 54 backcross derived lines (KS1-

650, KS1-299, KS1-649, KS1-1073, KS1-146) and the genotype (RWR 719) (Figure 2); this band was absent in the two recurrent parents and the negative control, NABE12C.



**Figure 1.** DNA amplification products obtained with markers SAB3 and SH18: L-DNA ladder, 1-G2333; 2-B93; 3-B133; 4-B157; 5-B160; 6-B189; 7-B264; 8-24-1; 9-6-2; 10-35-3; 11-35-5; 12-NAROBAN 3; 13-NAROBAN 4C; 14-NABE14



**Figure 2.** DNA amplification product obtained with the PYAA 19 marker: L- DNA ladder, 1-RWR719, 2-KS1-650, 3- KS1-299, 4-KS1-649, 5- KS1-1073, 6- KS1-146, 7-NAROBAN 3, 8-NAROBAN4C, 9-NABE 12C

In conclusion, the SAB3 and SH18 showed clear polymorphism between the NABE 14/G2333 backcross derived lines, G2333 and the recurrent parents, indicating that they can be used in the MAS backcrossing procedure to introgress the *Co-5* and *Co-4<sup>2</sup>* anthracnose resistance genes into NAROBAN 3 and NAROBAN 4C backgrounds. The PYAA19 marker also showed clear polymorphism between the NABE 12C/ RWR 719/G 2333/Mexico 54 backcross derived lines, RWR 719 and the recurrent parents, suggesting that it is suitable for use in the MAS backcrossing procedure to introgress the *Pythium* root rot resistance gene into the NAROBAN 3 and NAROBAN 4C backgrounds. Already the MAS backcrossing programme has been advanced through the first four backcross generations (BC<sub>1</sub>F<sub>1</sub> to BC<sub>4</sub>F<sub>1</sub>).

**ACKNOWLEDGMENTS:** We are grateful to the Kirkhouse Trust for facilitating the use of MAS in the NaCRRI bean breeding program.

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# SCREENING COMMON BEAN GERMPLASM WITH ENHANCED RESISTANCE TO ASHY STEM BLIGHT

Diego M. Viteri<sup>1</sup>, Angela M. Linares<sup>2</sup>, Zoralys Miranda<sup>1</sup>, and Roberto Vázquez<sup>1</sup>

<sup>1,2</sup> Department of Agro-environmental Sciences, University of Puerto Rico. <sup>1</sup>Isabela Research Substation, 2090 Av. Militar, Isabela, PR 00662; <sup>2</sup>Lajas Research Substation, Carr. 101 km 8.5 Barrio Palmarejo, Lajas, PR 00667.

**INTRODUCTION:** Under favorable weather conditions ashy stem blight [causal agent: *Macrophomina phaseolina* (Tassi) Goidanich] causes yield losses of over 60% in susceptible common bean (*Phaseolus vulgaris* L.) cultivars (Viteri and Linares, 2021). Low to high levels of resistance have been reported in the primary and tertiary gene pools (Méndez et al., 2017; Miklas et al., 1998a; Viteri and Linares, 2021). However, common bean breeding lines reported with ashy stem blight resistance (e.g., BAT 477, IPA 1, and XAN 176) can have susceptible scores to multiple *M. phaseolina* isolates (Viteri and Linares, 2017; Viteri et al., 2019). Our objectives were to (1) evaluate the levels of resistance of new developed breeding lines and (2) compare these new genotypes with other known sources of ashy stem blight resistance.

**MATERIALS AND METHODS:** Fourteen genotypes including the four breeding lines UPR-Mp-22, UPR-Mp-34, UPR-Mp-42, and UPR-Mp-48 developed at the University of Puerto Rico were evaluated in Isabela and Lajas in 2021 (Table 1). A randomized complete block design with four (greenhouse) and three (field) replications were used. Two inoculations of the PRI21 *M. phaseolina* isolate were carried out in each plant according to the methodology described by Viteri et al. (2019). Higher ashy stem blight scores were noted at 21 and 42 d after the second inoculation for the field and greenhouse, respectively. A 1-9 scale was used to score the disease severity where 1= no sign of *M. phaseolina* infection and 9= the fungus infection passed the third node above or below the point of inoculation. Genotypes with mean scores of 1-3 were considered resistant, 4-6 intermediate, and 7-9 susceptible (Viteri and Linares, 2017).

**RESULTS AND DISCUSSION:** Cultivars ‘Othello’ and ‘Verano’ were susceptible (mean scores  $\geq 7$ ) while Andean genotypes A 195, ‘Badillo’, ‘PC 50’, and PRA154 had an intermediate response (4-6), as expected (Viteri and Linares, 2017 and 2021; Viteri et al., 2019). Nonetheless, none of the breeding lines reported with resistance to ashy stem blight such as BAT 477, NY6020-4, TARS-MST1 and XAN 176 (Méndez et al., 2017; Miklas et al., 1998b; Porch et al., 2012; Viteri and Linares, 2017) had a resistant ( $\leq 3$ ) or intermediate response consistently. In fact, NY6020-4 and TARS-MST1 were susceptible in all the evaluations (Table 1). In contrast, all the new breeding lines had an intermediate and stable response to *M. phaseolina*. For instance, UPR-Mp-34 had significant lower scores than BAT 477 in all the environments with the exception of the field screening in Isabela (Table 1). Likewise, UPR-Mp-42 had higher levels of resistance compared to TARS-MST1 in both locations and to XAN 176 in Lajas (Table 1). Furthermore, the breeding line UPR-Mp-48 may be used to increase the levels of resistance of ‘Bella’, ‘Beníquez’, and ‘Verano’ which are the most common white beans planted in Puerto Rico and all of these cultivars were susceptible to *M. phaseolina* (this study; Viteri and Linares, 2017; Viteri et al., 2019). Similarly, UPR-Mp-22 should be used in combination with the other UPR-Mp breeding lines and/or complementary sources of resistance to incorporate the resistant genes/QTL into common bean cultivars of different market classes.

**Table 1.** Mean ashy stem blight disease scores of common bean (*Phaseolus vulgaris* L.) genotypes to PRI21 *Macrophomina phaseolina* (Tassi) isolate evaluated at 42 and 21 d after the second inoculation in the greenhouse and field, respectively at the University of Puerto Rico in 2021.

Genotype	Seed color or market class	Greenhouse		Field	
		Lajas (February)	Isabela (April)	Isabela (May)	Lajas (September)
<i>Susceptible cultivars</i>					
‘Othello’	Pinto	9.0 <sup>a</sup>	8.0	8.3	– <sup>b</sup>
‘Verano’	White	8.8	7.0	7.5	8.0
<i>Common bean genotypes reported with resistance to ashy stem blight</i>					
A 195	Canela	4.6	3.8	5.7	6.4
‘Badillo’	Light-red kidney	3.8	4.3	6.1	6.6
BAT 477	Cream colored	6.4	7.1	6.4	7.3
NY6020-4	White	7.3	7.0	6.7	7.8
‘PC 50’	Red-mottled	4.8	4.1	6.3	6.7
PRA154	Beige-mottled	4.4	4.1	5.1	4.6
TARS-MST1	Black	8.5	7.0	7.3	8.7
XAN 176	Black	7.1	6.2	6.1	7.5
<i>New breeding lines with partial resistance to ashy stem blight</i>					
UPR-Mp-22	Cranberry type	3.8	4.6	5.0	4.5
UPR-Mp-34	Cream colored	3.9	3.9	6.0	4.1
UPR-Mp-42	Black	5.4	5.3	5.0	4.9
UPR-Mp-48	White	4.4	4.2	5.9	4.7
Mean	...	5.9	5.5	6.2	6.3
LSD ( $P \leq 0.05$ )	...	1.2	1.4	1.0	0.9

<sup>a</sup> Ashy stem blight disease severity was scored on a 1 to 9 scale, where 1 to 3= resistant, 4 to 6= intermediate, and 7 to 9= susceptible.

<sup>b</sup> No-seed was available to include this susceptible check for the evaluation in the field at Lajas.

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# ASSOCIATIONS BETWEEN GERMINATION, PHYTIC ACID METABOLISM, AND COOKING TIME IN DRY BEANS

Hannah Jeffery<sup>1</sup>, Karen Cichy<sup>2</sup>

<sup>1</sup>Michigan State University, Dept. of Plant Soil and Microbial Sciences, East Lansing, MI 48824, USA

<sup>2</sup>USDA-ARS, Sugar Beet and Bean Research, East Lansing, MI 48824, USA

**INTRODUCTION:** Phytic acid is formed from an inositol precursor and six phosphate groups. It is predominantly found in bean cotyledons and catabolized during germination by phytases as a source of phosphorous (5). Phytic acid acts as antinutrient to humans by reducing the bioavailability of essential minerals, especially iron and zinc (4, 5, 6). Low phytic acid bean mutants have been reported to have reduced germination and longer cooking times (8). A greater understanding of the relationship between phytic acid, germination, and cooking time in bean seeds is needed. In this study, the germination rate, phytic acid content, and cooking times of four dry beans were measured in seeds soaked for 0 and 12 hours. The two different soaking times were explored because pre-soaking reduces the cooking times of beans significantly (1). The expression levels of phytic acid biosynthetic genes were measured in beans soaked for 0, 3, 6, 12, and 18 hours, as well. Finally, the relationships between these factors were explored.

**MATERIALS AND METHODS:** Two brown beans [TZ-27 (slow-cooking) and TZ-37 (fast-cooking)] and two yellow beans [PI527538 (slow-cooking) and Ervilha (fast-cooking)] were selected for this study because they have contrasting cooking times. For the RNA sequencing (RNA-seq), water uptake, and cooking time assays, the beans were analyzed as previously described (5). For the germination rate and phytic acid content assays, the genotypes were harvested at Montcalm, MI in 2020, and, for both assays, seeds were tested raw (unsoaked) and after soaking for 12 hours. The seed germination protocol by the University of Penn State Root Lab was followed (2). One-hundred-and-fifty beans were covered by a plastic bag and incubated at 28°C. The number of germinated beans was determined after four days. A bean was considered germinated if the tip of the primary root (radicle) had fully protruded through the seed coat. The number of germinated beans was divided by the number of seeds in the replicate to obtain the germination rate. Phytic acid was extracted from lyophilized bean flours and assayed according to the Megazyme kit manual (Bray, Ireland, 2019). Data was analyzed using the `lm` function in RStudio (v1.1.453) ( $\alpha=0.05$ ) (7). The principal component plot was created using the phenotypic data from these experiments with the `ggbiplot` package (9).

**RESULTS:** Principal component analysis of

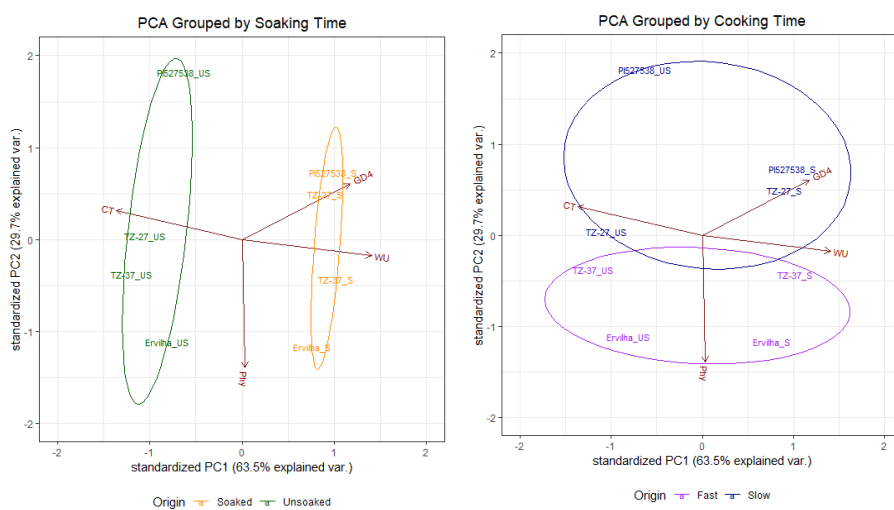


Figure 1: Principal component analysis plots grouped by the soaking times [unsoaked (US) or soaked (S)] (A) and cooking times (B) of the samples. CT: cooking time; WU: water uptake percentage; GD4: germination rate on day four of germination; Phy: phytic acid per mg of dry bean flour.

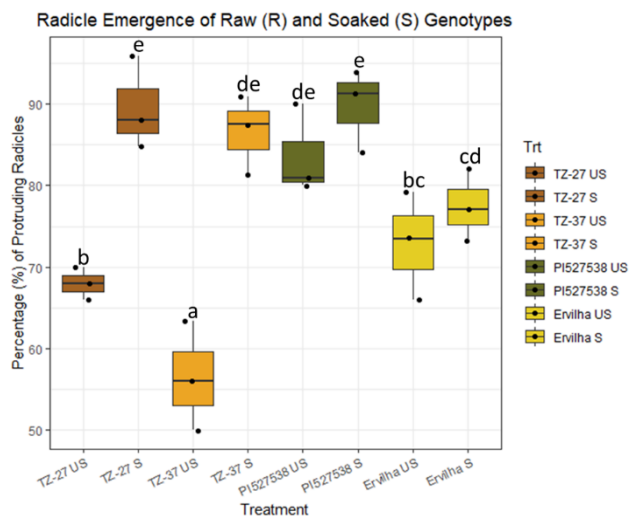


Figure 2: Percentages of unsoaked/raw (R) and soaked (S) beans with protruding radicles. Soaked beans were immersed in water for 12 hrs. Beans were considered germinated when the tip of the primary root (radicle) had fully protruded through the seed coat. The number of protruding taproots was counted after day four (4) of germination. Boxplots are an average of three replicates with 50 seeds each. Averages with the same letter are not significantly different (LSD,  $\alpha=0.05$ ).

the four genotypes grouped by soaking time (Figure 1A) and cooking time (Figure 1B) revealed PC1 separated the beans by soaking time and explained 63.5% of the variation and PC2 separated beans by fast and slow cooking genotypes and explained 29.7% of the variation. Soaking time (0 or 12 hours) had a positive association with water uptake percentage, whereas it had a negative association with cooking time. The germination rate of the beans increased after being soaked for 12 hours. Germination rates varied among the four genotypes, with the unsoaked brown beans having lower germination rates than the unsoaked yellow beans. Within each market class, the faster cooking beans had a reduced germination rate compared to their slower cooking counterparts (Fig. 2). The faster cooking yellow bean studied here also had higher levels of phytic acid both before and after soaking. Soaking, however, did not affect phytic acid levels in any of the genotypes (data not shown). It is possible that myo-

inositol, a precursor of phytic acid, affected germination rate (8). One gene related to phytic acid metabolism, Phvul.010G143500 (myo-inositol oxygenase 1), was upregulated in Ervilha relative to PI527538, and was found in a QTL for cooking time and water uptake (5). It catabolizes myo-inositol, possibly delaying germination. In TZ-27 and PI527538, Phvul.002G005300 (myo-inositol polyphosphate 5-phosphatase 2) was upregulated relative to TZ-37 and Ervilha, respectively, at hour six of soaking. Phvul.002G005300 recycles phytic acid precursors into myo-inositol, possibly promoting germination. In TZ-27, Phvul.009G121200 (phosphatidyl inositol-4-phosphate 5-kinase 2) is upregulated relative to TZ-37, and this gene acts upstream of Phvul.002G005300. It provides precursors for myo-inositol and phytic acid synthesis. No genes related to myo-inositol or phytic acid metabolism were upregulated in the fast-cooking beans (8).

**CONCLUSION:** In this study, slower germination rates and higher phytic acid levels were characteristic of faster cooking genotypes within a market class, particularly Ervilha. Genes identified as differentially expressed using RNA-seq may play important roles in germination and phytic acid metabolism in beans. Any genetic mechanisms will have to be explored further with larger data sets, though, before they can be confirmed.

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## POD STRINGS MAP TO REGION FLANKING *PvIND* ON Pv02 IN COMMON BEAN

Haidar Anwar Arkwazee<sup>1,2</sup>, Travis A. Parker<sup>3</sup>, Paul Gepts<sup>3</sup>, and James R. Myers<sup>2</sup>

<sup>1</sup>Horticulture Department, University of Sulaimani, Kurdistan Region, Iraq

<sup>2</sup>Department of Horticulture, Oregon State University, Corvallis, OR 97331, USA

<sup>3</sup>Department of Plant Sciences, University of California, Davis 95616, USA

**ABSTRACT:** Wild and domesticated dry beans produce fibrous pod strings, which provide a rigid structure that promotes pod opening in wild and dry beans. These fibers are undesirable in cultivars grown for pods as vegetables, and non-stringy “snap” varieties have become the market standard for this category. Attempts to genetically map pod strings have been inconsistent, with multiple chromosomes and multiple regions of Pv02 previously implicated. Here, we conducted QTL mapping for pod strings in a RIL population derived from a cross between dry bean (stringy) and snap bean (stringless) cultivars. The SNP nearest to *PvIND* on Pv02 showed the strongest association with pod strings, and phenotypes of all RILs matched at least one of the *PvIND* flanking SNPs, supporting the gene’s possible role in regulating pod string formation.

**INTRODUCTION:** Fibrous pod suture strings are the ancestral condition in *Phaseolus*, and these work in concert with wall fibers to provide the structure and torsion required for pod shattering (in wild beans) and threshing (in dry beans (1)). In snap beans, these pod strings create undesirable toughness and were once manually removed from all “string” beans. A dominant mutation leading to stringless pods (*St*) was identified by Calvin Keeney in the variety “Refugee Wax”, and this allele is now required in all commercial snap beans grown today. Other loci, such as *Ts*, may have a secondary effect hypostatic to *St*, leading to partial strings (2, 3). Mapping of pod strings has led to the identification of alleles on Pv06 (4) and Pv02 (5, 6). Gioia et al. (5) identified the candidate gene *PvIND* near the *St* locus, but found “recombinants” between the two at a distance of 7.8 cM (~2 Mb (7)). In contrast, Hagerty et al. (6) mapped *St* to a 500kb region on Pv02, bracketing *PvIND*. Here, we map pod strings in a new population to help resolve conflicting reports of *St*’s genomic position.

**MATERIALS AND METHODS:** A recombinant inbred population of 112 lines was developed from a cross between ‘A195’ (dry bean, stringy) and ‘6137’ (snap bean, stringless). Each of these were field-grown in Corvallis, Oregon, USA in three replicated plots. For each RIL, three full-sized pods at maximum fresh weight were sampled from each plot, for a total of nine pods per RIL. These were evaluated on a scale of 1-3: 1 (stringless), 2 (partial string), and 3 (full string).

Genotyping was conducted as described by Arkwazee (8). Briefly, DNA was extracted from leaf tissue of each line at the F<sub>6</sub> stage. Libraries were prepared for genotyping-by-sequencing using the *ApeKI* enzyme. SNP data were generated using the Tassel 5 analysis pipeline and imputation was conducted with Beagle4.1. Linkage maps were constructed in ASMap and QTL mapping was conducted with 5678 markers using rQTL.

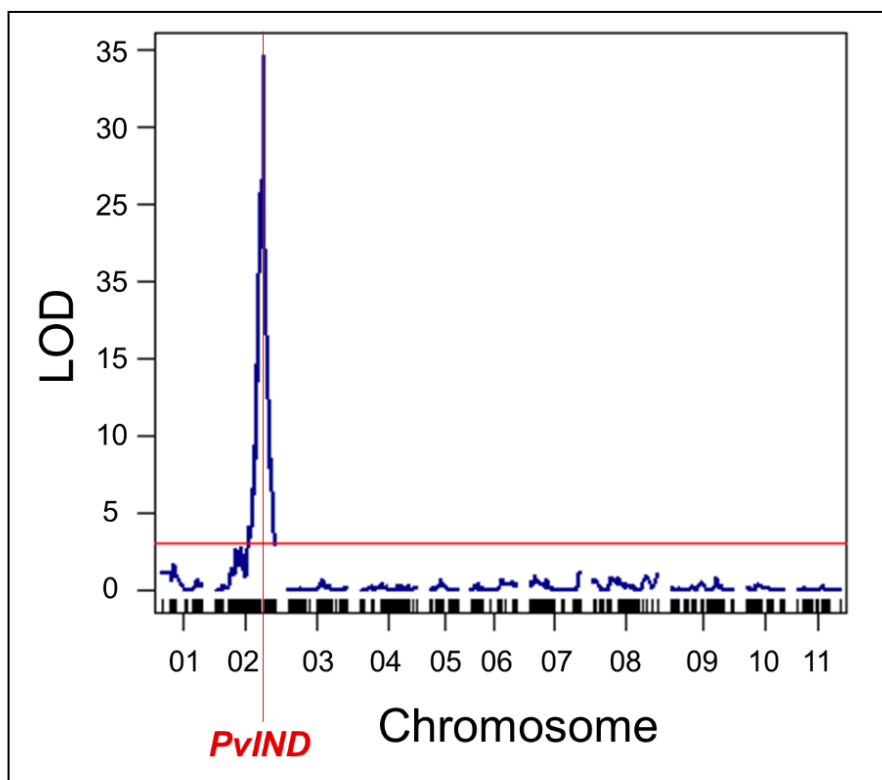
**RESULTS AND DISCUSSION:** A single major QTL for pod strings was identified (Fig. 1). The most significant SNP (LOD: 34.7) was also the closest in physical distance to *PvIND*, at Pv02 position 43,873,345 (G19833 v.1), 243 kb downstream of *PvIND*. The other flanking marker was at position 42,937,628, 692 kb upstream of *PvIND*. In all cases, the string phenotype corresponded to the genotype at one or both of the *PvIND* flanking markers, indicating that the major factor controlling strings is found between the two. Of the 15 RILs with recombination between the



flanking markers, phenotypes matched genotype of the downstream marker in ten and the upstream marker in five, closely matching expectations based on the physical spacing of *PvIND* between the markers (observed: 10:5; expected: 11:4;  $\chi^2$  test  $p=0.52$ ). This is consistent with the hypothesis

Our results are consistent with those of Hagerty et al. (6), who also found that flanking markers surrounding *PvIND* were most associated with pod strings. They contrast with studies which have mapped pod strings to other chromosomes (4), which may have segregated for secondary genes such as *Ts* (3). These results also contrast with studies finding considerable recombination between *PvIND* and *St* (e.g. 7.8cM; (5)), as our results indicate that the gene controlling *St* is somewhere from 3.9 cM upstream to 2.0 cM downstream of *PvIND* ( $\chi^2$  test boundaries for  $p<0.05$ ). Genotyping the population at *PvIND* will be an important step to confirm the relationship between *PvIND* and *St*.

**Fig. 1.** QTL mapping of pod strings in the A195/6137 population. A single major QTL was found on chromosome Pv02. The closest SNP to *PvIND* in physical distance was also the most significantly associated with pod strings. Phenotype of all lines matched the genotype at one or both of the SNP markers flanking *PvIND*. The horizontal red line represents the LOD threshold (3.03).



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# CHROMOSOME-SCALE GENOME ASSEMBLY OF THE COMMON BEAN GENOTYPE JaloEEP558 USING PACBIO HIFI AND HI-C

Juan C. Alvarez-Díaz<sup>1,2</sup>, Ariane Gratias<sup>1,2</sup>, Christophe Klopp<sup>3</sup>, David Latrasse<sup>1,2</sup>, Moussa Benhamed<sup>1,2</sup> and Valérie Geffroy<sup>1,2</sup>

<sup>1</sup>Université Paris-Saclay, CNRS, INRAE, Univ Evry, Institute of Plant Sciences Paris-Saclay (IPS2), 91405 Orsay, France; <sup>2</sup>Université de Paris, CNRS, INRAE, Institute of Plant Sciences Paris-Saclay (IPS2), 91405 Orsay, France ; <sup>3</sup>Plateforme Bioinformatique, Genotoul, BioinfoMics, UR875 Biométrie et Intelligence Artificielle, INRAE, Castanet-Tolosan, France.

**INTRODUCTION:** Pacific Biosciences (PacBio) high fidelity long reads sequencing, known as HiFi, and scaffolding technologies such as Hi-C, have allowed to produce assemblies with greatly improved contiguity and accuracy compared to short read assemblies (Burton et al., 2013; Lam et al., 2012). In common bean, several reference genomes have been published, such as for G19833 and BAT93 (Schmutz et al., 2014; Vlasova et al., 2016). However, BAT93 assembly presents low contiguity and is highly fragmented preventing structural variation analysis, including NLR gene evolution study, the largest class of disease resistance genes in plants (Richard et al., 2018). This prompted us to generate a chromosome scale genome assembly of BAT93 genotype using PacBio HiFi reads and Hi-C (Alvarez Diaz et al., 2021). BAT93 is one parent of a reference RILs population derived from a cross between BAT93 and JaloEEP558 (Freyre et al., 1998; Chen et al., 2010). Here, we generated a highly contiguous chromosome-scale genome assembly of genotype JaloEEP558 using PacBio HiFi sequencing and chromosome conformation capture data (Hi-C) for genome scaffolding.

**MATERIALS AND METHODS:** High molecular weight DNA from JaloEEP558 young trifoliolate leaves was sequenced using 1 SMRT cell of PacBio Sequel II system. In parallel, Hi-C chromosome conformation capture data were generated. Then, an assembly using HiFi long reads was generated and scaffolded using Hi-C data. Gene annotation was performed using homology and RNA sequencing evidence to build gene models integrated in EuGene software (release 4.2b) (Sallet et al., 2014). Finally NLR loci were predicted using the NLR-Annotator software (Steuernagel et al., 2020).

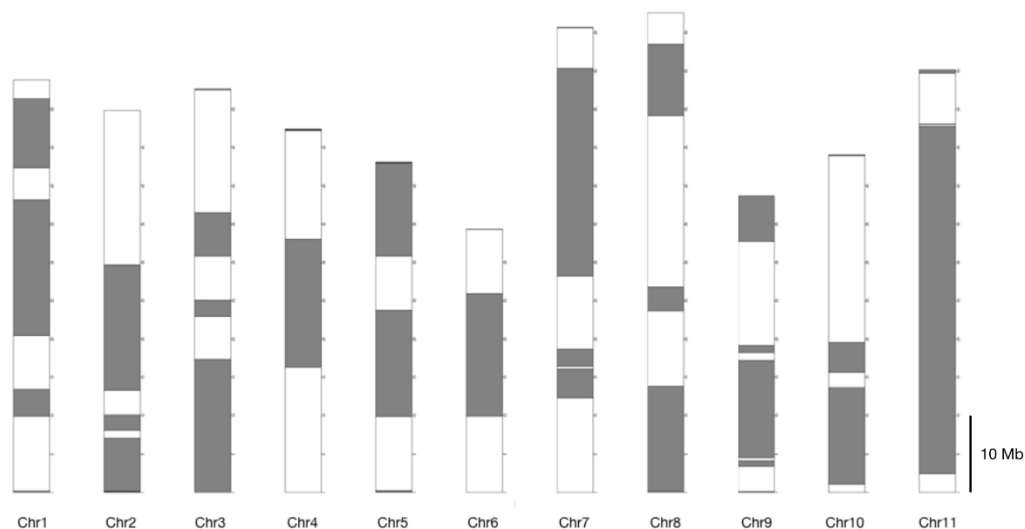
**Table 1.** Genome assembly and annotation statistics

Genomic features	Genotype	
	BAT93**	JaloEEP558
Assembly length in pseudo-molecules (Mb)	569.4	539.9
Contig number	1585	1422
Scaffold number	1441	1315
Scaffold N50 (Mb) / L50	52.3 /6	49.8/6
Longest contig/scaffold (Mb)	63.8	62.5
Number of annotated genes	28526	28129
Complete BUSCO* (%)	96.7	97
Number of predicted NLRs	439	383

Mb: Megabase, \*BUSCOs database: *Embryophyta* (n=1614), \*\* (Alvarez Diaz et al., 2021)

**RESULTS AND DISCUSSION:** HiFi long reads were assembled into 1422 contigs, corresponding to 606.5 Mb and 57X genome coverage. Strikingly, the total size of the contigs (606.5 Mb) is very close to the expected size of common bean genome based on flow cytometry (637 Mb) (Arumuganathan and Earle, 1991). We performed scaffolding using Hi-C, obtaining 11 chromosome-scale pseudomolecules with an N50 of 49.8 Mb and an assembly size of 539.9 Mb

(Table 1). The remaining unplaced scaffolds were very short (less than 1 Mb) and contained mostly repetitive sequences. Finally, 28129 genes were annotated and 383 NLR loci were predicted. Compared to BAT93 (439), JaloEEP558 presents less NLR genes (383), and dramatic differences were observed for several resistance gene clusters such as the *I* cluster, located at one end of chromosome 2.



**Figure 1.** Assembly overview of the 11 chromosome scale scaffolds of JaloEEP558 after Hi-C scaffolding. Chromosome numbers were assigned based on G19833 genome (Schmutz et al., 2014). White and grey blocks represent boundaries between contigs. Scale in Mb is presented to the right of the chromosomes.

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## BEYOND SCARS - A TABLE FOR SNPS AND INDELS CONVERTED TO TM-SHIFT ASSAYS

Alvaro Soler-Garzón<sup>1</sup>, L. Diaz<sup>2</sup>, B. Raatz<sup>3</sup>, G. Valentini<sup>4</sup>, M. Pastor-Corrales<sup>4</sup>, P. McClean<sup>5</sup>,  
T. Porch<sup>6</sup>, and P.N. Miklas<sup>1</sup>

<sup>1</sup>USDA-ARS, Prosser, WA; <sup>2</sup>CIAT Cali, Colombia; <sup>3</sup>Limagrain, la Menitre, France; <sup>4</sup>USDA-ARS, Beltsville, MD; <sup>5</sup>North Dakota State University, Fargo, ND; <sup>6</sup>USDA-ARS, Mayaguez, PR

Marker-assisted selection can be a useful tool for introgressing, transferring, combining and maintaining traits in a plant breeding program. Herein we introduce new SNP and InDel trait-linked markers for sharing with the common bean research community via a table in MS-Excel format within the Genetics section on the BIC website [http://www.bic.uprm.edu/?page\\_id=91](http://www.bic.uprm.edu/?page_id=91).

The ‘Beyond SCARs’ table (Table 1) is primarily meant to be an interactive resource for common bean researchers and breeders. We invite the community to submit markers with utility for MAS to add to the list. Feedback from users, concerning the effectiveness of any listed marker, will be key to the utility of this resource.

Currently, there are 42 markers linked to 27 loci influencing nine traits: resistance to BCMV/BCMNV, BCTV, BGYMV, anthracnose, common bacterial blight, rust, and white mold diseases, lectin genes and the gene for the slow darkening seed coat trait. Several loci (e.g. *I* gene, *Ur-3*, etc.) have multiple markers listed because recombination events to separate the markers in LD have not been identified yet or if found need further investigation. Other loci (e.g. *bc-2*) have multiple markers listed because each marker detects a different causal mutation within the candidate gene, a ~10,000 bp deletion in race Durango background (*bc-2*<sup>[U1111]</sup>) and point mutation in navy beans (*bc-2*<sup>[Robust]</sup>), or they detect variants within the candidate gene associated with different allelic effects (e.g. *bc-4*, *bc-4*<sup>2</sup>) (Soler-Garzón et al., 2021). Markers listed for some loci (e.g. *Co-4*<sup>2</sup>, *p*<sup>sd</sup>) were adopted from the literature.

All the SNP and Indels listed in the Beyond SCARs table have primers designed for detection of the marker using the Tm-shift assay (Wang et al., 2005). Briefly, each SNP or InDel variant has two forward allele specific primers with the 3’ base of each primer matching one of the alleles bases, and a reverse common primer. GC tails of different lengths were added to the 5’ end of allele specific primers. Fragments were amplified by standard PCR, with a fluorescent dye in each reaction, followed by a melting point analysis performed with a fluorescence-detecting thermocycler. Additionally, the resulting PCR products were evaluated for polymorphisms on 4% agarose gels in 1 × Tris-Borate-EDTA buffer on a gel electrophoresis system for 4h at 100 volts. A DNA dye was added to the gel for visualization of the fragments amplified on a UV imaging system.

About half of the markers in the Beyond SCARs table were incorporated in the CGIAR Excellence in Breeding (EiB), KASP low density genotyping platform <https://excellenceinbreeding.org/module3/kasp>, in collaboration with Intertek (Sweden), a genotyping service provider. The key information for converting a Tm-shift assay marker to KASPar is the sequence tag in column P of the table. Several markers (e.g. Pvvps4<sub>del</sub> for *bc-2*<sup>[U1111]</sup>, PvNAC1 for *bgm-1*) were not transferable to the Intertek KASPar platform. The Intertek platform is high-throughput, and can be used for outsourcing MAS, as leaf tissue can be sent to the lab and a suite of selected markers can be assayed across multiple plates of 384 genotypes.

<b>Table 1.</b> A transposed sample for the Beyond SCARS <a href="http://www.bic.uprm.edu/?page_id=91">http://www.bic.uprm.edu/?page_id=91</a> excel file on the BIC website 'Genetics' section showing information contained for a single marker.		
A*	Trait	BCMV
B	Gene/QTL	<i>bc-2</i> <sup>[Robust]</sup>
C	SNP marker	Pvmit-2 C del
D	Reference/Source	Soler-Garzón et al. 2021c
E	Intertek code	snpPV00165
F	Chromosome (G19833v2.1)	11
G	Position (G19833v2.1)	9278765 bp
H	Genetic Background	Robust
I	Check line	Sanilac
J	Gene Model	Phvul.011G092700
K	Annotation	AAA-type ATPase family protein
L	Variant	frameshift deletion
M	Alleles	C/-
N	Favorable Allele	-
O	primers Sense 5'→ 3'	+
P	Seq Tag (G19833v2.1)	....TCCTCGGCCCGACGCAGGTAATTTCTGCGTGATTGCCTC[C/-]TTCTTTCAT.....(this tag is purposely shortened for display here)
Q	Primer Fa	gcgggcagggcggcATTTCTGCGTGATTGCCTCT
R	Primer R	CTTCAAAACGCACCTCAAGTATGA
S	Primer Fb	gcgggcTCTGCGTGATTGCCTCC
T	Ta	55

\*Letters correspond to the column in the excel file [http://www.bic.uprm.edu/?page\\_id=91](http://www.bic.uprm.edu/?page_id=91)

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# DRY BEAN SUSTAINABILITY FROM INDUSTRY AND CONSUMER PERSPECTIVES

Mark A. Uebersax<sup>1</sup>, Karen Cichy<sup>2</sup>, Francisco Gomez<sup>1</sup>, Scott Bales<sup>1,3</sup> and Joe Cramer<sup>3</sup>

<sup>1</sup>Michigan State University, E. Lansing, MI 48824 (USA), <sup>2</sup>USDA-ARS, E. Lansing, MI 48824 (USA), <sup>3</sup>Michigan Bean Commission, Frankenmuth, MI 48734 (USA)

## INTRODUCTION

U.S. consumers have greatly intensified their interest in environmental sustainability and are seeking credible and reliable information on which to make purchase decisions<sup>(1)</sup>. Consumers are soliciting detailed information from all participants in the dry bean value chain (growers and elevator operators, food processors, wholesale distributors, retail marketing and sales companies and restaurant chains). This paper highlights the broad array of considerations that must be addressed to assure that dry beans will be adequately and appropriately positioned within “the agricultural / food sustainability” context.

*Dry Beans as a component of agricultural sustainability:* Dry beans are associated with several significant elements of sustainability as presented in Table 1.

**Table 1.** Role of Dry Beans in Sustainable Agriculture Systems

Genetic Potential & Innovations	Agronomic Practices & Outcomes	Sustainable Food & Nutrition Interventions	Global / Societal Impacts & Implications	Bean Industry Standards Implementation
<ul style="list-style-type: none"> <li>• Nitrogen fixation</li> <li>• Drought tolerance</li> <li>• Biodiversity and productivity</li> </ul>	<ul style="list-style-type: none"> <li>• Harvest efficiency</li> <li>• Field dehydration</li> <li>• Soil health &amp; IPM</li> <li>• Nutrient and energy cycles</li> <li>• Legumes in cropping systems</li> </ul>	<ul style="list-style-type: none"> <li>• Nutrient density</li> <li>• Healthy foods</li> <li>• Shelf-life and food waste</li> <li>• Plant-based protein sources</li> <li>• Chronic diseases mitigation</li> </ul>	<ul style="list-style-type: none"> <li>• Global food security</li> <li>• Gender equity</li> <li>• Infant and child mortality</li> <li>• Trade access</li> </ul>	<ul style="list-style-type: none"> <li>• Local, regional and international sustainability</li> <li>• Standards of practice</li> <li>• Documentation</li> </ul>

## Dry bean marketing and trade associated with dry bean sustainability

The U.S. dry bean industry markets dry beans and processed products to domestic and international venues. Consumer driven inquiries regarding a wide range of aspects about the ethical and safe business practices are increasingly routine. Specific details about ingredient origin and processes used to market final foods are transparently sought and answers expected. The details and expectations for “sustainable foods” are consumer driven aspects for product selection. The followings points are considerations that bean growers, traders and processors must be prepared to address.

*Consumers’ sustainability requests:* Most consumer product companies, including large and small food processors, are routinely receiving requests for background information about the “sustainability of their products.” Requests take the form of a need for general information or completion of complex and detailed questionnaires about the “sustainability” issues associated with the specified product. The definitions of “sustainable” are often focused on the aspects of the agricultural sector of ingredients (beans, peas etc.) and items such as soil fertility and erosion, pesticide and herbicide treatments, soil conservation practices, harvesting, handling and storage techniques. Further, much attention is given to the life cycle of packaging materials (note: canned beans generally get “high marks” for recyclable packaging). The scope of criteria is often far reaching and may include aspects of energy usage, total carbon footprint, labor workforce practices

(child labor practices etc.) and conservation efforts. Clearly these are consumer driven requests and are likely to increase and will be point of purchase drivers for many consumers. It is most apparent that bean processors need scientifically reliable data about production, harvest, storage, and transportation of dry beans. Improved authoritative descriptive communication messages associated with food safety are urgently needed. Topics for which parties in the value chain are seeking information to communicate to consumers and to identify areas for improvement include: overall environmental impact of products; water utilization and irrigation requirements; overall fertilizer usage; impact of crop production on deforestation; soil fertility practices; crop yield to carbon footprint ratio (also protein to yield relationships); on-farm health and safety practices impact on workers. The increased value consumers place on sustainability provides a great opportunity to emphasize the environmental impact of beans across the value chain, especially in comparison to other protein sources.

**Consumers seek “third party” endorsements:** Public sector researchers are positioned to provide meaningful publications that demonstrate important aspects of dry beans within sustainable agricultural systems. Consumers increasingly use external “accreditation” for accepting credible and sweeping validations for direct or implied sustainable claims associated with foods. Alignment with USDA’s *Climate Smart Agriculture Initiative*<sup>(2)</sup> is an example of a means for consumers to assess sustainability. Global trade is significantly influenced by sustainability. The European Union (EU) *Sustainable Food Systems Initiative*<sup>(3)</sup> focuses on criteria used to establish “sustainability.” The current U.S. trade policy agenda directed toward increased agricultural crop exports appears to be fully aligned with achieving expectations for enhanced sustainability requirements. This initiative is designed to accelerate and facilitate the transition to sustainable food systems (optimizing the production, distribution, and consumption of food to increase resource efficiency and reduce food waste) in the EU. Dry beans have great alignment potential under these programs.

## RECOMMENDATIONS

Further research and documentation of results suitable for adequate communication of the entire spectrum of topics associated with dry beans as a sustainable food crop are required. The U.S. Dry Bean Council (USDDB) recently formed a subcommittee to address the broad issues associated with dry bean sustainability. It is essential that all sectors need to demonstrate sustainability protocols throughout the dry bean value chain. Farmers/growers increasingly need to inform dealers, who in turn, sell to processors, who subsequently sell to retailers who finally sell directly to consumers. It is paramount that the sustainability standards address the inherent aspects of the dry beans, (multiple levels of genetics, agronomic and distribution practices, and food product utilization) that add value to sustainability. Such endeavours must be undertaken in a formal manner to assure credible data for each sector for the production, processing, and marketing of dry bean products. Thus, compiled information must be suitable for a broad array of audiences including the scientific community, trade organizations, and consumers. Collaborative efforts are needed to focus the diverse talent required to appropriately assess the scale and scope of sustainability that is evident within the dry bean community.

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## BIOMASS ACCUMULATION AND PARTITIONING IN MESOAMERICAN DRY BEAN LINES UNDER DROUGHT STRESS

Paul M. Kimani, Jackline Alufa, George Chemining'wa, Mary Mburu and John H. Nderitu

Dept. of Plant Science and Crop Protection, University of Nairobi, P.O Box 29053-00625  
Nairobi, Kenya

**INTRODUCTION:** Partitioning of shoot dry matter to pods and remobilization from pod wall to the developing grain is an important mechanism of adaptation to drought stress among dry bean genotypes (Rao et al., 2007). Yield under stress is enhanced through efficient assimilate redistribution in favour of grain production (Beebe et al., 2008). Drought tolerance genes have been incorporated into many small-seeded bean genotypes through intensive breeding (Beebe et al., 2008), but the mechanisms of adaptation to drought stress have not been adequately addressed. Such studies have yet to be reported in East and Central Africa. It is therefore important to understand these mechanisms and identify or develop bean varieties that efficiently accumulate dry matter, and partition and channel these photosynthates to the grain. This may contribute to yield stability of beans under the anticipated frequent and probably severe droughts associated with climate change and variability. The objective of this study was to determine if there is genotypic variation for physiological and phenological traits associated with enhanced drought tolerance in Mesoamerican dry bean genotypes.

**MATERIALS AND METHODS:** This study was conducted for two seasons at Kabete Field Station (1860m) of the University of Nairobi, and in a farmer's field in Mwea (1550m), Kirinyaga County. Eighty-five Mesoamerican dry bean genotypes which included three market classes: navy (DNB), small reds (DSR) and mixed colours (DMC), and local and international checks with contrasting drought responses, were tested under drought stress and non-stress conditions. The experimental design was a split-plot with three replications. Main plots were either irrigated (NS) or rainfed (DS). Genotypes were the subplots. The plot size was 3 m long planted with two rows each consisting of 30 plants at a spacing of 50 cm x 10 cm. Both DS and NS plots were initially grown under irrigation at 80% field capacity. Stress was induced by withholding water at pre-flowering to maturity for the DS treatment. Shoot, stem, leaf and pod biomass at mid-pod filling and stem, seed, pod wall and pod biomass were measured by destructive sampling of a 0.5 m row of plants at physiological maturity following procedures described by Rao et al (2007). Sampled plants were separated into their respective plant parts, oven dried at 60°C for 48 hours and dry weights recorded. Yield was measured by counting and harvesting the rest of the plants when fully dry and taking the seed weights. Soil moisture was monitored from the time of stress induction to maturity using the gravimetric method in order to determine moisture differences between the treatments. Genstat (13 edition) software was used for data analysis.

**RESULTS AND DISCUSSION:** The results indicated that under drought stress some genotypes such as DSR11-02, DSR11-21, DMC11-10, DMC11-11, DNB11-03, DNB11-07, as well as checks like SEA 15, KAT B1 and KATB9, exhibited a tendency to escape drought effects through accelerated reproductive development (Table 1). Drought stress reduced grain yield by over 30% and harvest index by 15% for most genotypes with the mixed colours recording the highest reduction. Under drought stress, grain yield ranged between 400 kg ha<sup>-1</sup> and 1,218 kg ha<sup>-1</sup>, while harvest index varied between 34 and 55%. Significant differences in dry matter partitioning among genotypes were observed with high yielding drought tolerant genotypes such as DSR11-08,



DMC11-10, DNB11-10 and SEA15 having higher harvest indices than the susceptible genotypes like DMC11-14, DMC11-20, DNB11-13 and GLP585. Stomatal conductance was low under drought stress conditions, and ranged between 36 and 206  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . There was a strong correlation between grain yield under drought stress and plant attributes such as pod harvest index ( $r=0.40^{***}$ ), pod partitioning index ( $r=0.89^{***}$ ) and stem biomass reduction ( $r=0.32^{***}$ ). Significant genotypic variation in drought tolerance existed among genotypes in the three market classes under drought stress and non-drought stress conditions with navy beans showing more drought tolerance and mixed colours the least.

**Table 1.** Plant traits<sup>#</sup> measured on 23 genotypes grown under irrigated (NS) and rainfed (DS) conditions.

GENOTYPE	PPI(%)		PHI(%)		HI(%)		SBR(%)		PWBP(%)		Yield in kg/ha	
	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS
DMC 11-10	54	95	69	62	38	33	-42	65	50	37	1302	1005
DMC 11-12	78	94	37	45	51	40	-24	73	62	55	841	716
DMC 11-22	61	77	64	67	58	47	-92	68	36	33	987	652
DMC 11-24	43	52	68	64	53	37	-80	86	35	31	1072	1027
DNB 11-06	57	73	65	63	40	24	-91	79	38	37	1213	924
DNB 11-07	67	81	68	58	49	45	-88	72	41	32	1508	1218
DNB 11-14	73	79	68	45	53	43	-33	48	54	32	952	894
DNB 11-15	65	80	67	57	47	31	-82	66	42	32	1286	1043
DSR 11-01	55	57	62	53	40	30	-36	34	48	46	876	810
DSR 11-03	68	95	66	55	50	33	-77	52	45	33	1281	868
DSR 11-09	79	97	71	64	52	47	-58	64	35	39	887	704
DSR 11-12	33	61	65	62	46	33	-29	65	44	37	1122	920
DSR 11-13	92	97	68	61	56	47	-12	76	58	38	1080	857
DSR 11-15	79	84	65	53	57	54	-84	72	54	46	978	880
DSR 11-18	74	83	61	55	56	44	-17	88	69	44	1029	812
DSR 11-24	79	96	68	64	38	32	-16	54	51	35	1040	858
GLPX92	69	92	69	63	36	25	-72	70	40	36	811	601
KATB1	80	84	69	68	33	23	-12	30	33	32	654	528
KATB9	23	41	68	68	47	31	-23	54	32	32	730	687
SEA15	47	91	67	52	2	17	-16	79	48	32	943	816
RCB231	66	67	67	63	54	43	-13	56	37	33	899	788
SEN56	87	90	62	41	37	28	-20	36	58	38	585	572
TIO CANELA	86	99	54	57	55	47	-12	47	45	42	773	771
<b>Mean</b>	<b>66</b>	<b>80</b>	<b>65</b>	<b>59</b>	<b>47</b>	<b>36</b>	<b>-46</b>	<b>64</b>	<b>46</b>	<b>37</b>	<b>938</b>	<b>770</b>
LSD(P<0.05)	<b>7.3</b>	<b>7.3</b>	<b>2.08</b>	<b>2.08</b>	<b>6.1</b>	<b>6.1</b>	<b>2.9</b>	<b>2.9</b>	<b>2.11</b>	<b>2.11</b>	<b>168</b>	<b>168</b>

<sup>#</sup>PPI= pod partitioning index; PHI= pod harvest index; HI=harvest index; SBR=stem biomass reduction; PWBP= Pod wall biomass proportion

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# **PARTICIPATORY SELECTION FOR DROUGHT TOLERANCE IN ANDEAN DRY BEAN GENOTYPES IN KENYA**

**Paul M. Kimani, Iddah Kabutbei, George Chemining'wa, Mary Mburu and John H. Nderitu**

Dept. of Plant Science and Crop Protection, University of Nairobi, P.O Box 29053-00625 Kenya

**INTRODUCTION:** Common bean (*Phaseolus vulgaris L.*) is the most important grain legume in east, central and southern Africa. However, its productivity is severely constrained by frequent droughts. Yield losses up to 60% are common. Okwiri et al., (2009) reported that farmers' ranked drought as the most important constraint in bean production. In this region, beans are mainly produced by smallholders who have limited access to seed of improved varieties and irrigation. Therefore, bean yield under on-farm conditions still remains below 500 kg ha<sup>-1</sup> while the potential yield is more than 1,200 kg ha<sup>-1</sup>. National bean programs in the Eastern Africa region have developed many drought tolerant genotypes whose impact has not been felt at the national level partly because they are lacking in other farmer preferred traits. Large seeded red mottled, red kidney and speckled sugar beans are the most important market class in east, central and southern Africa (Kimani, 2004; Wortmann et al, 1998). However, these market classes, which belong to race Nueva Granada of the Andean gene pool, are susceptible to drought. The involvement of farmers through participatory variety selection (PVS) is therefore important to introduce them to new potential genotypes that may be superior to their varieties and landraces. Participation of farmers during appropriate breeding stages can contribute to the identification of superior genotypes with farmer, consumer and market demanded traits which can enhance adoption. The objective of this study was to determine criteria used by farmers to identify and select high yielding drought tolerant Andean dry bean genotypes.

**MATERIALS AND METHODS:** Farmer participatory trials were conducted on-farm in Kirinyaga County, and on-station at Kabete Field Station, University of Nairobi during the short and long rain seasons. The experiment was a split plot design with three replicates. Irrigation treatments (stress and no stress) were the main plots. Eighty-eight Andean bean genotypes were the subplots. For the moisture stressed treatment, irrigation was withheld at flowering, but continued to maturity for the non-stress treatment. Twenty-seven farmers evaluated the on-farm trial at crop maturity during the first season, and 36 farmers participated in the selection during the second season. Thirty-four farmers evaluated the on-station trial during the second season. Both male and female farmers participated each season. The ribbon method was employed. Each farmer conducted individual selection using coloured ribbons. Male farmers used yellow to denote 'like', and red ribbons for 'dislike'. Female farmers used white ribbons to tag preferred genotypes, and black ribbon for 'dislike'. Numbers of 'likes' and 'dislikes' were recorded for each genotype. A focus group discussion followed to determine the criteria used for 'like' and 'dislike'. Yield data was subjected to analysis of variance using GENSTAT software. Least significant difference (LSD) test was used to compare the mean yields of the genotypes.

**RESULTS AND DISCUSSION:** The key characteristics used by farmers in the selection of the best genotypes were yielding ability, earliness, resistance to biotic and abiotic factors, seed color, growth habit, vigor, plant height, uniform maturity, marketability and the cooking ability. Genotypes with indeterminate growth habit were rejected due to their unsuitability for intercropping with maize. The local drought tolerant checks including GLP 92 and KAT 69 were

found to be inferior to the improved varieties in terms of drought tolerance. This study also showed that farmer criteria for selecting drought tolerant varieties overlapped with conventional scientific procedures as evident from the analysis of variance. Both approaches identified DRM 11-17, DRM 11-03, DRK 11-19 and DPC 11-09 as the genotypes with the best yield potential (Table 1). In contrast, 'Kenya Early' and 'Kenya Wonder,' which were the least preferred by farmers, also had the lowest yield in drought stressed conditions. There were gender differences in ranking of genotypes. Men preferred high yielding varieties irrespective of grain colour, while women preferred varieties with red grains. Men rejected climbing varieties as unsuitable when intercropped with maize and preferred varieties with high shoot biomass for fodder. Gender differences also emphasized the need to involve both men and women in PVS. New bean genotypes with better performance under drought than the local checks provide new opportunities for producers in drought prone production regions.

**Table 1.** Grain yield of bean genotypes in moisture-stressed and non-stress on-farm and on-station conditions and frequencies of selection by female and male farmers in Kenya.

Genotype	Grain yield (kg -ha <sup>-1</sup> )				Farmer selection frequency	
	On-station		On-farm		Positive	Negative
	DS	NS	DS	NS		
DRM 11-03	867.5	909.2	468.5	611.1	9	5
DRK 11-07	719.6	635.2	280.9	807.9	3	22
DRK11-22	837.8	671.2	421.1	449.1	17	0
DRM11-17	803.5	1226.7	341.0	852.7	29	4
DSS 11-17	740.9	769.8	250.8	145.2	1	12
DPC 11-02	694.9	720.7	84.2	248.9	1	21
DRK 11-18	623.5	649.5	379.2	843.4	21	0
DRK 11-10	867.7	927.3	92.2	409.1	15	14
KAT 69	555.6	767.1	289.2	632.6	4	18
DRK 11-19	590.4	711.2	491.1	725.3	18	5
DRK 11-16	502.6	860.0	453.6	837.3	31	2
DRM 11-13	517.1	1045.1	293.1	1017.0	17	1
DPC 11-09	630.6	563.1	249.1	304.1	18	1
DPC 11-06	482.5	936.7	336.0	751.5	19	11
DSS 11-01	519.2	570.2	346.3	431.3	21	0
Kabete Super	729.6	952.1	360.1	535.9	5	4
KAT 56	360.8	532.5	439.6	262.0	8	5
GLP 1004	454.7	700.1	297.6	471.7	5	3
DRK 11-15	462.0	567.2	476.8	746.1	51	0
DPC 11-01	263.0	604.3	723.9	544.8	4	5
Miezi Mbili	363.7	610.5	154.6	362.0	3	3
GLP 92	327.2	686.44	302.3	358.6	1	19
DPC 11-05	247.4	559.6	115.8	363.2	0	13
Kenya Early	201.4	1067	71.7	156.5	0	17
Kenya Wonder	259.0	671.52	164.3	362.4	0	15
Mean	530.4	757.9	297.7	508.4		
LSD 0.05 (treatments)		76.6		38.9		

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# SCREENING AND SELECTION OF DROUGHT TOLERANT COMMON BEAN FOR AN EQUATORIAL TROPICAL ENVIRONMENT: HEAT STRESS TOLERANCE TRAIT IS A PRE-REQUISITE

**David Appiah-Kubi<sup>1</sup>, James Asibuo<sup>1</sup>, Maxwell Lamptey<sup>1</sup>, Stephen Yeboah<sup>1</sup>, Patricia Amankwaa-Yeboah<sup>1</sup>, Emmanuel Asamoah Adjei<sup>1</sup>, Boaz Waswa<sup>2</sup> and Louis Butare<sup>2</sup>**

<sup>1</sup>CSIR-Crops Research Institute, P. O. Box 3785, Kumasi, Ghana; <sup>2</sup>CIAT-Pan Africa Bean Research Alliance (PABRA), Nairobi, Kenya

## INTRODUCTION

Food crops of legumes are important for Sub-Saharan Africa, providing cheaper sources of protein, vitamins and minerals for its growing population (Celmeli et al., 2018). Common bean is one of the important legumes for direct human consumption worldwide. Its cultivation in West Africa and in Ghana particular is new; which is partly due to erratic rainfall conditions, high temperatures (day and night), low soil phosphorus availability for which the environment is susceptible (Margaret et al., 2014). Common bean is a new crop in Ghana whose consumption among the middle class is increasing. It is mainly served as baked beans in hotels and restaurants. The CSIR-Crops Research Institute in collaboration with other partners has released four micronutrient rich varieties of common beans in Ghana, which were not drought tolerant. Climate change is predicted to increase global temperatures and reduce rainfall patterns, with adverse effects, particularly, at the critical stages of plant growth and development, resulting in significant yield losses. Rainfall under future climate change scenarios in SSA will either occur late or stop earlier than usual. It has become necessary to improve resilience of smallholder farmers and food security in the midst of adverse effects of climate change and global warming; which is a threat to a developing country like Ghana. This study evaluated common bean lines for drought tolerance and high yield potential.

## MATERIALS AND METHODS

About 116 common bean lines tolerant to drought with other stress trait attributes (multiple stress tolerance) received from the International Centre for Tropical Agriculture (CIAT) in its African Centre in Uganda were evaluated under field conditions for seed yield performance. Forty common bean lines selected based on yield output of 2.0 tons/ha and above were used for further evaluation in a screen house experiment to assess for drought and low-P tolerance. The set-up was a 2 x 2 x 40 factorial experiment at terminal drought in RCBD with three replications. The study was conducted during the dry season (December – February 2019). Temperature-humidity data logger (*Supco* ®. SL500TH. SN: 05151131CF) was set to read and record at three-hour intervals throughout the growth period.

## RESULTS AND DISCUSSION

The average day and night temperatures recorded during the growing period were 35.45 °C and 24.95 °C, respectively, with the maximum temperature recorded at 46.72 °C (Table 1). These temperature conditions experienced were higher and above the ideal day temperature condition of not more than 30.0 °C daytime, and not more than 20.0 °C at night (López-Hernández & Cortés, 2019). Higher temperatures reduces number of florets, causes susceptibility of flower abortion and subsequent yield reduction in common bean (Beebe et al., 2013; Rainey & Griffiths, 2005).

**Table 1.** Day and Night Temperatures Conditions in Screen-house

Variable	Night Temperature °C	Day Temperature °C
Average Temperature	24.95	35.45
Maximum Temperature	30.72	46.72
Minimum Temperature	18.78	23.08

**Table 2.** Day and Night Relative Humidity conditions at Screen-house

Variable	Night % Rel. Humidity	Day % Rel. Humidity
Average Relative Humidity	84.84	50.78
Maximum Relative Humidity	97.9	90.45
Minimum Relative Humidity	53.95	17.75

Among the total number of 478 experimental bean plant-accessions (two experimental plants were missing), 141 plants did not flower (29.5 %), 70 plants initiated flower buds but had flower-abortion (16.64 %), 168 plants initiated pods but later dropped (35.18 %), whilst 99 plants produced pod that reached physiological maturity with seeds (20.72 %). The heat stress experienced in the screen house identified a differential response to reproductive structural development in drought tolerant common bean accessions.

The study results identified four SEF bean lines (SEF 15, SEF 47, SEF 60 and SEF 62) as superior in their level of heat tolerance as shown in their high frequency of pod and seed formation under low-P and drought conditions. These lines are superior in their level of heat compared to the SMC, SMN and SMR bean lines.

If excessive warm temperature condition could disrupt reproductive structures of common bean plants from developing into seeds; then, it presupposes that effects of climate change and its associated global warming has serious consequences on food security. Therefore, climate resilient crops need to be developed, to curb the consequences of the phenomenon on food and nutrition security.

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# YIELD COMPONENTS AND GROWTH HABITS OF BEANS IN A WARM CLIMATE IN MEXICO

<sup>1</sup>Escalante-Estrada José Alberto Salvador, <sup>2</sup>Escalante-Estrada Yolanda Isabel, <sup>1</sup>Cid Aguilar Carpio and <sup>3</sup>L. Enrique Escalante Estrada

<sup>1</sup>Posgrado en Botánica, Colegio de Postgraduados. Campus Montecillo. Carretera México- Texcoco Km 36.5. Montecillo Mpio de Texcoco Edo. de Méx. México. CP; <sup>2</sup>Instituto de Investigación Científica, Área de Ciencias Naturales. Universidad Autónoma de Guerrero, Chilpancingo, Gro., México; <sup>3</sup>Colegio Superior Agropecuario del Estado de Guerrero

## INTRODUCTION

The bean (*Phaseolus vulgaris* L) is a basic crop of the Latin American diet. It presents different growth habits and cycle length (Escalante and Kohashi, 2015). Due to this, its adaptation and production can be variable in each region. In the state of Guerrero, irrigated autumn-winter sowings have an average production of 0.88 t ha<sup>-1</sup> (SIAP, 2021). However, this does not satisfy the requirement of the population. Thus, the search for better adapted cultivars and higher production in each region is justified. The objective of the study was to determine the cultivars with the highest yield and the components that determine it.

## MATERIALS AND METHODS

The study was carried out in Iguala Gro with a warm climate (Aw0, García, 2005) in a clay loam soil, pH 8.4, .MO 3.5 % and assimilable N of 45 kg ha<sup>-1</sup>. The sowing of the cultivars (CV): Bayomex and Cacahuete72 of determinate growth habit (GH) type I; Negro Chiapas, Negro Veracruz and Jamapa of indeterminate GH (IGH) type II; and Bayo 400, Flor de Mayo RMC and Pinto Nacional of IGH prostrate Type III, was on December 18, 2017 at 6.6 plants m<sup>-2</sup> (75 cm \* 20 cm). 9 irrigations were applied. Days to emergence, flowering (F) and physiological maturity (PM) were recorded. At PM grain yield (GY), grain size (GS), number of grains (GN) and pods (PN) and grains per pod (GP) were measured. An analysis of variance and the mean comparison test (Tukey  $\alpha = 0.05$ ) were applied to the variables under study with the statistical package SAS version 9.2.

## RESULTS AND DISCUSSION

During the development of the crop, the minimum and maximum mean temperature was 16 °C and 37 °C before F; 20 °C and 33 °C, from F to PM, respectively. Types II were later for F and PM; followed by types III and early type I (table 1). Regarding the GY and its components, the CVs under study presented significant changes (Table 1). The highest PN, GN and GY were found in type II. Within these Negro Chiapas showed the highest GY with 150 g m<sup>-2</sup>, followed by Negro Veracruz and Jamapa with 130 and 115 g m<sup>-2</sup>. Type III such as Pinto Nacional, Flor de Mayo RMC and Bayo 400 showed a GY of 89, 90 and 89 gm<sup>-2</sup>, respectively. The lowest was for type I such as Cacahuete 72 and Bayomex, which presented 72 and 66 g m<sup>-2</sup>, respectively. The highest GY (150 g m<sup>-2</sup>) was similar to that reported by Escalante *et al.* (2001) with 159 g m<sup>-2</sup> for cv. Michoacán 12A3 type II planted in the autumn in the region and higher than the regional average of 88 gm<sup>-2</sup> (SIAP, 2021). GY showed a high relationship with F ( $r = 0.78$  \*\*), PM ( $r = 0.91$  \*\*), GN ( $r = 0.96$  \*\*) and PN ( $r = 0.85$  \*\*). The later cultivars presented higher GN, PN and GY.

**Table 1.** Phenology, grain yield and its components, Iguala Gro. Mexico. Autumn-Winter 2017.

<b>Cultivar</b>	<b>Type</b>	<b>F (days)</b>	<b>PM (daYs)</b>	<b>GY (g m<sup>-2</sup>)</b>	<b>GS (mg)</b>	<b>GN m<sup>-2</sup></b>	<b>GP</b>	<b>PN m<sup>-2</sup></b>
<b>Negro Chiapas</b>	II	53 a	110 a	150a	223 d	672a	5 a	134 a
<b>Negro Veracruz</b>	II	53 a	108 a	130b	192 e	677 a	6 a	113 b
<b>Jamapa</b>	II	50 a	108 a	115 b	191 e	602 b	6 a	80 c
<b>Pinto Nacional</b>	III	45 b	101 b	89 c	299 c	298 d	6 a	100 b
<b>Flor de mayo RMC</b>	III	40 c	102 b	90 c	253 b	356 c	5 a	71b
<b>Bayo 400</b>	III	40 c	102 b	89 c	300 b	297 d	5 a	39 e
<b>Cacahuate 72</b>	I	38 c	90 c	72 d	360 a	200 e	5 a	59 d
<b>Bayomex</b>	I	37 c	93 c	66 d	304 b	217 d	5 a	43 e
<b>Mean</b>		44	101	100	265	415	6	80
<b>Tukey 0.05</b>		4	7	15	30	42	2	10

Within columns values with similar letters are statistically equal. F = flowering; PM = physiological maturity; GY = grain yield; GS = grain size; GN = number of grains; GP = grains per pod and PN = number of pods.

## CONCLUSIONS

Type II cultivars, and in particular Negro Chiapas, presented the highest yield, followed by type III. The lowest corresponded to type II cultivars. The changes in yield presented a high relationship with the days to physiological maturity and the number of grains, followed by the number of pods and the days to flowering.

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# YIELD COMPONENTS AS A FUNCTION OF POPULATION DENSITY OF BEANS OF DETERMINATE HABIT IN A WARM CLIMATE

<sup>1</sup>Escalante-Estrada José Alberto Salvador, <sup>2</sup>Escalante-Estrada Yolanda Isabel,  
<sup>1</sup>Cid Aguilar Carpio and <sup>3</sup>L. Enrique Escalante Estrada

<sup>1</sup>Posgrado en Botánica, Colegio de Postgraduados, Campus Montecillo, Carretera México-  
Texcoco Km 36.5, Montecillo Mpio de Texcoco Edo. de Méx., México; <sup>2</sup>Instituto de  
Investigación Científica, Área de Ciencias Naturales, Universidad Autónoma de Guerrero,  
Chilpancingo, Gro., México; <sup>3</sup>Colegio Superior Agropecuario del Estado de Guerrero.

## INTRODUCTION

The bean (*Phaseolus vulgaris* L.) is an important crop for the diet due to its nutritional and medicinal properties. In Mexico, 87% of the planted area is rainy, which makes it a crop highly dependent on weather conditions. The average yield of the Iguala Gro region is 0.91 g m<sup>-2</sup> (SIAP, 2021), which is not enough to supply local consumption. A strategy to increase yield is the management of plant population density (Escalante *et al.*, 2015). The objective of the research was to determine the effect of population density (PD) on biomass production, grain yield and its components in bean cultivars (*Phaseolus vulgaris* L.) of type I determinate growth habit in a warm climate.

## MATERIALS AND METHODS

The sowing of the cultivars (CV) Canario 107 and Cacahuate 72 with type I determinate growth habit was on July 15, 2017, in the rainy season in Iguala Gro with a warm climate (Aw0, García, 2005). The soil is clay loam, pH 8.4, MO 3.5% and assimilable N of 45 kg ha<sup>-1</sup>, under a randomized block design with four replications, two cultivars (larger plot) and four PD (plants m<sup>-2</sup>) (smaller plot) including: 4.16 (80 cm \* 30 cm); 8.3 (40 cm\*30 cm); 12.5 (40 cm\*20 cm) and 25 (40 cm\*10 cm). Days to emergence, flowering (F) and physiological maturity (PM) were recorded. At harvest (PM), biomass (TB, dry matter), grain yield (GY), grain size (GS), number of grains (GN) and number of pods (PN) and grains per pod (GP) were recorded. An analysis of variance and the comparison of mean (Tukey  $\alpha = 0.05$ ) were conducted with the SAS (version 9.2) statistical package.

## RESULTS AND DISCUSSION

The F and the PM were at 37 and 80 days for both CVs and the average minimum and maximum temperature during the cycle was 23 °C and 38 °C, respectively, and the sum of the pluvial precipitation was 750 mm. Except for PG, the remaining variables showed significant differences due to CV and PD. The TB, GY, GN, GS and PN were higher in Canario 107 in comparison with Cacahuate 72 (Table 1). The response to PD in TB, GS, GN and PN was of the quadratic type, where the highest values were found in the PD of 12.5 plants m<sup>-2</sup> (40 cm\*20 cm). Similar trends in Cacahuate72 beans in a temperate climate were reported by Escalante *et al.* (2015).



**Table 1.** Grain yield (GY) and its components in bean cultivars with type I determinate growth habit as a function of PD in Iguala Gro during the Summer of 2017.

Treatment	TB g m <sup>-2</sup>	GY g m <sup>-2</sup>	GN m <sup>-2</sup>	GS (g)	PN m <sup>-2</sup>	GP
<b>Cultivar</b>						
<b>Canario 107</b>	268 a	125 a	548 a	225 a	120 a	4.6
<b>Cacahuate 72</b>	219 b	102 b	409 b	249 b	91 b	4.5
<b>Media</b>	243	113	478	237	105	4.5
<b>Tukey 0.05</b>	40	19	50	20	20	1
<b>PD (plants m<sup>-2</sup>)</b>						
<b>4.16</b>	209 c	97 c	420 b	237 a	92 c	4.5
<b>8.3</b>	243 b	116 b	485 b	241 a	108 b	4.5
<b>12.5</b>	283 a	134 a	561 a	237 a	123 a	4.5
<b>25</b>	238 b	113 b	447 b	238 a	99 b	4.5
<b>Mean</b>	243	106	478	239	105	4.5
<b>Tukey 0.05</b>	30	15	52	20	15	1

In columns, values with similar letters are statistically equal according to Tukey,  $\alpha = 0.05$ .

## CONCLUSIONS

The days to flowering and physiological maturity did not show changes due to cultivar or population density. The cultivars under study showed differences in biomass, yield and its components and Canario 107 surpassed Cacahuate 72 in these attributes. The number of grains per pod was similar between cultivars. In both cultivars, biomass, yield and its components increased with increasing population density up to a maximum of 12.5 plants m<sup>-2</sup>. At higher densities, these attributes decreased. The number of grains per pod was not affected by changes in population density.

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## PINTO BEAN (*Phaseolus vulgaris* L.) PRODUCTION WITH ORGANIC FERTILIZATION IN NOMBRE DE DIOS, DURANGO, MÉXICO

Erika Cecilia Gamero-Posada, Marcelo Iván Hernández-Salinas, Sonia Valdez-Ortega  
Cynthia Adriana Nava-Berumen, Ixchel Abby Ortiz-Sánchez

Tecnológico Nacional del México campus Valle del Guadiana, km 22.5 Carretera Durango-México, Villa Montemorelos, Dgo., México. C. P. 34371. sonia.vo@vguadiana.tecnm.mx

**INTRODUCTION:** The cultivation of beans (*Phaseolus vulgaris* L.) has spread throughout the world and is considered part of the basic diet due to its nutritional contribution (24.7% protein, 69.4% carbohydrates and 1.7% lipids) (Lépiz and Ramírez, 2010). According to data from the Food and Agriculture Organization of the United Nations (FAO), in 2019 Mexico was the ninth largest producer of beans in the world with a total of 879,404 t (3% of world production). In Durango, from 2015 to 2019, a 228,000 ha area was planted with an average production of 103,000 t of grain; having an average yield under rainfed conditions of 460 kg ha<sup>-1</sup> (SIAP, 2020). The main factors that limit the production and nutritional quality of beans are climatological, soil fertility and the presence of pathogenic organisms that cause diseases and pests (Treviño and Rosas, 2013). To mitigate the above, chemical fertilizers and pesticides are applied, which cause negative effects on the environment and human health. To reduce these negative effects, the use of fertilizers of biological origin have been promoted using the microbial degradation and mineralization of organic remains from animals and vegetables (Monsalve et al., 2017). The objective was to evaluate the effects of organic fertilization on the production variables of Pinto Raramuri beans, in Nombre de Dios, Durango.

**MATERIALS AND METHODS:** The study was conducted in the Tuitán community of the Nombre de Dios municipality, Durango, Mexico. Pinto Raramuri beans were sown on an area of 2 ha with a precision seeder in the spring-summer 2021 cycle, leaving a distance of 10 cm between seeds and 76 cm between rows. The agronomic management was carried out in accordance with the Agricultural Technical Agenda of Durango and La Laguna (INIFAP, SAGARPA and COFUPRO, 2017) under rainfed conditions. Using a randomized block design, the response to 6 fertilization treatments was evaluated: magrolean fertilizer 100 L ha<sup>-1</sup> (Magro); magro fertilizer 100 L ha<sup>-1</sup> + foliar chemical fertilization with Bayfolan Forte® using an intermediate dose (Magro + ½ Chemical); commercial organic fertilizer NB-soil® 40 L ha<sup>-1</sup> (NB-soil); commercial organic fertilizer NB-soil® 40 L ha<sup>-1</sup> + foliar chemical fertilization with Bayfolan Forte® using an intermediate dose (NB-Soil + ½ Chemical); foliar chemical fertilization with Bayfolan Forte® at full dosage (2 L ha<sup>-1</sup>) (Chemical); and no fertilization (Control). The production variables evaluated were number of pods per plant, number of grains per pod, density (plants ha<sup>-1</sup>) and yield (t ha<sup>-1</sup>). An analysis of variance was performed on the data and a Tukey comparison test of means (alpha = 0.05) was performed using the statistical package InfoStat®.

**RESULTS AND DISCUSSION:** Foliar chemical fertilization generated the highest bean yield (3.47 t ha<sup>-1</sup>), together with the fertilization with Magro and a half dose of agrochemicals (3.26 t ha<sup>-1</sup>). The high yield obtained by these two treatments indicates a direct association with higher plant density (273,026.32 and 244,243.42 plants ha<sup>-1</sup>, respectively) and higher grain weight harvested in 0.76 m<sup>2</sup> (263.5 and 247.5 g), despite not being the treatments with the highest number of pods per plant (7.28 and 6.92) (Table 1). The rest of the organic treatments presented better yields than the unfertilized control (Table 1), and in the same way were associated with plant densities and grain

weight. NB-soil and NB-Soil + ½ Chemical produced the highest number of pods per plant (11.23 and 11.43), but their intermediate yields (2.11 and 2.25 t ha<sup>-1</sup>) were not associated with this variable. The number of grains per pod did not show a significant difference between the treatments.

**Table 1.** Raramuri Pinto bean production variables, in Nombre de Dios, Durango, for the 2021 spring-summer cycle.

Treatment	Variable				
	Pods per plant	Beans per pod	Grain weight in 0.76 m <sup>2</sup> (g)	Density (plants ha <sup>-1</sup> )	Yield (t ha <sup>-1</sup> )
Magro	9.12 b,c	4.01 a	141.00 b,c	232,456.14 a,b	1.86 b,c
Magro + ½ Chemical	6.92 c,d	4.00 a	247.50 a	244,243.42 a,b	3.26 a
NB-Soil	11.23 a,b	4.22 a	160.50 b	269,736.84 a	2.11 b
NB-Soil + ½ Chemical	11.43 a	3.87 a	171.00 b	212,171.05 b	2.25 b
Chemical	7.28 c,d	3.87 a	263.50 a	273,026.32 a	3.47 a
Control	6.87 d	3.99 a	94.50 c	166,666.67 c	1.24 c

<sup>a,b,c,d</sup> different literals in the same column denotes significant differences based on Tukey ( $P \leq 0.05$ ).

**CONCLUSIONS:** With the use of the organic fertilizers tested, either individually or in combination with intermediate doses of a chemical fertilizer, it is possible to increase the yield of Pinto Raramuri beans, compared to beans that are not fertilized and can even equal the yield obtained when complete chemical fertilization is used, as is the case with the treatment with Magro and an intermediate dose of chemical fertilizer.

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## SEED YIELD IN EARLY TO FULL-SEASON LIFE CYCLE IN COMMON BEAN BREEDING LINES SELECTED IN DURANGO, MÉXICO

Rigoberto Rosales-Serna<sup>1</sup>, Saúl Santana-Espinoza<sup>1</sup>, Ramón Garza-García<sup>2</sup>, and Cynthia Adriana Nava-Berumen<sup>3</sup>

<sup>1</sup>INIFAP – Campo Experimental Valle del Guadiana. Durango, Dgo., México. <sup>2</sup>INIFAP – Campo Experimental Valle de México, Coatlinchán, Texcoco, Edo. de Méx., México. <sup>3</sup>Tecnológico Nacional de México – Campus Instituto Tecnológico del Valle del Guadiana, Dgo., México.

**INTRODUCTION:** Irregular duration in growing season has been observed through the years in the state of Durango mainly caused by late frosts, changes in the start of the rainy season and the amount of rain and distribution patterns. The use of full-length life cycle common bean cultivars has been reinforced due to their high yield potential when late rains (October) are registered, also causing the grain discoloration in early to intermediate pinto bean cultivars. Trends for the selection of early to intermediate life cycle improved pinto bean cultivars has been practiced recently due to actual agrometeorological conditions, mainly observed under rainfed systems. The best fit in the available growing season and thus high yielding cultivar has been Pinto Saltillo, recommended to be sown before July 15<sup>th</sup>, but then, the grain quality is damaged by the late rains. Improved pinto germplasm has been developed by using multiple and diverse parents to obtain intermediate to full-season cultivars with higher yield and premium seed quality. Improved common bean lines need to be evaluated to determine yield potential, commercial seed quality and maturity under the actual growing season. The objective was to evaluate seed yield for early to full-season life cycle pinto common bean lines selected in Durango, México.

**MATERIALS AND METHODS:** Improved pinto lines (510) were sown in a field trial established under irrigation in Durango, México. The Pinto Saltillo cultivar was included as a commercial check. Improved lines were sown on June 29<sup>th</sup>, 2021, in one row 5 m in length and 0.81 m apart. Fertilizer was incorporated into the soil at the rate of 35-50-00 (for N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O). Irrigation was applied once to avoid severe water stress in plants and insecticide (Dimethoate) was applied three times to control the bean beetle (*Epilachna varivestis*) and the bean pod weevil (*Apion* sp.). At maturity, plant samples were taken in each improved line for seed yield determinations. Plant samples consisted of one row 4 m in length by 0.80 m in width (3.24 m<sup>2</sup>). Days to first flower and physiological maturity (CIAT, 1987) were registered as days after sowing (DAS), as well as disease response, seed yield and 100 seed weight. The analysis of the results was obtained by using descriptive statistics (mean).

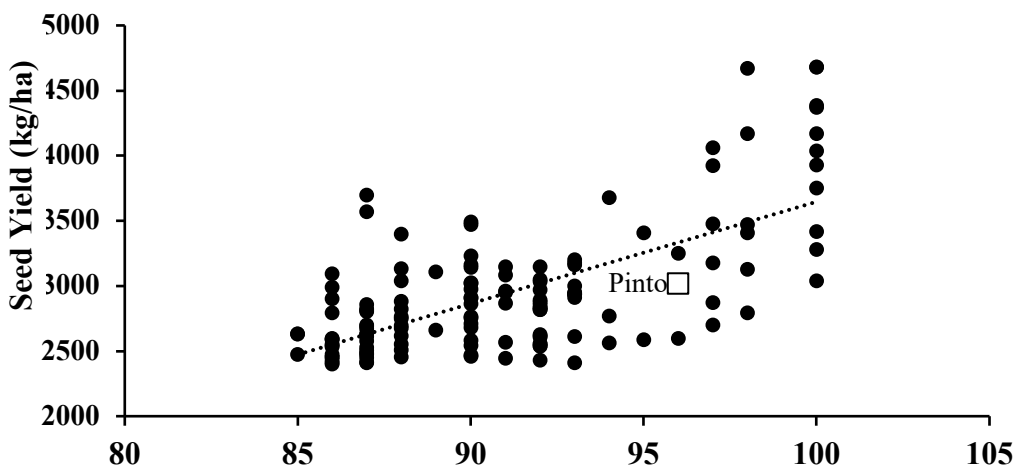
**RESULTS AND DISCUSSION:** The amount of accumulated rain in the 2021 growing period (786 mm) surpassed the historic local average (357 mm) (Medina *et al.*, 2005). Outstanding rain events were registered in October (65 mm) causing significant damage to seed quality in pinto bean cultivars sown in late June and early July. The number of days to flowering were higher in the selected pinto bean cultivars (44-45 DAS), compared to group average (39 DAS) and the check (40 DAS) (Table 1). Symptoms (1) of anthracnose and rust were not observed, while intermediate (4-5) symptoms were observed for common bacterial blight (CBB), which is the most frequent disease in Durango. Intermediate to full-season maturity was registered in pinto beans (97-100 DAS) compared to early (< 96 DAS) life cycle germplasm. High yielding improved lines had yields of 4,037 to 4,683 kg/ha, surpassing the check (3,010 kg/ha) and the group average (2,388 kg/ha). High seed yield observed at this location was due to the high amount of rain, irrigation water and soil fertility. Seed weight in all the lines (38.3 to 42.1 g/100 seeds) exceeded Pinto Saltillo,

enhancing the commercial acceptance of the improved germplasm. Trends in seed yield were observed in relation to germplasm life cycle (Figure 1). The genetic improvement of common bean in irrigated areas and the adoption of improved cultivars will contribute to a significant increment in seed yield and production stabilization, also reducing the use of water. Common bean is an useful plant species under irrigation and its cultivation increases yield, reduces the number of irrigations (compared to corn) and increases the economic benefits for farmers.

**Table 1.** Improved lines selected in the pinto common bean seed class. Durango, 2021.

Code	<sup>1</sup> DF	A	R	CBB	DM	Yield kg ha <sup>-1</sup>	100Seed Wt (g)
<sup>2</sup> PT21001	44	1	1	4	100	4683	41.0
PT21002	44	1	1	4	100	4683	40.8
PT21003	45	1	1	4	98	4673	40.3
PT21004	45	1	1	4	100	4387	41.8
PT21005	44	1	1	4	100	4375	42.1
PT21006	44	1	1	4	98	4172	41.1
PT20007	45	1	1	4	100	4171	38.2
PT20008	43	1	1	4	97	4063	39.2
PT20009	44	1	1	4	100	4037	40.5
PT Saltillo (check)	40	1	1	5	91	3010	33.2
Average	39				89	2388	34.6

<sup>1</sup>DF= days to first flower, A= antrachnose, R= rust, CBB = common bacterial blight, DM= days to physiological maturity; <sup>2</sup>PT = pinto seeds.



**Figure 1.** Influence of the days to maturity on the yield of improved common bean pinto lines, compared to the commercial check, under irrigated conditions in Durango, México.

**CONCLUSIONS:** Yield performance was related to the life cycle in selected pinto seeded common bean germplasm and high yielding lines showed intermediate to full-season life cycle.

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## YIELD AND GRAIN QUALITY IN NEW IMPROVED COMMON BEAN CULTIVARS GROWN IN COMMERCIAL PLOTS IN DURANGO

Rigoberto Rosales-Serna<sup>1\*</sup>, Saúl Santana-Espinoza<sup>1</sup>, Cynthia Adriana Nava-Berumen<sup>2</sup>, Ramón Garza-García<sup>3</sup> and Sonia Valdez-Ortega<sup>2</sup>

<sup>1</sup>INIFAP – Campo Experimental Valle del Guadiana, Dgo., México; <sup>2</sup>Tecnológico Nacional de México – Campus Instituto Tecnológico del Valle del Guadiana, Dgo., México; <sup>3</sup>INIFAP – Campo Experimental Valle de México, Coatlínchán, Texcoco, México.

**INTRODUCTION:** Common bean (*Phaseolus vulgaris* L.) is an important crop due to its multiple benefits on soil fertility, economic income for farmers, and nutritive traits, mainly related to the seed protein content (Yadav and Raverkar, 2021). Cooking time of the grain is another important trait for which lower values are preferred reducing gas and firewood use. PID 1 and NOD 1 are improved common bean cultivars released in 2020 mainly for irrigation areas in the state of Durango, México (Rosales *et al.*, 2020a; 2020b). Other high yielding improved lines (PT14053) have been developed and validation need to be performed in commercial plots to establish their adoption possibilities by producers and consumers. Opaque black common bean cultivars, such as NOD 1, Jamapa and Negro Michigan, are considered as preferable only for the export markets, as well as Negro San Luis the most popular full-season cultivar with black shiny seeds. Evaluation of cultivar adoption and preference is need to increase the efficiency of technology transfer programs. The objective was to evaluate yield and grain quality in some new improved common bean lines and cultivars grown in commercial plots at Durango, México.

**MATERIALS AND METHODS:** A commercial plot (1 ha) was established in Durango and La Soledad, in the state of Durango, México. Four cultivars (Pinto Saltillo, PID 1, NOD 1 and Negro San Luis) were sown on July 10<sup>th</sup> (Durango) and 13<sup>th</sup> 2020 (La Soledad). Two lines (PT14053 and PT14036) were also included in the Durango plot. Cultivars were sown in strips, consisting of 24 to 40 rows, 100 m in length and 0.81 m apart. Fertilizer was mechanically incorporated at the rate of 35-50-00 (for N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O) and foliar fertilizer was also sprayed during the flowering period and pod set. Irrigation was applied one to three times to avoid severe water stress in plants. Insecticide (Dimethoate) was sprayed twice for the control of common bean pod weevil (*Apion* sp.). Data was taken for seed yield, using five to six plant samples harvested in each cultivar. Plant samples consisted of two rows, 5 m in length by 0.81 m in width (8.1 m<sup>2</sup>). At the laboratory the content of protein (P), ash (A), crude fiber (CF), fat (F) and nitrogen free extract (NFE) was also determined according to the AOAC (1990) recommendations. For each cultivar/line twenty-five soaked seeds (18 h) were placed in duplicate on a Mattson cooker apparatus, cooked in boiling distilled water, and the average of 100% pin drop time was recorded as the cooking time. The analysis of variance was obtained by location using a completely randomized design with 3 to 6 replications and mean comparisons were performed using the Tukey's test ( $p \leq 0.05$ ), in both cases using the SAS ver. 9.4® computer program.

**RESULTS AND DISCUSSION:** Significant differences ( $p \leq 0.05$ ) were detected among cultivars for seed yield in Durango, and for seed content of protein, fiber and NFE in both locations (Table 1). The mean yield was higher in Durango (2,768 kg/ha) compared to La Soledad (1,080 kg/ha). The highest yield was observed in the improved line PT14053 (4,423 kg/ha) in Durango, while NOD 1 showed the highest yield in La Soledad (1,421 kg/ha). Improved lines (PT14053) derived from the crosses including multiple and diverse parents (PTBayacora/Maverick///PTClaro

/PTSalttillo//PTSalttillo/PTVilla-2-6) favored seed yield potential. This line will be released as the PID 2 cultivar for Northern México. Seed ash varied between 3.8 to 4.5% in Durango, and from 4.2 to 4.6% in La Soledad. All the cultivars reached statistically similar levels for mineral absorption related to the ash content. Protein content was higher in Durango (20.7%), and varied from 17.6% (PID 1) to 25.1% (Negro San Luis). In La Soledad protein content varied from 16.9% (PID 1) to 21.4% (Negro San Luis). This full-season cultivar Negro San Luis registered higher values for protein content and this trait was related to its full-length life cycle (115 to 125 days after sowing: DAS) favoring the accumulation of elaborated substances; while in early pinto cultivars (<96 DAS) high content of NFE was related to soluble carbohydrates.

Low values for fat were found ranging from 0.9 % to 2.3% in Durango and from 0.9 to 2.6% at La Soledad. Significant differences between cultivars for fat content was not found due to high levels of variation between replications. In Durango, most of the cultivars were statistically similar for fiber content with values from 2.7% to 3.5% and PID 1 showed the lowest value. In La Soledad, Pinto Saltillo had the highest value surpassing all the other cultivars. The highest proportion of the seed was observed for the nitrogen free extract, with values ranging from 67.3% (Negro San Luis) to 75.9% (PID 1) in Durango and from 68.8 (Pinto Saltillo) to 75.4% (PID 1) at La Soledad. Negro San Luis showed lower NFE values in both locations, mainly due to higher levels for protein content.

In Durango, cooking time varied from 60 min in PT14053 to 70 min in PID 1 and Negro San Luis; while in La Soledad it ranged from 60 min NOD 1 to 69 min (PID 1). High seed yield was observed in improved germplasm, specifically PT14053, which also showed low values for cooking time. Higher protein content and lower cooking time were observed for Negro San Luis, considered as a preferred landrace grown in the high-yielding areas of Durango and Zacatecas.

**Table 1.** Yield and seed quality in common bean lines and cultivars grown in Durango, México.

Cultivar/Line	Seed Yield	Ash	Protein	Fat	Fiber	<sup>1</sup> NFE	Cooking
Durango							
<sup>2</sup> PID 1	2873 <sup>bcd</sup>	3.8	17.6 <sup>b</sup>	0.9	1.9 <sup>b</sup>	75.9 <sup>a</sup>	70 <sup>a</sup>
PT14053	4223 <sup>a</sup>	4.1	20.5 <sup>ab</sup>	1.5	2.7 <sup>ab</sup>	71.2 <sup>ab</sup>	60 <sup>b</sup>
NOD 1	2084 <sup>e</sup>	4.5	21.4 <sup>ab</sup>	2.3	2.9 <sup>ab</sup>	68.9 <sup>ab</sup>	66 <sup>ab</sup>
PT14036	3185 <sup>bc</sup>	4.0	20.8 <sup>ab</sup>	1.0	2.7 <sup>ab</sup>	71.5 <sup>ab</sup>	67 <sup>ab</sup>
Negro San Luis	1891 <sup>e</sup>	3.9	25.1 <sup>a</sup>	0.9	2.9 <sup>ab</sup>	67.3 <sup>b</sup>	70 <sup>a</sup>
PT Saltillo	2883 <sup>bcd</sup>	4.0	18.9 <sup>b</sup>	1.2	3.5 <sup>a</sup>	72.4 <sup>ab</sup>	66 <sup>ab</sup>
<b>Average</b>	<b>2768</b>	<b>4.1</b>	<b>20.7</b>	<b>1.3</b>	<b>2.9</b>	<b>69.9</b>	<b>67</b>
La Soledad							
PID 1	729	4.3	16.9 <sup>b</sup>	0.9	2.6 <sup>b</sup>	75.4 <sup>a</sup>	69
NOD 1	1421	4.6	18.7 <sup>ab</sup>	1.5	3.0 <sup>b</sup>	72.2 <sup>ab</sup>	60
Negro San Luis	1194	4.6	21.4 <sup>a</sup>	1.4	3.0 <sup>b</sup>	69.7 <sup>b</sup>	62
PT Saltillo	976	4.2	19.1 <sup>ab</sup>	2.6	5.4 <sup>a</sup>	68.8 <sup>b</sup>	62
<b>Average</b>	<b>1080</b>	<b>4.4</b>	<b>19.0</b>	<b>1.6</b>	<b>3.5</b>	<b>71.5</b>	<b>63</b>

<sup>1</sup>NFE = nitrogen free extract, <sup>2</sup>PT = pinto.

**CONCLUSIONS:** A massive evaluation program of improved lines is required to establish adaptability and impact on seed yield, lower cooking time and market quality in Northern México. Negro San Luis is an important landrace showing high seed yield, seed quality and nutritional quality.

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## STORABILITY OF COMMON BEAN SEEDS IN SOUTHERN GHANA

Emmanuel Asamoah Adjei<sup>1</sup>, Maxwell Lamptey<sup>1</sup>, James Asibuo<sup>1</sup>, Stephen Yeboah<sup>1</sup>, David Appiah-Kubi<sup>1</sup>, Patricia Amankwaa-Yeboah<sup>1</sup>, Boaz Waswa<sup>2</sup> and Louis Butare<sup>2</sup>

<sup>1</sup>CSIR-Crops Research Institute, P. O. Box 3785, Kumasi, Ghana

<sup>2</sup>CIAT-Pan Africa Bean Research Alliance (PABRA), Nairobi, Kenya

Correspondence; proyeboah@yahoo.co.uk

### INTRODUCTION

Physiological, biochemical and health changes occur during seed storage, and the rate at which the process takes place depends on the ability of the seed to resist degradation and protection mechanisms, which are specific for each plant species (Shaban, 2013). The major aim of seed storage is to preserve the initial seed qualities for subsequent planting, and the conditions of the storage environment affect these qualities (Hendges *et al.*, 2017). Effective management of storage conditions namely temperature, relative humidity (RH) and oxygen are fundamental to preserve physiological, biochemical and health qualities of seeds and to promote longevity. Usually, bean seeds stored in inappropriate conditions, packaging materials and moisture content result in qualitative and quantitative losses. In the Ashanti region, which is in southern Ghana, the weather conditions are not conducive for seed storage, especially for legume seeds due to high temperature and relative humidity (Asiedu *et al.*, 2005). This challenge in the cultivation of legumes for seed results in scarcity and high price for good quality seeds. Thus, there was a need to determine how to manage the seed quality in storage for short to medium term storage periods. Research was conducted to determine the appropriate seed moisture content, packaging material and storage conditions to maintain the initial seed quality over an eight-month period.

### MATERIALS AND METHODS

Seeds were dried to 11 and 8% moisture contents, packaged in plastic containers and polythene bags, and stored under ambient and cold storage conditions. Seeds were sampled after 8 months for physiological, biochemical and health analyses.

### RESULTS AND DISCUSSION

Results on the physiological quality in this research effort over the period showed that the germination percentage ranged from 78-85%, TSW from 22-30 g, root length from 9.4-12.3 cm, shoot length from 22-27 cm, seedling vigour II from 260-430, and tetrazolium test from 93-100%. For biochemical quality the seed contained 4.1-4.9% ash, 202-281 EC, 2080-2879 ppm K, 10.3-12.7% MC, 229-296 ppm P, and 23.4-25.1% protein. The predominant fungi species included *Aspergillus* spp., *Rhizopus* spp., *Cladospermum* spp., and *Penicillium* spp.



Figure 1. Germination Test of common bean seeds



Seeds of common bean variety *Nsroma* can be dried to 11% moisture content, packaged in 0.2 mm polythene bags and stored under ambient storage conditions to maintain good seed quality for an 8-month period in the Ashanti region. This recommendation is economical compared to storage under cold conditions that involves high electricity and facility maintenance costs.

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# RESPONSE OF TEPARY BEAN BREEDING LINES AND ENTRIES OF THE TEPARY DIVERSITY PANEL (TDP) WHEN INFESTED WITH THE COMMON BEAN WEEVIL (*ACANTHOSCELIDES OBTECTUS*)

Timothy G. Porch<sup>1</sup> and James S. Beaver<sup>2</sup>

<sup>1</sup>USDA-ARS Tropical Agriculture Research Station, 2200 P.A. Campos Ave, Suite 201, Mayagüez, PR 00680; <sup>2</sup>Agricultural Experiment Station, Univ. of Puerto Rico, P.O. Box 9000, Mayagüez, Puerto Rico 00681-9000.

**INTRODUCTION:** In the Tropics, the common bean weevil (*Acanthoscelides obtectus* [Say]) can cause significant postharvest losses to the common bean (*Phaseolus vulgaris* L.). The wild tepary bean (*Phaseolus acutifolius* L.) germplasm accession G40199 has been successfully used to develop common bean breeding lines with enhanced levels of resistance to this pest (Kusolwa et al., 2016). The objective of this research was to screen the Tepary Diversity Panel (TDP) for reaction to infestation with the common bean weevil to identify additional sources of resistance.

**MATERIALS AND METHODS:** Thirty-four cultivated and 122 wild tepary bean genotypes from the TDP were screened for resistance. Twenty adult weevils were placed in 118 cm<sup>3</sup> plastic containers containing 20 seeds of each genotype. Ten adults were used in containers having fewer seed. The tepary bean breeding lines TARS-Tep 23, Tep 90 and Tep 93 were included as susceptible checks. At 60 d after infestation (DAI), the number of seed with perforations and the number of soft seed were counted. Initial seed weights and seed weights at 60 DAI were measured after the second evaluation to calculate percentage seed weight loss. The first and second evaluations were conducted during the summer and fall months of 2021.

**RESULTS AND DISCUSSION:** All the cultivated tepary bean genotypes and breeding lines were susceptible to common bean weevil damage at 60 DAI. Only seven wild genotypes had ≤ 10% damaged or soft seed for both the first and second evaluations (Table 1). Percentages of loss of initial seed weights ranged from 0.0 to 3.0% for the resistant wild tepary bean genotypes. These results suggest that domestication of tepary bean may have resulted in the loss of valuable genetic diversity for bruchid resistance. Four of the resistant genotypes were collected in the United States, two were collected in Mexico and the source of one is unknown. Although Shade et al. (1987) reported that PI 310803 was resistant to the common bean weevil, as expressed by a longer development time, it was not resistant in this study. Correlations from the second evaluation between the traits used to measure weevil damage were positive and highly significant for the number of visibly damaged and the number of soft seed (0.92) and correlations between % initial seed weight loss and numbers of both soft and damaged seed were 0.89. Hundred seed weights of the resistant wild genotypes were low, ranging from 1.5 to 7.1 g (Table 1). Nevertheless, there were numerous wild genotypes within that range of seed types that were susceptible to the common bean weevil. The recent identification of bridging-parents should facilitate interspecific crosses and accelerate the introgression of valuable traits such as enhanced levels of common bean weevil resistance from tepary into common bean (Barrera et al., 2020).

**Table 1.** Response of tepary bean breeding lines and entries of the Tepary Diversity Panel (TDP) when infested with the common bean weevil *Acanthoscelides obtectus*

TDP #	ID, Type, County of origin	First evaluation			Second evaluation				
		60 d # soft seeds	60 d # damaged seeds	Total # seeds	60 d # soft seeds	60 d # damaged seeds	Total # seeds	100 seed weight (g)	% loss of initial weight
61	G40078, Wild, Texas	1	0	20	0	0	20	2.4	0.2
68	G40087, Wild, Durango, Mexico	0	0	20	0	0	20	7.1	3.0
173	G40196, Wild, Texas	1	1	20	0	1	20	6.4	0.0
194	G40214, Wild, Arizona	2	0	20	0	0	20	4.3	8.1
235	G40253A, Wild, Mexico	0	0	20	0	0	20	3.3	0.2
334	PI 661753, Wild, Unknown	1	0	20	0	0	10	1.5	0.0
341	PI 640989, Wild, Arizona	2	0	20	1	0	20	3.1	0.0
372	PI 310803, Cultivated, Nicaragua	6	6	10	20	18	20	18.7	57.8
318	Bawi, PI 440799, Cultivated, Arizona	9	9	10	20	18	20	21.1	53.4
312	TARS-Tep 23 <sup>1</sup> Breeding line	20	20	20	20	9	20	20.6	40.0
NA	TARS-Tep 90 Breeding line	20	20	20	20	12	20	22.0	46.4
NA	TARS-Tep 93 Breeding line	20	20	20	20	5	20	16.6	49.0

<sup>1</sup> Porch et al. (2021) Release of tepary bean TARS-Tep 23 germplasm with broad abiotic stress tolerance and rust and common bacterial blight resistance. *J. Plant Reg.* 16:109-119.

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## 2022 MEMBERSHIP LIST

Director of Research (Bean Research Group)  
Alemaya Univ. of Agriculture  
P.O. Box 138  
Dire Dawa  
ETHIOPIA

Mirka Maily Acevedo Romero  
Andador Playa Roqueta # 316 Fracc.  
Canelas Durango, Dgo.  
MEXICO  
6181223389  
mirkamar.itvg@hotmail.com

Jorge A. Acosta Gallegos  
Cerro del aire 127  
Col. Colinas del Climatarario  
Queretaro, Queretaro 76090  
MEXICO  
461-661-5023 ext. 164

Gabriel Aimé Diasso  
Institute de L'Environnement  
Et de Recherches Agricoles - INERA  
01 BP 476, Ouagadougou 01  
BURKINA FASO  
diassogabriel@yahoo.fr

Manuel Amane  
National Bean Program Coordinator  
IIAM  
AV. FPLM 2698, Maputo  
MOZAMBIQUE

Berhanu Amsalu Fenta  
Bean Coordinator, EIAR  
Melkassa Research Center  
P.O. Box 436, Adama  
ETHIOPIA  
berhanufenta@gmail.com

Chad Anderson  
North Dakota Crop Improvement and  
Seed Association  
chad@ndcropimprovement.com

Hery Andriamazaoro  
Chief, Programme Legumineuses  
DRA FOFIFA  
B.P. 1444 Ambatobe, Antananarivo  
101  
MADAGASCAR  
hery.andriamazaoro@gmail.com;

Rodrigo Anschau  
Univerisidade Estadual de Londrina  
Londrina - Parana 86020-000  
BRAZIL  
55 43 99619-6411  
rodrigo\_anschau@hotmail.com

Gene Arganosa  
University of Saskatchewan  
gene.arganosa@usask.ca

Demerson Arruda Sanglard  
ICA - UFMG  
Avenida Universitaria 1.000 Bairro  
Universitario  
Montes Claros - MG- CEP 39.404-457  
BRAZIL  
sanglard@ufmg.br

James Asibuo  
Senior Research Scientist  
CSIR - Crops Research Institute  
P.O. Box 3785, Kumasi  
GHANA  
jyasibuo@gmail.com

Ahsan Asif  
Chickpea Breeding Australia,  
Department of Primary Industries,  
NSW, Australia  
ahsan.asif@dpi.nsw.gov.au

Ignacio Aspiazú  
Av, Reinaldo Viana 2630 - Bico da  
Pedra  
Janaúba - MG - 39440-000  
BRAZIL  
ignacio.aspiazu@unimontes.br

Fabio Aurelio dias Martins  
Santa Rita 153  
Jardin Gloria  
Lavras - MG 37200-000  
BRAZIL  
fabioaureliod@gmail.com

Ömer Avican  
May Seed Company  
omer.avican@may.com.tr

Parthiba M. Balasubramanian  
Agriculture & Agri- Food Canada  
Lethbridge Research Centre  
5403 – 1 Ave., S. PO Box 3000  
Lethbridge, Alberta T1J 4B1  
CANADA  
Parthiba.Balasubramanian@AGR.GC.  
CA

Scott Bales  
9923 Krueger Rd.  
Frankemuth, MI  
48734, USA  
balessco@msu.edu

Marco Barelli  
Dept. of Agronomy  
Cidade Universitária, Campus de  
Cáceres  
Av. Santos Dumont, Cáceres - MG  
CEP 78-200  
mbarelli@unemat.br

Abdullah Al Bari  
North Dakota State University  
md.bari@ndsu.edu

Amber Bassett  
1066 Bogue St., RM A364  
East Lansing, MI 48824  
(865)384-9657  
basset31@msu.edu

Messias Jose Bastos de Andrade  
Departamento de Agricultura  
Universidade Federal de Lavras  
Cx. P. 3037, CEP 37200-000 Lavras-  
MG  
BRAZIL  
mandrade@ufla.br

Vanet Batista de Souza  
Osvaldo Cruz St, 603  
Maringa  
BRAZIL  
55 44 997588482  
vanetbatista@yahoo.com.br

Bean Research Group  
Awassa Research Center  
P. O. Box 6  
Awassa  
ETHIOPIA

Bean Research Group  
KARLO-EMBU  
Regional Research Center  
P.O. Box 27 , EMBU  
KENYA

Bean Research Group  
KARI-Katumani  
Dryland Farming Research Center  
P.O. Box 340, Machakos  
KENYA  
karanjadr@yahoo.com

James S. Beaver  
Dept. of Crop and Agro-Environmental  
Univ. of Puerto Rico, Mayaguez  
P.O. Box 9000, Mayaguez, PR 00681  
PUERTO RICO  
320-200-8787  
j\_beaver@hotmail.com

Steve Beebe  
CIAT  
7343 NW 79th Terrace  
Medley, FL 33166-2211  
650-833-6625  
s.beebe@cgiar.org

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Casper Beneke  
P.O. Box 14466  
Bredell, Kempton Park, Gauteng 14366  
SOUTH AFRICA  
casper@starkeyres.co.za

Tchabana Bere  
Institute Togolais de Research  
Agronomique  
ITRA / CRASS  
P, O, Box 129, Kara  
TOGO  
beretchabana@gmail.com;  
tchabanab@yahoo.fr

Shannon Berndt  
Northern Pulse Growers Association  
berndt@northernpulse.com

Erika Berghauer  
Seminis Vegetable Seed  
7202 PORTAGE ROAD  
DEFOREST, WI 53532  
(608) 842-1435  
erika.mary.berghauer@monsanto.com

Kirstin Bett  
Dept. of Plant Sciences  
University of Saskatchewan  
51 Campus Dr. Saskatoon, SK S7N  
5A8  
CANADA  
306-966-4947  
k.bett@usask.ca

Gilberto Bevilaqua  
Embrapa CPACT  
Pelotas - RS 96010-280  
BRAZIL  
gilberto.bevilaqua@embrapa.br

Harbans Bhardwaj  
AGRIL. Research Station  
BOX 9061  
Virginia State University,  
Petersburg, VA 23896  
HBHARDWJ@VSU.EDU

Papias Binagwa  
P.O. Box 6024  
Arusha  
TANZANIA  
hongera1984@yahoo.com

Mariana Vaz Bisneta  
Universidade Estadual de Maringá,  
Departamento de Agronomia, Maringá,  
Brazil  
marianavazbisneta@hotmail.com

Fred A. Bliss  
214 Inca Pl.  
Davis, CA 95616  
530-756-5154  
Fbliss@dcn.org

Jeffrey Boersma  
U112, 3912-77th Avenue  
Leduc, Alberta T9E 0B6  
CANADA  
jeff97boersma@yahoo.com.au

Tayah Bolt  
Plant Sciences  
UC Davis  
tmbolt@ucdavis.edu

Joao Bosco dos Santos  
Departamento de Biologia  
UFLA, C.P. 3037  
CEP 37200-000 Lavras-MG  
BRAZIL  
35 3829 1357  
jbsantos@dbi.ufla.br

Sandra Branham  
1025 Birchdale Drive  
Charleston, SC 29412  
sebranh@clemson.edu

Mark A. Brick  
Dept. of Soil & Crop Sciences  
Colorado State University  
Fort Collins, CO 80524  
970-491-6551

Robin Buruchara  
CIAT  
c/o ICIPE, Duduville Complex  
Off Kasarani Rd, P.O. Box 823-00621,  
Regards, Nairobi  
KENYA  
r.buruchara@cgiar.org

Louis Butare  
CSIR-Crops Research Institute  
P.O. Box 3785, Kumasi  
GHANA  
l.butare@cgiar.org

Ana Campa Negrillo  
SERIDA  
Apdo.13, 33300 Villaviciosa  
SPAIN  
acampa@serida.org

Jacqueline Campbell  
2604 Stange Road, Apt 3  
Ames, IA 50010  
515-441-2857  
jdjax@iastate.edu

Steve Cannon  
1017 Crop Genome Informatics Lab  
Wallace RD  
Iowa State University, Ames, IA  
50010  
515-294-6971  
steven.cannon@usda.gov

Carolyn Caron  
University of Saskatchewan  
carolyn.caron@usask.ca

Ivon Cerda-Hurtado  
Centro de Biotecnologia  
Genomica-IPN  
Blvd. Del Maestro esq. Elias Pina  
Col. Narcisco Mendoza,  
88710 Reynosa, Tamaulipa  
MEXICO

Simon Chang  
Syngenta Seeds, Inc.  
6338 HWY 20 - 26  
Nampa, ID 83687  
208-465-8538  
simon\_jc.chang@syngenta.com

Syama Chatterton  
Lethbridge Research Center AAFC  
5403 1 Ave South  
POB 3000 Lethbridge, AB T1J 4B1  
CANADA  
403-317-2226  
Syama.Chatterton@agr.gc.ca

Antonio Chicapa Dovala  
National Bean Coordinator  
Instituto de Investigacao Agronomica  
11ª AV. Deolinda Rodrigues KM5,  
C.P. 2104,  
Luanda, ANGOLA  
achicapa@hotmail.com

Chief  
Programme Haricot  
ISABU  
B.P. 795, Bujumbura  
BURUNDI  
nduweric2003@gmail.com

Rowland Chirwa  
Coordinator, SABRN  
Chitedze Res. Stat.  
P. O. Box 158, Lilongwe  
MALAWI  
781-182-76722  
r.chirwa@cgiar.org

Virginia Chisale  
National Bean Coordinator - DARS  
Chitedze Res. Stat.  
P. O. Box 158, Lilongwe  
MALAWI  
vchisale@yahoo.com

Karen Cichy  
USDA-ARS  
434 Plant & Soil Sciences Bldg.  
Michigan State University, East Lansing,  
MI 48824-1325  
517-355-0271x210  
karen.cichy@usda.gov

Jean Claude Rubyogo  
CIAT Regional Bean Programme  
P.O. Box 2704, Arusha  
TANZANIA  
j.c.rubyogo@cgiar.org

Cornell University Library  
110 Olin Library  
Ithaca, NY 14853

Henry Cordoba Novoa  
McGill University  
henry.cordoba@mail.mcgill.ca

Paul Cornwell  
Hensall District Cooperative  
Ontario, Canada  
pcornwell@hdc.on.ca

Caio Correa  
University of Guelph  
caio@uoguelph.ca

Stephanie Cosme Reyes  
USDA-ARS-TARS  
Mayaguez, Puerto Rico  
stephanie.cosme@usda.gov

Vicky Crone  
USDA National Agric. Library  
Current Serial Records, Room 002  
10301 Baltimore Ave., Beltsville, MD  
20705  
301-504-5210  
vicky.crone@usda.gov

Fernando da Silva Rocha  
Institute de Ciencias Agrarias - ICA  
UFMG Campus Regional de  
Montes Claros  
Avenida Universitaria 1.00  
Montes Claros - MG 39404-547  
BRAZIL  
rochafspant@yahoo.com.br

Helene Davidson  
Laney.Davidson@dpi.nsw.gov.au

Régis de Araujo Pinheiro  
Rua Uruguay 1888 apartamento 3  
Pelotas - RS 96010-630  
BRAZIL  
regispinheiroagro@gmail.com

Carlos Alessandro de Freitas  
431, Dr. José Adriano Arrobas Martins Ave,  
Jardim Nova Aparecida  
Jaboticabal-SP, 14883-300  
BRAZIL  
carloscaf77@gmail.com

Marcelo Mueller de Freitas  
40 Vitorio st. apt 204  
Jaboticabal-SP, 14883-360  
BRAZIL  
5.5169819559e+012  
freitasmm@hotmail.com

Trazilbo Jose de Paula, Jr.  
EPAMIG  
Vila Gianetti 47  
Vicosa, MG 36570-000  
BRAZIL  
trazilbo@gmail.com

Antonio M. de Ron Pedreira  
Mision Biologica de Galicia  
El Palacio-Salcedo  
SPAIN  
34-986-854800  
amderon@mbg.csic.es



Maria Jose del Peloso  
Doutora em Genetica e Melh.  
de Feijao Comum  
EMBRAPA Arroz e Feijao  
C. P. 179 75 375-000  
Santo Antonio De Goias, BRAZIL  
mariajose.peloso@embrapa.br

Daniel G. Debouck  
CIAT  
7343 NW 79th Terrace  
Medley, FL 33166-2211  
650-833-6625  
danieldebouck@outlook.com

Jessica Delfini  
Agronomic Institute of Paraná  
Londrina, Paraná  
BRAZIL  
jessica\_delfini@hotmail.com

David DeYoung  
Michigan State University  
deyoun59@msu.edu

Fábio Aurélio Dias Martins  
Rua Santa Rita, 153  
Barrio Jardim Glória  
Lavras-MG 37200-000  
BRAZIL  
fabioaureliod@gmail.com

Patern Diatta  
Institut Senegalais de  
Recherches Agricoles  
ISRA-CDH  
P.O. Box 3120, Dakar  
SENEGAL  
djilessso@yahoo.fr

Christine Diepenbrock  
Plant Sciences  
UC Davis  
chdiepenbrock@ucdavis.edu

Siba I. Dopavogui  
Legumineuses Alimentaires Institut  
De recherche Agronomique de Guinee  
IRAG CRA-K, P.O Box 1523, 163  
Kindia-Guinee  
Guinea Conakry  
siba1dop@yahoo.fr

José dos Santos Neto  
Agronomic Institute of Paraná -IAPAR  
Area of breeding and plant genetics  
Rod. Celso Garcia Cid, km 375.  
Londrina, Paraná BRAZIL  
(51) 43 998075063/ 43 33762495  
js.neto@iapar.br

Robert Duncan  
Dept. of Plant Science  
222 Agriculture Bldg, 66 Dafoe Rd  
University of Manitoba Winnipeg,  
MB R3T 2N2  
CANADA  
202-474-6076  
duncanrw@cc.umanitoba.ca

Malaika Ebert  
North Dakota State University  
malaika.ebert@ndsu.edu

Gamal Elkheir Khalifa  
National Bean Coordinator  
ARC - Hudeiba Research Station Eldamer  
P.O. Box 31, Eldamer  
SUDAN  
gamalhrs@yahoo.com

Hayley Ellen Wilson  
hayley.wilson@dpi.nsw.gov.au

Emmalea Ernest  
University of Delaware  
Carvel Research & Education Center  
16483 County Seat Hwy, Georgetown,  
DE 19947  
302-856-7303  
emmalea@udel.edu

J. Alberto Escalante Estrada  
Colegio de Postgraduados  
Campus Montecillo  
km 36.5 Carretera, Montecillo,  
Mex 56230, MEXICO  
595-2-0247  
jasee@colpos.mx

Consuelo Estevez de Jensen  
Dept. of Crop and Agro-Environmental  
Sciences  
University of Puerto Rico  
consuelo.estevez@upr.edu

Sydney Everhart  
Department of Plant Pathology  
University of Nebraska  
Lincoln, NE 68583-0722  
(402) 472-2879  
everhart@unl.edu

Kathryne Everts  
27664 Nanticoke Rd.  
Salisbury, MD 21801  
410-742-8788  
keverts@umd.edu

Felipe Favoretto Furlan  
Universidade Estadual de Londrina  
Parana CEP 86200-000  
BRAZIL  
55 43 999730764

Juan Jose Ferreira  
SERIDA  
Apdo.13, 33300 Villaviciosa  
SPAIN  
34 985 890066  
jjferreira@serida.org

Anatercia Ferreira Alves  
Fitotecnia- Biotecnologia e  
Melhoramento de Plantas (UFV)  
Universidade Estadual do Maranhão-CESI  
Gurupi - Tocantins 77405-090  
BRAZIL  
anaterciaa@yahoo.com.br

Iraja Ferreira Antunes  
Embrapa Clima Temperado - C.  
Postal 403  
Pelotas, Rio Grande do Sul 96001-970  
BRAZIL  
53-275-8434  
iraja.antunes@embrapa.br

Eric Fedosejevs  
Agriculture and Agri-Food Canada  
eric.fedosejevs@agr.gc.ca

Allison Fletcher  
Saskatchewan Pulse Growers  
afletcher@saskpulse.com

Hilario Flores Gallardo  
Carretera Durango  
El Mezquital Km 4.5  
Durango CP34170  
MEXICO  
618 826 0433  
flores.hilario@inifap.gob.mx

Dimitri Fonseca  
North Dakota State University  
lakshan.fonseka@ndsu.edu

Deidre Fourie  
Dry Bean Producers Organization  
P. O. Box 15587  
Lynn East, 0039, Plot 20  
Zeekoegat  
Pretoria  
Tel: +27 12 819 8100  
deidre@beans.co.za

Thais Freitas Santos  
Rua Anicuns  
Quadra 02 Lote 08,  
Iporá, Goiás  
BRAZIL  
64 999035179  
thaisfreitassantos26@gmail.com

Carmen García-Fernandez  
SERIDA (Regional Agrifood Research  
and Development Service)  
cgarcia@serida.org

Valérie Geffroy  
Institut de Biologie des Plantes  
Université Paris Sud  
Bat 630  
FRANCE  
33 1 69 15 33 65  
valerie.geffroy@u-psud.fr

Dimitar Genchev  
Dobroudja Agricultural Institute  
9520  
General Tochevo  
BULGARIA  
359-58-653-234  
genchev@dai-gt.org;  
dd\_genchev@abv.bg

Fleur Geoghegan  
Kirkhouse Trust  
fleur.geoghegan@kirkhoustrust.org

Paul Gepts  
Dept. of Plant Sciences/MSI  
1 Shields Avenue  
University of California,  
Davis, CA 95616  
530-752-7743  
plgepts@ucdavis.edu

Thiago A.S. Gilio  
316 Lyric Lane  
Silver Springs, MD 20901  
thiago\_gilio@hotmail.com;  
thiago.sgilio@gmail.com

Manpartik Gill  
Plant Sciences Department  
Rothamsted Research  
manpartik.gill@rothamsted.ac.uk

Chris Gillard  
Ridgetown College  
120 Main St., E.  
University of Guelph Ridgetown,  
ON N0P 2C0, CANADA  
519-694-1632  
cgillard@ridgetownc.uoguelph.ca

Humberto Godoy Androcioli  
Celso Garcia Cid Road, Km 375  
Londrina, PR 86001-970  
BRAZIL  
55 43 3376-2298  
handrocioli@iapar.br

Deidrah Goldoff  
Harris Moran Seed Co.  
1677 Muller Road  
Sun Prairie, WI 53590  
608-772-9799

Francisco Gomez  
Department of Plant, Soil and  
Microbial Sciences  
Michigan State University  
gomezfr1@msu.edu

Maria Celeste Goncalves Vidigal  
Av. Colombo 5790-cep:87020-900  
Univ. Estadual de Maringa  
Maringa, Parana, 87020-900  
BRAZIL  
442635036  
mcgvidigal@uem.br

Juan Manuel Gonzalez Prieto  
Centro de Biotecnologia Genomica  
Instituto Politecnico Nacional  
Reynosa, Tamaulipas, CP 88710  
MEXICO

Raymond Glahn  
USDA-ARS  
Ithaca, NY  
raymond.glahn@usda.gov

Neroli Graham  
NSW Department of Primary Industry  
neroli.graham@dpi.nsw.gov.au

Michael Grusak  
USDA-ARS RRVARC  
1605 Albrecht Blvd N  
Fargo, ND 58102  
701-239-1371  
mike.grusak@usda.gov

Azalea Guerra Garcia  
University of Saskatchewan  
a.guerra@usask.ca

Lucas Haag  
Kansas State University  
lhaag@ksu.edu

Donna Harris  
University of Wyoming  
Donna.Harris@uwyo.edu

John Hart  
Plant Breeder  
EarthWork Seeds  
Cell/text: 1 (607) 342-7772  
jhart@earthworkseeds.com

Miranda J Haus  
Department of Horticulture  
Michigan State University  
hausmira@msu.edu

Jerry Haynes  
Jack's Bean Company LLC  
402 N. Interocean Ave  
Holyoke, CO 80734-1000  
970-854-3702  
office@jacksbean.com;  
jerry@jacksbean.com

Jim Heitholt  
Dept Plant Sciences - 3354  
1000 E University Ave  
University of Wyoming,  
Laramie, WY 82071  
307-766-3104  
jim.heitholt@uwyo.edu

Luiz Henrique Campos de Almeida  
Rua Alexander Graham Bell 560  
3701 Parue Jamaica  
Londrina - PR 86063-250  
BRAZIL  
caluizhenrique@msn.com

Sanjuana Hernandez-Delgado  
Instituto Politecnico Nacional  
Reynosa  
MEXICO  
shernandezd@ipn.mx

Becky Higgins  
Dept. of Plant Pathology  
1875 No. 38th, 406 PSH  
UN-L East Campus, Lincoln, NE  
68583-0722

Kristy Hobson  
New South Wales Department of  
Primary Industries  
Australia  
kristy.hobson@dpi.nsw.gov.au

Azize Homer  
1115 Reynolds St.  
Laramie, WY 82072  
307-742-5161  
ademirbas@hotmail.com

Claudia Canales Holzeis  
Kirkhouse Trust  
claudia.canales@kirkhoustrust.org

George L. Hosfield  
208 Artists Alley  
Blowing Rock, NC 28605-9615  
828-295-6727  
georgehosfield@bellsouth.net

Khwaja G Hossain  
SB 108  
330 3rd Street, NE  
Mayville State University, Mayville, ND  
58257  
701-788-4728  
k.hossain@mayvillestate.edu

Anfu Hou  
Unit 100-101  
Route 1Y5  
Morden, Manitoba R6M 1Y5  
CANADA  
204-822-7228  
houa@agr.gc.ca

Benjamin Hughey  
Pure Line Seeds, INC  
P.O. Box 746  
Warden, WA 98857  
608-438-2554  
bhughey@purelineseed.com

Jodi Humann  
Washington State University  
jhumann@wsu.edu

Oscar P. Hurtado  
USDA-APHIS  
Bld 580, BARC-EAST  
Beltsville, MD 20705  
ophurtado@gmail.com; oscar.hurtado-  
gonzales@usda.gov

Francisco Ibarra-Perez  
CE Cotaxtla, INIFAP  
Carretera Veracruz-Cordoba km 34.5  
Medellin de Bravo, Veracruz 94270  
MEXICO  
011 52 229 262 2233  
fcojip@hotmail.com

Molly Irvin  
Michigan State University  
Irvinmol@msu.edu

Maki Ilunga  
Southern D.R. Congo  
INERA Kipopo  
P.O. Box 224, Lubumbashi  
D.R. CONGO  
243 810727569  
ilunga.meschac@gmail.com

Ousseini Issaka Salia  
Washington State University  
o.issakasalia@wsu.edu

Shalu Jain  
Syngenta  
shalu.jain@syngenta.com

Hannah Jeffery  
Michigan State University  
jeffer90@msu.edu

Bosen Jia  
University of Ottawa  
bjia029@uottawa.ca

Magdalena Julkowska  
Boyce Thompson Institute  
Ithaca, NY  
mmj55@cornell.edu

Carmen Jacinto-Hernandez  
Tepetlaoxtoc Mna-5,  
L-2. Fracc. Lomas de Cristo,  
Texcoco, Estado de México. CP 56253  
MEXICO  
595-4-2877  
carmenj9@yahoo.com

María Jurado-Cañas  
Agri-Food Research and Development  
Regional Service (SERIDA)  
Asturias, Spain  
mjurado@serida.org

Jagroop Kahlon  
Alberta Pulse Growers  
jkahlon@albertapulse.com

Venugopal Kalavacharla  
205 Baker Annex  
Delaware State University  
1200 N DuPont Hwy, Dover, DE  
19901-2277  
302-857-6492  
vkalavacharla@desu.edu

Kelvin Kamfwa  
Department of Plant Science  
University of Zambia  
P.O. Box 32379, Lusaka  
ZAMBIA  
2.6097360256e+011  
kelvinkamfwa@gmail.com

Kris Kappenman  
ADM-Seedwest  
PO Box 1470, Decatur, IL 62525-1820  
217-451-4707  
kappenman@adm.com

Alexander Karasev  
University of Idaho  
Dept of PSES, AgSci Rm. 242  
875 Perimeter Dr. - 2339,  
Moscow, ID 83844-2339  
208-885-2350  
akarasev@uidaho.edu

Olga Khmel'nitsky  
Boyce Thompson Institute  
Ithaca, NY  
ok84@cornell.edu

Chris Kelley  
Kelley Bean Company  
1520 Ave "B"  
Scottsbluff, NE 69361  
308-633-7333  
ckelley@kelleybean.com

James D. Kelly  
1066 Bogue St  
Michigan State University  
East Lansing, MI 48824  
517-353-0169  
kellyj@msu.edu

Michael Kilango  
Min. of Agric. Research and  
Training Inst. (MARTI)  
Uyole  
P.O. Box 400, Mbeya  
TANZANIA  
michaelkilango@yahoo.com;  
michaelakilango@gmail.com

Paul Kimani  
Dept of Crop Science-Kabete  
University of Nairobi  
P. O. Box 30197, Nairobi  
KENYA  
pmkimani@uonbi.ac.ke

Ken Kmiecik  
714 Seneca Pl.  
Madison, WI 53711  
608-698-5198  
kakmiecik@sbcglobal.net

Josue Kohashi-Shibata  
Centro de Botanica. Col. De Postgrad  
Montecillo, Edo. De Mexico  
C.P. 56230  
MEXICO  
595-95-20200  
jkohashi@colpos.mx

George Korontzis  
Syngenta  
george.korontzis@syngenta.com

David Kramer  
DOE-Plant Research Laboratory  
S220 Plant Biology Building  
Michigan State University,  
East Lansing, MI 48824  
kramerd8@msu.edu

Paul Kuin  
Pop Vriend Seeds  
pkuin@popvriendseeds.nl

Paul Kusolwa  
Sokoine U. of Agriculture  
Department of Crop Science  
Tiba Road, P.O. Box 3005,  
Morogoro, Tanzania  
kusolwap@gmail.com

Nicholas Larkan  
Agriculture and Agri-Food Canada  
nicholas.larkan@agr.gc.ca

Jamie Larsen  
Agriculture and Agri-Food Canada  
jamie.larsen@agr.gc.ca

Calvin Lietzow  
HM. Clause  
calvin.lietzow@hmclause.com

Mylene Corzo Lopez  
University of Guelph  
corzolom@uoguelph.ca

Giovanni Lorenzo  
USDA-ARS-TARS  
Mayaguez, PR  
giovanni.lorenzo@usda.gov

Maike Lovatto  
Universidade Estadual de Maringá  
maikelovatto2@gmail.com

Regina Lucia Ferreira Gomes  
Rua Manoel Felicio de Carvalho  
1864, Ininga  
Teresina - PL 64-49-690  
BRAZIL  
86-3215-5754  
r.lfgomes@hotmail.com

Alice MacQueen  
University of Texas  
Austin, TX 78759  
alice.macqueen@gmail.com

Domenico Magnifico  
Tera Seeds SRL Cons.  
Via della Rotaia 4/5 47035  
Gambettola (FC), ITALY  
139-547653884  
dmagnifico@teraseeds.com

Nicholas Manana  
Malkens Research Station  
P.O. Box 4, Malkens  
SWAZILAND  
manananicho@yahoo.com

Puneet Mangat  
Washington State University  
puneet.mangat@wsu.edu

Bijula Mankara Sureshbabu  
bijula.mankarasureshbabu  
@jacks.sdstate.edu

Frédéric Marsolais  
Southern Crop Protection & Food  
Res Centre AAFC  
1391 Sandford St. London,  
ON N5V 4T3 CANADA  
519-953-6718  
Frederic.Marsolais@agr.gc.ca

Mark Massoudi  
AG BIOTECH INC.  
9701 Blue Larkspur Lane  
Suite A, Monterey, CA 93940  
831-324-0585  
info@agbiotech.net

Netzahualcoyotl Mayek-Perez  
Centro de Biotecnologia Genomica-IPN  
Blvd. Del Maestro esq. Elias Pina  
Col. Narcisco Mendoza, 88710 Reynosa,  
Tamaulipa, MEXICO  
nmayek@ipn.mx

Michael Mazourek  
248 Emerson Hall  
Cornell University  
Ithaca, NY 14853  
607-254-7256  
mm284@cornell.edu

Phil McClean  
Department of Plant Sciences,  
NDSU Dept # 7670  
PO Box 6050, 270B Loftsgard  
North Dakota State University,  
Fargo, ND 58108-6050  
701-231-8443  
phil.mcclean@gmail.com

Cirano Cruz Melville  
Universidade Estadual Paulista Jaboticabal,  
SP CEP: 14883-900  
BRAZIL  
ciranomelville@outlook.com

Phil Miklas  
USDA-ARS-IAREC  
24106 No. Bunn Road  
Washington State University,  
Prosser, WA 99350-9687  
509-786-9258  
phil.miklas@usda.gov

Wezi Mkwaila  
Dept of Horticulture  
LUANR  
P.O. Box 219, Lilongwe  
MALAWI  
265 0 998331376  
wezimkwaila@gmail.com

Vania Moda-Cirino  
IAPAR  
Rod. Celso Garcia Cid (PR-445), Km 375  
Londrina - Paraná  
BRAZIL  
+55 (43) 3376-2123  
vamoci@iapar.br

Odireleng Molosiwa  
Bean Research Coordinator  
DAR, P.B. 0033  
Content Farm Sebele, Gaborone  
BOTSWANA  
omolosiwa@gov.bw

Bertrand Monsimier  
Vilmorin-Mikado SAS  
Route Du Manoir  
49250 La Menitre  
FRANCE  
bertrand.monsimier@vilmorinmikado.com

Mario Morales  
North Dakota State University  
mario.morales@ndsu.edu

Emily Morneau  
Agriculture and Agri-Food Canada  
emily.morneau@agr.gc.ca

Kennedy Muimui  
Misamfu Regional Research Cntr.  
PO Box 410055  
Kasama  
ZAMBIA  
kmuimui04@yahoo.co.uk

Clare Mukankusi  
Country Coordinator CIAT  
Kawanda Agric. Research Institute  
P.O. Box 6247, Kampala  
Uganda  
c.mukankusi@cgiar.org

Augustine Musoni  
Chief, Programme Legumineuses  
RAB, Rubona  
B.P. 138, Butare  
RWANDA  
afmusoni2016@gmail.com

Bruce Mutari  
Bean Coordinator, Agron. Inst.  
Dept. of Research & Spec. Serv.  
PO Box CY-550, Causeway, Harare  
ZIMBABWE  
bruceMutari@gmail.com

James R. Myers  
Dept. of Horticulture, ALS 4017  
Oregon State University  
Corvallis, OR 97331  
541-737-3083  
myersja@hort.oregonstate.edu

National Bean Programme  
Coordinator  
Selian Agriculture Research  
Institute - SARI  
P.O. Box 6024, Arusha  
TANZANIA  
edithkadege@gmail.com

National Bean Coordinator  
C/O Ministry of Agriculture Forestry,  
Tourism, Animal Resources...  
Juba  
REPUBLIC OF SOUTH SUDAN

Felix Navarro  
Seneca Foods Corporation  
fnavarro@senecafoods.com

Susan Nchimbi-Msolla  
Dept. of Crop Science and  
Production  
Sokoine University of Agriculture  
P.O. Box 3005, Chuo Kikuu  
Moragoro, Tanzania  
nchimbi@suanet.ac.tz;  
smsolla@yahoo.com

Tim Neefjes  
Pop Vriend Seeds tneefjes@popvriendseeds.nl

Berlin Nelson  
Dept. of Plant Pathology #7660  
Walster Hall 306  
NDSU, Fargo, ND 58105-6050  
701-231-7057  
berlin.nelson@ndsu.edu

Martin Nguiguim  
Institut De Recherche Agricole  
Pour Le Developpement  
IRAD  
P.O. Box 2067, Yaounde  
CAMEROUN  
mnguiguim@gmail.com

Alessandro Nicoli  
Instituto de Ciencias Agrarias  
ICA / UFVJM  
Vereador João Narciso Avenue  
Unai 1380 - MG  
BRAZIL  
55 38 3677-9952  
agronicoli@yahoo.com

Ekta Ojha  
North Dakota State University  
ekta.ojha@ndsu.edu

Dâmiany Pádua Oliveira  
Rua Lasmar 116  
Vista Alegre  
Perdoes - Minas Gerais,37260-000  
BRAZIL  
damy\_agro84@hotmail.com;  
damiany.padua.oliveira@gmail.com

Marina Borges Oliveira Silva  
Rua Alfonso Pena  
São Gonçalo  
Janaúba MG 39440-000  
BRAZIL  
55 38 38211457  
mariunim@yahoo.com.br

Juan M. Osorno  
Dept. of Plant Science  
NDSU Dept. 7670, P.O. Box 6050  
North Dakota State University, Fargo, ND  
58108-6050  
701-231-8145  
juan.osorno@ndsu.edu

Travis Parker  
Plant Sciences  
University of California, Davis  
trparker@ucdavis.edu

Steve Noffsinger  
1246 Ivy St  
DeKalb, IL 60115  
334-737-1766  
steve.noffsinger@gmail.com

Barry Ogg  
Dept. of Soil & Crop Sciences  
Colorado State University  
Fort Collins, CO 80523-1170  
970-491-6354  
Barry.Ogg@colostate.edu

Sebastia Oliveira  
Rua Dona Inacia 171  
Lavras, MG 3700-000  
BRAZIL

Carla Olson  
Plant Germplasm Introduction and  
Testing Research Program  
USDA-ARS  
carla.olson@usda.gov

Esteban S. Osuna Ceja  
km 32.5 Carretera. Ags.-Zac.  
C.P. 20660 , A.P. 20  
Pabellon de Arteaga, Ags.  
MEXICO  
01495-65-8-01-67  
osuna.salvador@inifap.gob.mx

Talo Pastor-Corrales  
Soybean Genomics and Improvement  
Laboratory  
Bldg. 006, Room 118, BARC-West  
10300 Baltimore Ave,  
Beltsville, MD 20705  
301-504-6600  
talo.pastor-corrales@usda.gov

Luciano Nogueira  
Rodovio GO 330, km 241  
Ipameri GO 75780-000  
BRAZIL  
55 64993211556  
lucianonogueiraagro@gmail.com

Atena Oladzadabbasabadi  
Department of Plant Sciences, NDSU  
Dept # 7670  
PO Box 6050, Loftsgard  
North Dakota State U., Fargo, ND  
atena.oladzad@ndsu.edu

Eli Carlos Oliveira  
Rua Luiz Lercos, 399  
Ap # 705 Torre # 01  
Londrina – Paraná 86047 – 610  
BRAZIL  
55 43 9631 6040  
elioliveira.agro@gmail.com

Arie Oppelaar  
Monsanto Holland BV  
Wageningse Afweg 31  
NETHERLANDS  
31317468364  
arie.oppelaar@monsanto.com

James Palmer  
Michigan Crop Improvement Assoc.  
P.O. Box 21008  
Lansing, MI 48909  
517-332-3546  
palmerj@michcrop.com

Magno Antonio Patto Ramalho  
Dept. de Biologia - UFLA  
Cx. Pos. 3037  
37200-000 Lavras, M.G  
BRAZIL  
035-829-1352  
magnoapr@ufla.br



Peter Pauls  
44 James St W  
Guelph Ontario N1G 1E4  
CANADA  
ppauls@uoguelph.ca

R Varma Penmetza  
University of California, Davis  
rvpenmetza@ucdavis.edu

Waldo Penner  
Agriculture and Agri-Food Canada  
waldo.penner@agr.gc.ca

Georgina Penilley  
NSW Department of Primary Industries  
Australia  
georgina.pengilley@dpi.nsw.gov.au

Stéphanie Pflieger  
Université Paris-Saclay  
stephanie.pflieger@universite-paris-  
saclay.fr

Sherrilyn Phelps  
Saskatchewan Pulse Growers  
sphelps@saskpulse.com

Philip Pinheiro  
Kirkhouse Trust  
philip.pinheiro@kirkhoustrust.org

Alexis Plouy  
Crites Seed Inc.  
16500 Rd. 5 NW  
P.O. Box 8, Quincy, WA 98848  
208-329-0335

Tim Porch  
USDA-ARS-TARS  
2200 P.A. Campos Ave., Suite 201  
Mayaguez, PR 00680  
787-831-3435 x254  
timothy.porch@usda.gov

Bodo Raatz  
Limagrain Vegetable Seeds  
3 Rue de Manoir  
49250 la Menitre  
France  
+33 6 37 48 91 27

Waltram Ravelombola  
Texas A&M AgriLife Research  
waltram.ravelombola@ag.tamu.edu

John Rayapati  
ADM  
4666 Faries PKWY 050  
Decatur, IL 62526  
john.rayapati@adm.com

Guiherme Renato Gomes  
Rua Alexander Graham Bell 560  
Londrina CEP 86069-250  
BRAZIL  
55 43 984257925  
guilhermerenatogomes@hotmail.com

Jaap Reus  
Syngenta  
jaap.reus@syngenta.com

Otsuyula Reuben Masheti  
KARLO-Kakamega  
Regional Research Centre  
P.O. Box 169, Kakamega  
KENYA  
rmotsyula@yahoo.com

Hendrik Rietman  
Storm Seeds  
Heidebloemstraat 2-1  
Belgium  
31-6-1996-406  
hendrik@rietman.nu

Dale Risula  
Government of Saskatchewan  
Dale.risula@gov.sk.ca

Charlene Robast  
Vilmorin-Mikado SAS  
Route Du Manoir  
49250 La Menitre  
FRANCE  
02 4179 4179  
charlene.robast@vilmorinmikado.com

Maria Teresa Rodriguez Gonzalez  
Colegio de Postgraduados  
Campus Montecillo  
km 36.5 Carretera, Montecillo  
MPIO. De Texcoco 56230  
MEXICO  
01-595-95-20200  
mate@colpos.mx

Rigoberto Rosales Serna  
Encinos 158  
Residencial Los Pinos  
Durango, Dgo. Mex. 34162  
MEXICO  
rigoberto\_serna@yahoo.com

Janice M.W. Rueda  
ADM  
4666 Faries Parkway  
Decatur, IL 62526  
217-451-7722  
Janice.Rueda@adm.com

Ana Saballos  
University of Illinois  
saballos@illinois.edu

Gopesh C. Saha  
Brotherton Seed Company, INC.  
451 S. Milwaukee Ave  
Moses Lake, WA 98837  
509-750-2756  
gopesh@brothertonseed.com

Ehsan Sari  
Agriculture and Agri-Food Canada  
ehsan.sari@agr.gc.ca

Yakende Rodrigue Prosper  
Institut Centrafrican de Recherche  
Agriconomique  
ICRA  
P.O. Box 1762 , Bangui  
CENTRAL AFRICA REPUBLIC  
yakendero@yahoo.fr

Juan Carlos Rosas  
EAP/ZAMORANO  
Residencial La Hacienda,  
P.O. Box 93, Tegucigalpa,  
HONDURAS  
504-2287-2000 ext 2314  
jcrossas@zamorano.edu

Kijana Ruhebuza  
CIEF D'ANTENINE  
PNL/INERA MULUNGU (D.R. Congo)  
BP 327, Cyangugu  
RWANDA  
kijanaruhebuza@yahoo.fr

Rie Sadohara  
Michigan State University  
sadohara@msu.edu

Nubia Andrea Villota Salazar  
Instituto Politécnico Nacional, CBG  
Reynosa Tamps,  
MEXICO  
andreavillota17@yahoo.com.mx;  
nvillotas1000@alumno.ipn.mx

Madalyn Scanlan  
USDA-ARS  
East Lansing, Michigan  
madalyn.scanlan@usda.gov

Leticia Rodrigues Sousa  
Rua dos Imigrantes Q. 2 L. 10  
Bairra Mato Grosso Iporá- GO  
BRAZIL  
(+55) 064 9.9653-2770  
letyrodriques21@gmail.com

Jayanta Roy  
North Dakota State University  
jayanta.roy@ndsu.edu

Ivan A.  
Russkikh  
Belarus State University  
Nezavisimosti Prospect, 4  
220030 Minsk  
BELARUS  
375 447193920  
russkikh@bsu.by

Jeff Safe  
Crites Seed Inc.  
16500 Rd. 5 NW  
P.O. Box 8, Quincy, WA 98848  
509-787-1446  
jeff@critesseed.com

Helton Santos Pereira  
Rodovia GO-462 (Goiânia –  
Nova Veneza), km 12  
zona rural (Embrapa Arroz e Feijão)  
Santo Antônio de Goiás Goiás,  
75375-000, BRAZIL  
helton.pereira@embrapa.br

Jim Schild  
Scotts Bluff County Extension  
4502 Avenue I  
Scottsbluff, NE 69361  
308-632-1480  
Jschild1@unl.edu

Todd F Scholz  
USA Dry Pea & Lentil Council  
toddscholz@usapulses.org

Howard F. Schwartz  
C205 Plant Sciences  
Dept. of Bioagr. Sci. & Pest Mgmt.  
Colorado State University,  
Fort Collins, CO 80523-1177  
970-491-6987

Lesole Sefume  
Department of Agricultural Research -  
DAR  
P.O. Box 100, Maseru  
LESOTHO  
sefumelesole@gmail.com

Serials ACQ Dept.  
Iowa State University  
204 Parks Library  
Ames, IA 50011-2142

Serials Dept  
Penn State University  
126 Paterno Library  
University Park, PA 16802-1808

Luke Shellenberger  
Pro Vita  
PO Box 628  
Kuna, ID 83634  
208-463-7624  
ron@provita-inc.com

Matt Shellenberger  
Pro Vita  
PO Box 628  
Kuna, ID 83634  
208-463-7624  
Matt@Provita-Inc.com

Gerald Smith  
Texas A&M AgriLife Research  
g-smith@tamu.edu

Thomas H. Smith  
Crop Science Building  
University of Guelph  
50 Stone Rd. E. Guelph, ON, N1G 2W1  
CANADA  
519-824-4120 ext 58339  
thsmith@uoguelph.ca

Svetla Sofkova-Bobcheva  
Massey University  
Palmerston North  
NEW ZEALAND  
svetla.sofkova@gmail.com

Alvaro Soler-Garzon  
Prosser, Washington  
alvaro.solergarzon@wsu.edu;  
al.solerg@gmail.com

Claire Spickermann  
University of California, Davis  
cspickermann@ucdavis.edu

Fabricia Sousa Silva  
Avenida Rio XII, no. 742  
Iporá, Goiás  
BRAZIL  
64 99964-1558  
fabriciasosilva@outlook.com

Elaine Souza  
Departamento de Biologia  
UFLA, C.P. 3037  
CEP 37200-000 Lavras-MG  
BRAZIL  
35 3829 1354  
easouza@dbi.ufla.br

Maria da Conceição Martiniano  
de Souza  
Rua Capitão Rui Lucena 71  
Apto 903  
Boa Vista Recife PE 50070-080  
BRAZIL  
mariamartiniano@hotmail.com

Thiago Souza  
Embrapa Rice and Beans  
GO-462, km 12, Zona Rural  
Santo Antonio de Goiás, GO CEP:  
75375-000  
BRAZIL  
55 (62) 3533-2129  
thiago.souza@embrapa.br

Kathy Stewart-Williams  
Idaho Crop Improvement Association  
2283 Wright Ave, Suite C  
Twin Falls, ID 83303  
208-733-2468  
kathysw@idahocrop.com

Morgan Stone  
Clemson University  
mstone9@clemson.edu

Robert Stonehouse  
robstonehouse@gmail.com

Akiko Sugio  
INRAE - National Research Institute  
for Agriculture, Food and the  
Environment (France)  
akiko.sugio@inrae.fr

Hayley Sussman  
Boyce Thompson Institute  
Ithaca, NY  
hs995@cornell.edu

SUWECO CZ, s.r.o.  
Sestupna 153/11  
CZECH REPUBLIC  
poustkova@suweco.cz

John Theuws  
Kempenlaan 7  
B-3600 Genk  
BELGIUM  
32-89-85-2931  
johntheuws@telenet.be

Henry J. Thompson  
Colorado State University  
Cancer Prevention Lab  
1173 Campus Delivery,  
Fort Collins, CO 80523-1173  
970-491-7748  
henry.thompson@colostate.edu

Joseph Michel Tohme  
C I A T  
7343 NW 79th Terrace  
Medley, FL 33166-2211  
415-833-6625  
j.tohme@cgiar.org

Yohari Torres  
Dept. of Crop and Agro-  
Environmental Sciences  
University of Puerto Rico  
yohari.torres@upr.edu

Oscar H. Tosquy-Valle  
CE Cotaxtla, INIFAP  
Carretera Veracruz- Cordoba km 34.5  
Medellin de Bravo, Veracruz 94270  
MEXICO  
tosquy.oscar@inifap.gob.mx

Kadiatou Gamby Toure  
Fruits and Legumes Institute  
D'Economie Rurale IER CRRA BP 262  
Sotuba  
MALI  
kadidiatou5@yahoo.fr

Jennifer Trapp  
Opus Seed  
1532 11th Ave E  
Twin Falls, ID 83301  
509-521-5507  
molassesface@hotmail.com

Shing-Jy Tsao  
1184 Fynes Ct.  
San Jose, CA 95131  
408-332-1955  
jocelyn@ntu.edu.tw

Mark A. Uebersax  
2846 West Braden Road  
Perry, MI 48872  
517-204-2723  
uebersax@tds.net

Dr. Michael Ugen  
Bean Program Coordinator  
NARO-NACRRI  
P. O. Box 7084, Kampala  
UGANDA  
256-41-567635  
tamusange@gmail.com

University of California Library  
Bioscience & Natural Res.  
2101 VLSB #6500  
Berkeley, CA 94720-0001

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St Lucia Campus  
St Lucia QLD 4072  
AUSTRALIA

Carlos Urrea  
Panhandle Research & Extension  
Center  
4502 Avenue I  
University of Nebraska,  
Scottsbluff, NE 69361  
308-632-0556  
Currea2@unl.edu

Sonia Valdez Ortega  
Calle Esmeralda no. 303  
C.P. 34237  
Durango, Dgo.  
MEXICO  
52 (618)2998168  
sonia\_valdez@hotmail.com

Giseli Valentini  
4416 Tonquil Pl.  
Beltsville, Maryland  
20705  
240-615-1333  
giselivalentini@hotmail.com

Arlene Valmadrid  
East-West Seed Co., Inc.  
Km 54 Cagayan Valley Road  
Sampaloc, San Rafael, Bulacan  
3008, Philippines  
(044) 766-4952 to 57  
arlene.dionglay@eastwestseed.com

Gerthon van de Bunt  
Pop Vriend Seeds B.V.  
P. O. Box 5  
NETHERLANDS  
31-22859-1462  
gvandebunt@popvriendseeds.nl

Shanice Van Haeften  
Queensland Alliance of Agriculture and  
Food Innovation Institute (UQ)  
s.vanhaeften@uqconnect.edu.au

Ana Vargas  
Dept. of Plant Sciences  
51 Campus Drive, U. of Saskatchewan  
Saskatoon, SK S7N 5A8  
CANADA  
anavargaspal.2@gmail.com;  
agv937@mail.usask.ca

Greg Varner  
MI Dry Bean Res. Board  
8439 N. Blair Road  
Breckenridge, MI 48615-9726  
989-751-8415  
varnerbean@hotmail.com

Elise Vendevre  
Vilmorin-Mikado SAS  
Route Du Manoir  
49250 La Menitre  
FRANCE  
elise.vendevre@vilmorinmikado.com

Rogério Faria Vieira  
Grain Legume Researcher  
EPAMIG - Vila Gianetti 47  
Vicoso, MG 36571-000  
BRAZIL  
55-31-3891-2646  
rfvieira@epamig.br

Pedro Vidigal Filho  
Av. Colombo 5790-cep:87020-900  
Univ. Estadual de Maringa  
Maringa,Parana,87020-900  
BRAZIL  
442635036  
vidigalfilhop@gmail.com

Diego Viteri  
Condominios Laderas del Mar 202  
Aguadilla, PR 00603  
dviterid@hotmail.com

Eric vonWettberg  
University of Vermont  
Eric.Bishop-Von-Wettberg@uvm.edu

Oswaldo Voysest  
1225 Bushnell St  
Beloit, WI 53511-6430  
608-313-8606  
ovoysestv@aol.com

Dan Wahlquist  
Syngenta Seeds, Inc.  
6338 HWY 20 - 26  
Nampa, ID 83687  
208-465-8510  
dan.wahlquist@syngenta.com

J. G. Waines  
Botany and Plant Sciences  
University of California  
Riverside, CA 92521-0124  
951-682-3838  
giles.waines@ucr.edu

Jenn Walker  
Alberta Pulse Growers  
jwalker@albertapulse.com

Zoe Wall  
University of Wyoming  
ZWall@uwyo.edu

Lyle Wallace  
3405 NW Orchard Ave  
Apt 252  
Corvallis, OR 97330  
847-942-2849  
LW2671@gmail.com

Ning Wang  
Canadian Grain Commission  
ning.wang@grainscanada.gc.ca

Weijia Wang  
Michigan State University  
wangwe33@msu.edu

Jim Weller  
University of Tasmania  
Australia  
Jim.Weller@utas.edu.au

Ivo Eduardo Wellington  
Thereza Cristina de Jesus Julião,  
nº740  
Jardim Nova Aparecida  
Jaboticabal, SP CEP: 14883-296  
BRAZIL  
wellington\_ic@hotmail.com

Molly Welsh  
P.O. Box 6  
Colton, WA 99113  
509-330-0546  
wandh@pullman.com

Jeffrey White  
2414 West Shawnee Drive  
Chandler, AZ 85224  
USA  
480-772-0145  
jeff.white.az@gmail.com

Jason Wiesinger  
USDA Robert Holley Center for  
Agriculture and Health  
Cornell University  
Ithaca, NY 14853  
607-255-8002  
jaw456@cornell.edu

Jennifer Wilker  
University of Wisconsin-Madison  
Madison, Wisconsin  
jilwilker@wisc.edu

Martin Williams  
USDA-ARS  
Urbana, Illinois  
martin.williams@usda.gov

Donna Winham  
Food Sci. and Human Nutrition  
Iowa St. Univ.  
Ames, IA 50010  
515-294-5040  
dwinham@iastate.edu

Andi Woolf  
Idaho Bean Commission  
821 W. State St.  
Boise, ID 83702  
208-334-3520  
andi.woolf@bean.idaho.gov

Evan Wright  
Michigan St. Univ.  
4450 Beaumont Rd.  
Lansing, MI 48910  
517-355-2287  
wrigh294@msu.edu

Stephen Yeboah  
CSIR-Crops Research Institute Kumasi,  
Ghana [proyeboah@yahoo.co.k](mailto:proyeboah@yahoo.co.k)

BaiLing Zhang  
Agriculture and Agri-Food Canada  
[bailing.zhang@agr.gc.ca](mailto:bailing.zhang@agr.gc.ca)

**2021 FINANCIAL STATEMENT  
BEAN IMPROVEMENT COOPERATIVE**

**BALANCE AS OF January 1, 2021** **\$ 38,409.64**

**INCOME**

	<b>2021</b>
2021 Membership dues and Registrations	\$ 10,400.00
BIC Meeting Reimbursement (from NAPIA)	\$ 7,473.96
BIC Meeting Sponsorships	\$ 9,700.00
Bank Interest	\$ 0
<b>TOTAL INCOME</b>	<hr/> \$ 27,573.96

**EXPENSE**

Labor charges	\$ 0
Postage, Copy Charges and Office Supplies	\$ 29.00
Pdf & Book editing and publishing fees	\$ 543.80
BIC-NAPIA virtual meeting platform	\$ 26,789.90
BIC Student Awards	\$ 600.00
PayPal Fees	\$ 303.94
Bank Fees	\$ 133.00
<b>TOTAL EXPENSE</b>	<hr/> \$ 28,399.64

**BALANCE AS OF December 31, 2021** **\$ 37,583.96**