ANNUAL REPORT OF THE

BEAN IMPROVEMENT COOPERATIVE

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

> VOLUME 63 2020

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THE LXIII

Report of The

BEAN IMPROVEMENT COOPERATIVE

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

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Lúcia S. A. Takahashi¹

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Cover: Drought experiments in Mitchell, NE (photo taken by Eduardo Valentin Cruzado)

THE 63nd ANNUAL REPORT OF THE BEAN IMPROVEMENT COOPERATIVE

In these trying times with the pandemic, we hope that each one of you is safe and that your families and friends are healthy. The dramatic shift in the workings of the world has caused us to reassess aspects of our lives and the interconnectedness of humanity. Our work as bean breeders, pathologists, entomologists, agronomists, and physiologists becomes more important considering our fundamental contributions to maintaining the food supply through our research in this ever-changing world landscape. The research collaboration that characterizes the bean community is both a hallmark of this community and its major strength. This publication is an example of that collaboration and the productivity that results from it. Thank you for these important contributions.

Dr. Phillip Miklas has completed his term as President of the Bean Improvement Cooperative, from 2010 to 2019, and we thank him for his exemplary service to this community during this last decade! He continues his unabated contributions to research, training, and cultivar development, through his example of excellence in research. We value his continued guidance and support, his friendship, and his dedication to this community.

The Bean Improvement Cooperative (BIC) 30th Biennial Meeting took place from November 3 through November 6, 2019, in Fargo, North Dakota. We would like to thank the local BIC meeting organizing committee for their hospitality and admirable meeting organization including, Juan Osorno, Phil McClean, Julie Pasche, and Mike Grusak. For those not able to attend, the meeting agenda is available on the BIC website: <u>http://www.bic.uprm.edu/</u>.

We want to give special recognition and congratulations to those receiving awards during the 2019 BIC Meeting Award Banquet, including Juan Carlos Rosas and James Beaver for their receipt of the **Frazier-Zaumeyer Distinguished Lectureship** recognizing their exceptional contributions. This special award is in memory of the founding members of the BIC. The **BIC Meritorious Service Award** was awarded to James R. Myers of Oregon State University and to Sara F. Rose of Bush Brothers Company. The **BIC Achievement Award** was awarded to Frédéric Marsolais of Agriculture and Agri-Food Canada. The **BIC Technical Merit Award** was awarded to Albert Jody Vander Wal of North Dakota State University. Please read the descriptions of their contributions in this Report.

Please share information about the BIC with interested colleagues who might like to attend the next meeting in 2021 in Saskatchewan, Saskatoon, Canada 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. A recent suggestion that we are planning to implement is an update to the Research Techniques page of the BIC website. Your contributions to update these methods and to include new methods are much appreciated.

The BIC continues to conduct business by email, postings on the webpage, and through the online publication of this Annual Report. A site for members to download the report will be provided and older issues are posted on the BIC webpage. Members are asked to ensure that email addresses are current and to periodically review the web page for information on meetings, deadlines and critical dates. We are always open to new ideas to make the BIC a more effective organization and any suggestions can also be shared with members of the Coordinating Committee.

Warm regards, **Tim Porch, BIC President**

BIC COMMITTEE MEMBERSHIP - 1957 to 2020

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

Awards Committee:

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

Genetics Committee

2004 Bassett (Chair), Beaver, Blair, Gepts, McClean, Miklas, Welsh (ex officio)

2005 Beaver (Acting Chair), Blair, Gepts, McClean, Miklas, Porch, Welsh (ex officio)

2007 Beaver, Blair, Gepts, McClean, Miklas, Porch (Chair), Welsh (ex officio)

2008 Bett, Blair, Gepts, McClean, Miklas, Porch (Chair), Urrea, Welsh (ex officio)

2014 Bett (Chair), Ferreira, Gepts, Goncalves-Vidigal, Kalavacharla, Kelly, Kisha (ex officio), McClean, Osorno, Porch, Urrea

2018 Bett, Ferreira, Gepts, Goncalves-Vidigal, Kalavacharla, Kelly (Chair), Kisha (ex officio), McClean, Osorno, Porch, Urrea

2020 Bett, Ferreira, Gepts, Goncalves-Vidigal, Kalavacharla, Kelly, McClean, Miklas (Chair), Osorno, Porch, Urrea

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

BIC Genetics Committee Meeting Minutes During the 2019 BIC Meeting

Meeting location:	Hotel Radisson Downtown Fargo, Fargo ND
Date:	Wednesday Nov 6. 2019, 11:15-12:30

Committee Members and Guests present:

Avican, Omer (omer.avican@may.com.tr) – May Seed, Turkey Baetsen-young, Amy (amy.baetsen-young@syngenta.com) – Syngenta Seeds Beebe, Steve (s.beebe@cgiar.org) – CIAT, Colombia Cichy, Karen (Karen.cichy@ars.usda.gov) – USDA-ARS, East Lansing, MI Emmalea, Ernest (emmalea@udel.edu) – University of Delaware Gang, David (gangd@wsu.edu) – Washington State University Glahn, Ray (Raymond.glahn@usda.gov) - USDA-ARS Gomez, Francisco (gomez225@umn.edu) – University of Minnesota Goncalvez Vidigal, Maria C. (mcgvidigal@gmail.com) – UEM, Brazil Grusak, Mike (mike.grusak@ars.usda.gov) – USDA-ARS, Fargo, ND Haus, Miranda (hausmi@msu.edu) – Michigan State University Heitholt, Jim (Jim.Heithlot@uwyo.edu) – University of Wyoming Hellier, Barbara (Barbara.heliler@usda.gov) – USDA-ARS, Pullman, WA Hoyos-Villegas, Valerio (Valerio.hoyos-villegas@mcgill.ca) – McGill University Hou, Anfu (anfu.hon@canada.ca) - Ag-Canada Humann, Jodi (jhumman@wsu.edu) – Washington State University- pulsedd.org Hyten, David (David.hyten@unl.edu) – University of Nebraska Kelly, Jim (kellyj@msu.edu) – Michigan State University Mazourek, Michael (mm284@cornell.edu) – Cornell University McClean, Phillip (Phillip.mcclean@ndsu.edu) – North Dakota State University Munoz-Amatriain, Maria (maria.munoz amatriain@colostate.edu) - Colorado State University Miklas, Phil (phil.miklas@ars.usda.gov) – USDA-ARS, Prosser, WA Osorno, Juan (juan.osorno@ndsu.edu) – North Dakota State University Porch, Tim (timothy.porch@ars.usda.gov) – USDA-ARS, TARS, Mayaguez, PR Pastor-Corrales, Talo (talo.pastor-corrales@ars.usda.gov) – USDA-ARS, Beltsville, MD Raatz, Bodo (b.raatz@cgiar.org) - CIAT, Colombia Trapp, Jennifer (jtrapp@senecafoods.com - Seneca Foods Safe, Jeff (jeff@critessed.com) – Crites Seed, Inc. Saha, Gopesh (gopesh.saha@brothertonseed.com) – Brotherton Seed Co. Scholz, Todd (tscholz@usapulses.org) – American Pulse Association Shi, Ainong (ashi@uark.edu) – University of Arkansas Souza, Thiago (Thiago.souza@embrapa.br) – EMBRAPA, Brazil Uebersax, Mark (uebersax@msu.edu) – Michigan State University (retired) Vidigal Filho, Pedro (vidigal.filhop@gmail.com) - UEM, Brazil Urrea, Carlos (currea2@unl.edu) – University of Nebraska Vaz Bisneta, Mariana (marianavazbisneta@hotmail.com) – UEM, Brazil Wahlquist, Dan (<u>dan.wahlquist@syngenta.com</u>) Syngenta Seeds Wiesinger, Jason (Jason.wiesinger@usda.gov) – USDA-ARS Woolf-Weibye, Andy (bean@bean.idaho.gov) – Idaho Bean Commission

1. Old Business:

Approval of the Genetics Committee meeting minutes from 2017

Decision: The Genetics Committee minutes from the meeting held at the Kellogg Center, Michigan State University, East Lansing, MI on Nov 1, 2017 were approved. Motion by Miklas – seconded by Porch - AIF.

Introduction to Bean Genes List

The introduction to Bean Genes List should be updated with the new genomic tools we now have available providing links for all the reference genome materials: G19833 V2.1, BAT 93, OAC REX, UI-111 (soon to be available), gene annotation lists and other genomic materials that could be shared.

Decision: Phil McClean will provide a draft narrative for the Bean Genes List – Introduction section that can then be edited.

Update the 2010 SCAR table and transform into a SNP table

Decision: Those involved in generating SNPs/Indels for important traits will provide those SNPs/Indels to Phil Miklas before publication and for inclusion in an accessible SNP table for the BIC webpage. This resource will make the SNPs available for use with economical external laboratories, e.g. Intertek, for genotyping by the bean community at large.

Naming of the *bc-u* gene (item from Phil Miklas)

An item for discussion is the naming of the bc-u locus (loci) given that two linked loci have been found that are about 3 Mb apart and that interact separately/differentially with bc-2/bc-2(2) locus. Actually, there are no alleles for bc-2 in the Durango market class. The bc-2 gene of Michelite, Sanilac, Robust origin is different and still being sorted out. bc-u(A) with bc-2 is resistant to NL-4 (PG VII) and susceptible to NL-3, while bc-u(B) with bc-2 is susceptible to NL-4 and resistant to NL-3 (this latter interaction has always been characterized as the bc-2(2) allele.

Decision: No decision was made at the meeting. Instead this discussion will continue by email. Some ideas included: using bc-u and bc-s for unspecific and specific reaction, respectively; or naming the additional locus bc-4. Other ideas included investigating the system used for recessive genes in cucurbits or for the *pto* disease resistance locus in tomato.

Updating text for the photoperiod gene (*Ppd*) in the Bean Genes List (item from Jim Kelly)

The text currently states: *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+ for short day vs. neu for day neutral flowering response to length of day of Rudorf (1958).

Decision: Add the following text to the Bean Genes list for *Ppd*: "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)."

Kamfwa, K., K.A. Cichy and J.D. Kelly. 2015. Genome-wide association study of agronomic traits in common bean. The Plant Genome 8: doi:10.3835/plantgenome2014.09.0059.

Weller, J. L., J. K. Vander Schoor, E. C. Perez-Wright, V. Hecht, A. M. González, C. Capel, F. J. Yuste-Lisbona, R. Lozano, and M. Santalla. 2019. Parallel origins of photoperiod adaptation following dual domestications of common bean. J. Exp. Botany 70:1209-1219.

Also a lookup table, or an http link in the pdf to phytosome would be helpful that links the symbol with the gene sequence.

Request for a consensus about an appropriate QTL symbol for quantitative resistance to rust, e.g. Ua3.1, Ur3.1, Ru3.1, BR3.1. (item from Phil Miklas) Decision: No decision was made at the meeting, but several ideas were presented including BR, RU, RST, RUST, RUS.

Molecular evidence for new gene symbol in rules section (item from Jim Kelly)

This item concerns the Coordination of Genes and Gene Symbol Nomenclature of BIC Genetics Committee and a requirement for physical map positions. Currently the evidence must include: "2. iv. molecular marker data and genetic linkage map and physical map (preferred) positions when available." The suggestion is to make the physical map position mandatory.

Decision: No decision was made, instead the committee has decided to send this item out to the BIC community at large for recommendations about making the physical map position information mandatory, and about what to do with cases of gene clusters.

2. New Business

Membership: Election of new Genetic Committee Chair

Decision: The Committee has selected Dr. Phillip Miklas as the new Genetics Committee Chair and thanks Dr. James Kelly for his service in this position.

3. Next meeting:

Decision: The next Genetics Committee Meeting will be held in conjunction with the W-3150 meeting to be held in mid to late-August 2020 in Scottsbluff, NE and hosted by Carlos Urrea.

2019 BIC AWARD RECIPIENTS THE BEAN IMPROVEMENT COOPERATIVE

Proudly Presents the

Frazier – Zaumeyer Distinquished Lectureship

to

JAMES BEAVER

Professor Dept. of Agroenvironmental Sciences, University of Puerto Rico Mayaguez, Puerto Rico

and

JUAN CARLOS ROSAS

Emeritus Professor Zamorano University Zamorano, Honduras

the

Distinguished Achievement Award

to

FRÉDÉRIC MARSOLAIS

Agriculture and Agri-Food Canada London, Ontario, Canada

and the

Meritorious Service Award

to

JAMES R. MYERS

Oregon State University Corvallis, Oregon

and

Meritorious Service Award

SARA F. ROSE

Bush Brothers Company Knoxville, Tennessee

and the

Technical Merit Award

to

ALBERT JODY VANDER WAL

North Dakota State University Fargo, North Dakota

JAMES BEAVER

Dr. James Beaver was born in Noblesville, Indiana, completed his B.S. at Purdue U., and his Masters and PhD at the U. of Illinois on soybean. He served in the Peace Corps in Brazil and integrated the focus of service in his career as a professor and plant breeder at the U. of Puerto Rico starting in 1981, along with his wife Dr. Linda Beaver, a squash breeder and educator.

Dr. Beaver's remarkable contributions to common bean breeding have resulted in synergistic advances in disease, abiotic and insect resistance in cultivars throughout the Caribbean and Central America, resulting in a total of over 50 released cultivars and germplasms. BGYMV is a critical constraint to common bean production in the Caribbean and Central America, and was a focus of his early efforts, followed by the release of multiple virus resistant cultivars, combining BGYMV, BCMV, and BCMNV resistance. His methodical approach to pyramiding resistance genes through use of a broad diversity of germplasm, developing and applying existing conventional screening techniques, and advancing and integrating marker assisted selection, has resulted in multiple disease and abiotic stress resistant cultivars that have brought increased food security to the region. Recent collaborative releases such as 'Sankara' in Haiti, 'Bella' and 'Beniquez' in Puerto Rico, and 'Lenca Precoz' in Honduras, represent great advances in pyramiding multiple disease resistances such as BCMV, BCMNV, BGYMV, CBB, rust, ashy stem blight and web blight, as well as heat, drought and low fertility tolerance. His collaboration with Dr. Juan Carlos Rosas has resulted in cultivars with some of the broadest and most durable genetic resistance available that are key genetic resources for other production zones.

Through the Master's program at the U. of Puerto Rico, Dr. Beaver trained over 50 scientists who are now in key faculty, industry and government positions internationally, with many serving in the common bean research community. The training of new scientists that returned to their National institutions helped to foster long-term collaborations with National Programs and led to the development of active plant breeding programs. Dr. Beaver worked closely with the National Seed Service of Haiti and collaborators in the development and release of 'Sankara', a multiple virus resistant black bean cultivar. Considering the susceptibility of the seed sector to natural disasters, he supported an Idaho-based seed production and dissemination initiative that resulted in Idaho-grown certified 'Sankara' seed distribution to Haiti following Hurricane Matthew in 2016.

In addition to the genetic diversity available within common bean, Dr. Beaver pursued the use bruchid resistance from tepary bean (P. acutifolius) using germplasm developed from Oregon State U. and Sokoine U. in Tanzania. This seminal work on bruchid resistance is a game changing postharvest achievement as it alleviates rapid degradation of seed quality in the tropics and subtropics, particularly affecting poor farmers. The germplasm developed from these efforts, AO-1012-29-3-3A, represent the first release, while Dr. Beaver continues to introgress this resistance into additional classes, pyramided with multiple disease resistance. The impact of Dr. Beaver's scholarship resulted in the publication of over 130 refereed journal papers. In addition, his regional approach to breeding, collaboration, and training has resulted in long-term, substantial, and broad impact in the knowledge-base in the Caribbean and Central America, and more recently in Sub-Saharan Africa. Among his awards, Dr. Beaver (and Dr. Rosas) received the 2017 BIFAD Scientific Award for Excellence in a Feed the Future Innovation Lab recognizing the impact of their individual and collaborative contributions, the 2015 Certificate of Recognition from the House of Representatives of Puerto Rico for Bean Research Contributions, and the Bean Improvement Cooperative's Meritorious and Distinguished Achievement Awards. He was also named a Fellow of the Crop Science Society of America in 2011.

JUAN CARLOS ROSAS

Dr. Juan Carlos Rosas is an emeritus professor and bean breeder at the Zamorano University in Honduras and has a long record of achievements that have contributed to agricultural development throughout Latin America. Born in Peru, Dr. Rosas obtained his Agronomy title from National Agrarian Univ. La Molina in Lima, Peru. After graduation, he worked as Research Assistant at the Agricultural Research Institute in Peru and then in the Bean program at CIAT. Then he moved to the U.S. and obtained both his M.S. and PhD in Plant Breeding and Plant Genetics from University of Wisconsin- Madison.

Dr. Rosas is a leader for bean research in Central America and the Caribbean (CA/C). He coordinates the evaluation of small red and black bean regional trials that include the most promising bean breeding lines from Zamorano, CIAT and Central American and Caribbean breeding programs. Dr. Rosas participated in the development and release of more than 70 cultivars with enhanced levels of resistance to diseases, abiotic stresses, nutritional value, and biological nitrogen fixation. The disease resistant and heat tolerant cultivar Amadeus 77 has been the most popular small red bean planted in Central America. Other important varieties include Tio Canela 75, CENTA Pipil, CENTA EAC, ICTA Sayaxché, INTA Centro Sur, and XRAV 40-4. It is estimated that ~200,000 small-scale bean producers in CA/C currently plant bean cultivars developed by Dr. Rosas' bean breeding program. He has employed modern breeding methods such as marker-assisted selection, controlled disease/pest evaluation methods, and precision soil fertility treatment to accelerate his breeding efforts and for agronomic and genetic elucidation of these traits. He has author/co-authored more than 100 refereed and non-refereed publications and is an active participant in the annual meetings of the PCCMCA, which brings together scientists from CA/C and Mexico. As a professor at Zamorano, Dr. Rosas has served as academic mentor for more than 80 students.

Dr. Rosas has also contributed to institutional capacity building and strengthening by organizing numerous workshops for agronomists from CA/C covering a wide array of topics. In recognition of his contributions to teaching and research, Dr. Rosas received the 2014 Gamma Sigma Delta Distinguished Achievement in Agriculture Award. Even a teaching auditorium for 200 students at Zamorano was named in his honor. He works with NGOs and farmer associations to deliver improved bean cultivars and Rhizobium inoculant. He uses participatory plant breeding techniques to improve local landraces and develop locally adapted cultivars, allowing many local communities in Central America to produce and store high-quality bean seed, which has led to improved food security.

In recognition of his contributions to bean research, Dr. Rosas has received both the Meritorious and Distinguished Achievement Awards from the BIC. More recently, Dr. Rosas (along with Dr. James Beaver) was the recipient of the 2017 Board for International Food and Agricultural Development (BIFAD) Scientific Award for Excellence in a Feed the Future Innovation Lab recognizing the impacts of their synergistic bean breeding collaboration. Dr. Rosas remains active in research activities at Zamorano and as the Director of the new Master's Degree Program. His record exemplifies what a faculty member at an institution of higher learning in a developing country can do to contribute to research, teaching and the dissemination of technology to increase food security and impact smallholder farmers.

JAMES R. MYERS

Dr. James R. Myers is an Indiana and Missouri native who received his BS degree at Kansas State University in 1978 in horticulture. He then attended the University of Wisconsin where he received MS (1981) and PhD (1984) in Plant Breeding and Genetics. Following a postdoctoral fellowship and research specialists positions at the University of Kentucky (1984-1987), Jim was an assistant and associate professor at the University of Idaho from 1987-1996 where he focused on dry bean research. During that tenure Jim was instrumental in releasing 14 bean cultivars in the Cranberry, Dark Red Kidney, Black, Small White, Navy, Great Northern, Pinto, Pink, and Small Red market classes. Since 1996 Jim has been the Baggett-Frazier Endowed Professor of Vegetable Breeding and Genetics at Oregon State University where he primarily focuses on snap bean breeding, breeding for white mold resistance and the genetics of flavor. His bush blue lake cultivar OSU 5630 occupies about 80% of the snap bean processing acreage in the Pacific Northwest. He continues dry bean research with breeding for bruchid resistance, and breeding and release of Mayocoba market class cultivars.

From a service perspective, Dr. Myers organized the Cooperative Dry Bean Nursery each year of his tenure at the University of Idaho. This is a critical nursery open to public and private breeders to collect multi-location performance data on their advanced breeding lines to ensure their lines meet the standards for modern improved varieties. Jim has served the Bean Improvement Cooperative as a member of the Coordinating Committee since 1996. Jim assisted the BIC byhosting the 1993 BIC meeting in Boise, Idaho and the 2013 BIC meeting in Portland, Oregon. Jim served from 1993-2002 on the BIC Genetics Committee (chairman 1993-1998). Since 1996, Jim has been a member of the Phaseolus Crop Germplasm Committee, and from 2002-2007, he served as president of the committee. Jim has also been a member of the Western Regional USDA W-3150 committee (and its progenitor committees) since 1987. Jim was a major contributor to USAID Bean/Cowpea CRSP projects from 1993-2007, and served in several capacities as research advisor (1997-2000; chair 1999-2002), technical committee member (1998-2002; chair 2000-2002), and regional facilitator (2002- 2007). Jim also provided service to the community from 2009-2014 as a member of the USDA Common Bean Coordinated Agricultural Project (BeanCAP) executive committee. As an educator, Jim has advised or co-advised 10 Ph.D., 13 M.S. and 24 undergraduate students.

Applied and basic common bean research has been strengthened by the presence of Dr. Myers's research program. Kusolwa and Myers (2011, 2012) significantly contributed to improve smallholder farmers' livelihoods. Bruchids are a major storage pest, and the efforts of these two researchers culminated in the introgression of a strong resistant gene, from tepary bean, into modern dry bean germplasm used in both Central America and Africa. This locus was mobilized in a recently released red kidney cultivar for distribution in the Americas and Africa (Kusolwa et al. 2015). Dr. Myers is the leading snap bean geneticists in the world. A recent publication of Jim's research group clarified the relationship of snap beans and dry beans. Wallace et al. (2018) confirmed the long-held belief that snap beans were primarily, but not exclusively, of Andean origin. From this publication, it is now known that Middle American snap beans are a distinct group, as are a group that shows admixture between the two gene pools. Among the various market classes of beans, this admixture is distinct to snap beans. Dr. James Myers has made many significant service and research contributions to the world common bean community, and the common bean community would be distinguished by awarding him the BIC Meritorious Service Award.

SARA F. ROSE

Sara Rose is a talented and capable individual who has long served as an active advocate for improved consumption of dry beans within the U.S. diet. Her advocacy for beans as a nutritious food has had many dimensions and has served the professional bean science community and the general public in many creative and tangible means. She came to this position with highly specialized credentials which have served the entire bean community. She graduated from Vanderbilt University (Bachelor of Arts, magna cum Laude, Phi Beta Kappa English major). She furthered her formal education through attaining an MBA from Indiana University, Evanston, IL. Her exceptional communication skills and sound business acumen have been directly channeled into improved study and promotion of dry beans.

Ms. Rose joined Bush Brothers and Company (Knoxville, TN) in 1996 and established a full career that culminated with retirement in 2018. During this period, she served in various roles including: Director of Research; Vice President and Director of Strategic Business Development; Vice President and Director, Industry and Government Affairs. She has been a champion and facilitator for securing publicly funded bean research appropriations.

In her role as vice president she directed Bush Brothers and Company Health Initiative which had many positive impacts on the use of beans in school lunch programs and other broad-based public nutrition policies. She is the named principle investigator on U.S. patents designed to reduce flatulence and enhance the digestibility of dry beans.

Ms. Rose provided long-term leadership through extensive communications and documentation with the Food and Drug Administration (FDA) regarding the health benefits of beans in the U.S. diet. These efforts resulted in the approval of the Bean Dietary Guidance Message (DGM) as follows: "A diet including beans may reduce your risk of heart disease and certain cancers."

Her sustained efforts with leadership in the bean industry's Beans for Health Alliance resulted in a joint effort between USAID and industry to enhance educational awareness of the health benefits attributed to dry beans. This work resulted in documented promotion of "BEANS...the vegetable with more." Further, numerous publicly funded peer reviewed research projects focused on nutritional and health attributes of dry beans were awarded and administered under her leadership.

Ms. Rose provided the inspirational leadership for the United Nations declaration of the Year of the Pulse (2016). She served as primary coordinator of the conference "Little Beans, Big Opportunities: Realizing the Potential of Pulses to Meet Today's Global Health Challenges" held at the Sackler Institute for Nutrition Science at the New York Academy of Sciences, November 19, 2015. This nutrition conference program was the kickoff event for the International Year of the Pulse.

Ms. Rose has been a regular contributor to BIC meetings and has actively supported numerous research programs and individual researchers. She has been a career long servant on the various bean policy bodies including the U.S. Dry Bean Council, American Pulse Association, and as a board member of Pulse Canada. Sara F. Rose is a most worthy candidate for the prestigious BIC Meritorious Service Award.

FRÉDÉRIC MARSOLAIS

Dr. Frédéric Marsolais was born on March 24, 1968 in Jonquière, Québec, Canada. He is currently a Research Scientist at the London Research and Development Centre of Agriculture and Agri-Food Canada (AAFC) and Adjunct Research Professor, Department of Biology of the University of Western Ontario in London, Ontario, Canada. Dr. Marsolais graduated with a B.Sc. in Microbiology (1992) and an M. Sc. in Biology (1995) from Laval University, and a Ph. D. in Biology from Concordia University (2001). Prior to joining AAFC, he was an NSERC Visiting Fellow at Duke University and the University of North Carolina at Chapel Hill (2001-2003).

Dr. Marsolais' research focuses on the protein chemistry of pulses, particularly of common bean, including the relationship between seed protein composition and protein quality. His laboratory also investigates the metabolism of sulphur and amide amino acids, and its link with seed protein accumulation. Between 2014 and 2018, Dr. Marsolais was also responsible for the dry bean breeding program at the Harrow Research and Development Centre. During this time, navy bean cultivars AAC Argosy and AAC Shock were commercialized. Dr. Marsolais is an Editorial Board Member of the new Wiley journal Legume Science. To date he has published 49 articles in peer-reviewed journals and nine book chapters. He recently co-edited a book on the Common Bean Genome (published by Springer in 2017).

Dr. Marsolais has played a nearly unique and certainly important role in the bean community as a protein biochemist, given the important nutritional role of seed proteins in common bean. He discovered that the levels of nutritionally essential sulfur amino acids, methionine and cysteine, can be significantly increased in common bean genotypes lacking the 7S globulin phaseolin and lectin phytohemagglutinin, resulting in improved protein quality. This change happens mainly at the expense of an abundant non-protein amino acid, Smethylcysteine, which cannot substitute for methionine or cysteine in the diet. The absence of phaseolin and phytohemagglutinin is compensated by an increased concentration of multiple sulfur-rich proteins, including the 11S globulin legumin, basic 7S globulin-2, albumin-2, albumin-1, trypsin and Kunitz trypsin inhibitors, and defensin D1. An investigation of the genetic basis of differences in protein composition identified several types of polymorphism, including single nucleotide changes affecting cisregulatory elements in the promoters of phaseolin and lectin genes.

Furthermore, transcript profiling revealed a coordinated regulation of sulfate transporters, sulfate assimilatory enzymes, and other enzymes involved in cysteine and methionine metabolism. The differential gene expression of sulfur-rich proteins preceded that of sulfur metabolic enzymes, suggesting a regulation by demand from the protein sink. Recently, using stable isotope labeling combined with liquid chromatography and high-resolution tandem mass spectrometry, the biosynthetic pathways of S-methylcysteine were identified, providing information on potential targets for genetic improvement.

As a plant protein biochemist, Dr. Marsolais plays a role complementing those of breeders, nutritionists, and molecular biologists. As beans are touted as amore sustainable and healthy alternative protein source, the information developed by Dr. Marsolais on the molecular and biochemical basis of bean seed proteins is of increasing importance. Furthermore, he played an important role as a member of the Ontario, Canada, bean genome team, which developed genomic resources for common bean for the benefit of bean breeding. Both as a scientist and a team player in the bean community, he is a most deserving recipient of the Distinguished Achievement Award of the BIC!

ALBERT JODY VANDER WAL

Mr. Albert Jody Vander Wal, or as he prefers, Jody, is a research technician in the dry bean breeding program at North Dakota State University (NDSU). This is Jody's 38th year working in the dry bean breeding program, which is a great accomplishment by itself. However, this recognition is not just because of his long tenure at NDSU, but also because of his contributions to the program, the bean industry and to the people who have worked with him. Jody is a native of Linton, a town in south central North Dakota.

Jody obtained a B.S. in Zoology from NDSU in 1981 and right after graduation, he started working in the NDSU dry bean breeding program, the youngest public dry bean breeding program in the U.S., with Dr. Ken Grafton as the lead and Jody as the research technician. During the first year of the program, 3.5 acres of experiments at 3 locations were grown. In 2007, Dr. Juan Osorno was hired as the second dry bean breeder when Dr. Grafton moved on to administrative responsibilities at NDSU. Today, the breeding program grows ~40 acres of field experiments across 7 locations every year. Thanks to their hard work, the NDSU dry bean breeding program evolved to become one of the largest and most important programs in the country. In addition, North Dakota became the largest producer of dry beans in the country as well, which gave even more relevance to the breeding program at NDSU. Consequently, during many years and even today, many dry bean varieties grown in the region originated from the NDSU dry bean breeding program. Jody was actively involved in the development of well-known varieties such as Norstar, Frontier, Arthur, Hatton, Maverick, Eclipse, Lariat, Stampede, Avalanche, Talon, Rosie, and ND-Palomino, among others. Similarly, Jody has contributed to the development of improved germplasm with resistance to diseases and other important agronomic traits. In addition to these contributions, Jody has significantly assisted in the research, formation and training of more than 20 M.S. and Ph.D. students and numerous undergraduate students whom have worked as summer workers, interns, or visiting scholars in the program. Thanks to those contributions, Jody is the co-author of many variety releases as well as other research articles. As mentioned by some students, Jody has the innate ability to be a friend, teacher, and supervisor all at the same time.

Jody is responsible for handling thousands of lines/genotypes every year in both the field and greenhouse. Jody is also the curator of all the breeding records for all this genetic material. Each year, Jody is in charge of making all the crosses in the greenhouse where he creates ~200 new parental combinations. Thanks to his recent efforts, all of the bean breeding program has been digitalized into a relational database, so he never stops learning and using technology to be more efficient. Jody frequently gets calls from growers and cooperators asking questions about dry bean production and varieties. Jody has gained the respect of the entire Plant Sciences department and the local dry bean industry because of his diligence, hard work and discipline, organization, people skills, and quality performance. Jody's career and work performance could be summarized in three words: discipline, responsibility, and independence. Jody's hard work and contributions have been crucial for the success of the NDSU dry bean breeding program, the bean industry, and the bean scientific community.

IN MEMORY OF LESLIE L. "BILL" DEAN

Leslie L. "Bill" Dean passed away August 29, 2019 at the age of 100. Bill was born in Twin Falls, Idaho on February 4, 1919. He grew up on the Salmon Tract in Southern Idaho, and graduated from Hollister High School in 1937. Bill received his B.S. (1942) and M.S. (1947) from the University of Idaho. His Master's thesis was titled 'A New Mosaic of Beans.' Bill then served in the U.S. Navy in World War II. He returned to school after the war and received his doctoral degree from Purdue University in 1951.

Bill's entire career focused on beans. He began in 1940 as a college student working on the University of Idaho bean improvement project. He returned to Idaho from Indiana in 1950 as the University of Idaho Assistant Plant Pathologist for Bean Diseases. When Bill retired as Professor Emeritus of Plant Sciences in 1975, he had released 18 dry edible and snap bean cultivars, including UI 36 and UI 37 small reds and UI 111 and UI 114 pintos. These pinto cultivars are considered cornerstones of the North American pinto bean industry. In fact, UI III was recently used to generate a reference genome for the Durango Race of beans. Bill also performed groundbreaking work on the persistent color trait which he introduced from haricot vert beans into snap beans. This trait produces an attractive, uniformly colored pod and is now in about 40% of snap bean cultivars grown in North America.

Following retirement from the University of Idaho, Bill founded the Idaho Seed Bean Company, where he continued an active dry bean breeding program. Successful cultivars released by Idaho Seed Bean Company include Fiesta and Apache pinto, ISB 462 pink, and Admiral and Aspen navy. Bill was a boots-on-the-ground breeder, and continued to inspect his seed fields and breeding nurseries himself until he was 96.

Bill helped lead the campaign for zero tolerance of bacterial bean diseases in Idaho seed production fields, and helped craft the first Idaho Bacterial Blight Control Order. He was awarded the Governor's Lifetime Achievement Award for Technical Innovation in 2009 for his contributions to the Idaho bean industry and his efforts helping shape Idaho seed laws. The Idaho bean industry honored Bill with a Lifetime Meritorious Service Award in 2007.

Bill was among the founding members of the Bean Improvement Cooperative (BIC). He served the organization diligently as a member of the Coordinating Committee and Awards Committee. He received the BIC Meritorious Service Award in 1973, and presented an invited paper at the 1993 BIC meeting in Boise, Idaho.

Memories would be incomplete without mentioning a glass of single malt scotch, a whiff of good pipe tobacco, a sunny day where you can see new snow on top of Pomerelle, walking through single plant rows of beans with the South Hills in the distance. He was generous with his time and his stories; we learned so much, laughed so hard, and occasionally shared a few tears with Bill. Treasured memories, indeed.

IN MEMORY OF JOSEPH RENNIE STAVELY

Dr. Joseph 'Rennie' Stavely of Silver Spring, MD, passed away October 13, 2019. He was 80. Dr. Stavely was born May 28, 1939, in Wilmington, DE. After graduating in 1957 from Newark High School, he earned a B.S. degree in Entomology and Plant Pathology from the University of Delaware in 1961. He then attended the graduate program at the University of Wisconsin where he received his M.S. in 1963 and Ph.D. in 1965 in Plant Pathology. In August of the same year, he married Nancy Carol Gall. They enjoyed water skiing on Lake Mendota and all that Madison had to offer. After a year of postdoctoral research on the nature of powdery mildew resistance in red clover at the University of Wisconsin, he joined the USDA-ARS in 1966 as a Research Plant Pathologist working on tobacco at the Beltsville Agricultural Research Center.

Luckily for the bean industry, Rennie transferred to the Molecular Plant Pathology Laboratory also at Beltsville in 1980 to work primarily on bean rust caused by the pathogen Uromyces appendiculatus until his retirement in 2000. It did not take Rennie long to become an internationally recognized authority on bean rust. In 1983, Dr. Stavely led the organization of an international Bean Rust Workshop in Puerto Rico that standardized a 1 to 6 grading scale for characterizing rust reactions, and developed an international set of differential cultivars for race identification. Dr. Stavely monitored yearly changes in the virulence of the rust pathogen across U.S. bean production regions, and led the identification of approximately 70 races of the rust pathogen from worldwide collections. He also developed and published a rapid technique for the inoculation of common bean plants with up to eight races of the bean rust pathogen. Many researchers visited Rennie over the years to learn this technique. This methodology was crucial for the development of dry and snap beans cultivars with multiple genes conferring broad resistance to all known races of the hypervariable bean rust pathogen. The program resulted in the release of 41 dry bean (20 Pinto, 12 Navy and 9 great northern) and 75 snap bean (43 processing and 24 fresh market) germplasm lines with resistance to all known strains of the bean rust fungus. Several of these lines also contained genes conferring resistance to three viral pathogens BCMV, BCMNV, and BGYMV. His germplasm has been used extensively by breeders in the U.S., and worldwide, to develop rust resistant dry and snap bean varieties.

The screening protocol also enabled Dr. Stavely to discover in 1984 the first cluster of resistance genes in common bean for the Ur-5 gene block, and to realize that the Ur-3 and Ur-11 genes were tightly linked. Rennie quickly embraced DNA marker technology, and he supplied the segregating near-isogenic line population for the Ur-4 gene, which led to the development of the first resistance linked DNA marker in common bean in 1993. Dr. Stavely also conducted research on biological control of the rust fungus which lead to a patent for a strain of Bacillus subtilis, and he identified conditions favorable for rust hyper parasitism by Verticillium lecanii. Dr. Stavely was a longtime member of the American Phytopathological Society and served as President of the Society's Potomac division. Over the years he received several awards including the George M. Worrilow Award from the University of Delaware Ag Alumni Association, the American Phytopathological Society's Distinguished Service Award, and the Bean Improvement Cooperative's Meritorious Service Award. Rennie's other interests included genealogy and family research. He also enjoyed gardening and traveling. He was a member of the Toast Masters Club in Beltsville, MD, and a lifelong member of St. Philips Episcopal Church in Laurel, MD, where he served on the vestry for two terms. Dr. Stavely is survived by his wife Nancy Carol Gall Stavely and his son Joseph Carl Stavely and daughter-in-law Sandi, as well as nieces, nephews and cousins.

IN MEMORY OF MICHAEL D.J. THUNG

Michael D.J. Thung was born on 29 September 1941 in Indonesia. Michael's father managed a rubber plantation, and introduced Michael to the concept of nutrient use efficiency in a tree-crop – a concept that Michael applied later in his career.

Michael obtained his B.Sc. degree at Bogor University in Indonesia (1965), and later was awarded M.Sc. (1969) and Ph.D. degrees (1975) at Justus von Liebig University in Germany in soil science, carrying out his field research in Turkey.

He joined CIAT in 1975 as post-doctoral fellow working on cassava-bean associated systems, and in 1978 was incorporated as a full-time staff into the Bean Program where he initiated evaluations of advanced breeding lines for aluminum tolerance and low phosphorus adaptation.

In 1982 he joined the Bean Team of EMBRAPA in Goiania, Brazil as CIAT's out-posted representative, and there he spent the rest of his professional career. Michael participated in the release of many of the important bean cultivars in Brazil, including some for niche markets such as Andean types. His colleagues in Brazil remember him as an example for all who worked with him, both for his dedication to work and for his cheerful personality. His can-do attitude inspired everyone.

He was well known for smoking cigars, and everyone knew when he was in his office when they could smell the smoke up and down the corridors, and they would drop-in to chat. Throughout his wide-ranging career Michael acquired seven languages, starting with his native Indonesian, and ending with Portuguese. He is survived by his daughter Tonya and his son Martin.

BEAN PRODUCTION AND IMPROVEMENT IN CENTRAL AMERICA

Juan Carlos Rosas

Zamorano University, Honduras

Common beans (*Phaseolus vulgaris* L.) are an essential component of the daily diet of rural and low-income families in urban areas of Central America. Beans are grown mostly by small-scale farmers on hillsides and marginal soils, using few or low levels of inputs and having limited access to technology. According to FAOSTAT (2017), 692,892 tons of beans were harvested in Central America from 785,847 ha with an average yield of 882 kg/ha. The average *per capita* consumption in Central America was 12.4 kg/person/year (FAOSTAT 2013).

Traditionally, more than 60% of beans in Central America are produced during the second (postrera) planting season (Sep-Oct) in monoculture or in relay intercropping systems after maize. Because the beans are planted during the latter part of the rainy season, crop development and yield is often limited by terminal drought. Production during the first (primera) planting season (May-Jun) has increased in recent years in response to higher prices for beans. In humid regions, such as the northern coast of Honduras and eastern Nicaragua, beans are planted in a late second (apante) season to avoid waterlogging conditions or excessive rainfall during the harvest.

Bean production in Central America is limited by several diseases, including *Bean common mosaic virus* (BCMV) and *Bean golden yellow mosaic virus* (BGYMV), angular leaf spot, anthracnose, common bacterial blight, rust and web blight (Beaver et al. 2003). An increased incidence of charcoal rot, that infects the stem of common beans, could become an important disease problem in Central America under more frequent drought periods and higher temperatures. In addition, several pests, including whiteflies, aphids, leafhoppers, pod borers and bruchids affect bean production and storage.

Most soils where beans are produced in Central America have low levels of organic matter and essential nutrients, which reduces seed yield potential. In general, bean growers lack resources to use fertilizer and soil amendments to improve soil fertility. It is possible to produce beans on larger farms at lower altitudes that have better soil fertility and irrigation facilities in rotation with some export crops. However, increasing temperatures due to effects of climate change in lowland regions result in poor agronomic performance of traditional bean cultivars.

Collaborative bean research activities in Central America were initially conducted by scientists from the International Center of Tropical Agriculture (CIAT) and researchers of the National Agricultural Research Institutes (NARs), through the ProFrijol Network funded by the Swiss Development Corporation. The research was initially focused on the development of cultivars with BCMV and BGYMV resistance. Zamorano University (J.C. Rosas) and the University of Puerto Rico (J. Beaver) were invited in the 1990's to join the ProFrijol Network to participate in the screening of bean germplasm for drought and heat tolerance, disease resistance and *Rhizobium* inoculation.

After the end of the ProFrijol Network in 2002, the Bean Research Program (BRP) of Zamorano assumed the responsibility of coordinating the Central American and Caribbean Bean Research

Network (CAC-BRN). Since then, the BRP-Zamorano continued the distribution of small red and black bean breeding lines through the VIDAC adaptation nursery and ECAR yield and adaptation trial, as the mechanism for testing and dissemination of improved germplasm. Since 2010, regional trials to screen advanced breeding lines for specific traits including angular leaf spot and web blight resistance, tolerance to heat, drought, low fertility and multiple abiotic stress factors and enhanced biological nitrogen fixation (BNF) have been distributed.

This paper attempts to identify some of the most important bean research achievements in Central America during the past 30 years. Activities carried out by the BRP-Zamorano in collaboration with NARs researchers of the CAC-BRN, the Collaborative Program of Participatory Plant Breeding for Mesoamerica (PPB-Mesoamerica), CIAT, the Universities of Puerto Rico and Nebraska, Pennsylvania, Michigan and North Dakota State Universities and USDA/ARS-TARS of Puerto Rico are described.

The development of disease resistance cultivars has been a major research objective of the BRP-Zamorano. Research was initially focused on breeding for resistance to BCMV and BGYMV and at least one other important disease in target countries (Rosas, 2013; Beaver et al., 2003; Rosas et al., 2000a; Rosas et al., 2000b). Multiple virus resistant small red and black bean cultivars were developed more recently in response to the arrival of BCMNV in the Caribbean and due to its potential to threaten bean production in Central America (Beaver et al., 2014; Beaver et al., 2015). The multiple virus resistant black bean cultivar 'Sankara' was released in Haiti in 2013, and 'Azabache 40' and 'Lenca Precoz' were released in Honduras in 2016. Small red advanced lines with multiple virus resistance are available for on-farm testing in Central America.

Breeding for resistance to other diseases such as angular leaf spot, anthracnose, common bacterial blight, rust and web blight have received attention in Central America in the last two decades. For example, three cycles of recurrent selection to improve resistance to web blight (Beaver et al., 2002; Rosas et al., 2018) and pyramiding Andean and Mesoamerican genes for resistance to angular leaf spot (Rodriguez et al., 2014), have been conducted in collaboration with the UPR. The web blight resistant black bean cultivars 'ICTAZAM' and 'ICTA Sayaxche' were released in 2010 in Guatemala in collaboration with ICTA and the small red angular leaf spot resistant cultivar 'Tolupan Rojo' was released in Honduras in 2019. The majority small red and black lines currently distributed for regional testing in Central America have multiple disease resistance.

Central America and the Caribbean are one of the most vulnerable regions worldwide due to the threat of climate change to agriculture and natural ecosystems (Hannah et al. 2013). Bean production in Central America is affected by increasing temperatures in the lowlands, more frequent drought periods and soil degradation. Increased temperature will reduce crop production and will facilitate the proliferation of pests and weeds. Changes in rainfall patterns including frequent periods of drought increase the probabilities of failures in harvest and production. The most affected in Central America are small farmers on hillsides and other marginal regions who have limited resources to implement practices to cope with the effects of climate change.

In many bean production regions, such as in the Pacific 'dry corridor', more than one abiotic factor (e.g. drought and low fertility) can limit bean yield, especially on small-scale farms. Cultivars with improved heat tolerance and disease resistance are needed to guarantee bean production in the lowlands (Porch et al., 2007). A few improved bean cultivars adapted to lowland regions of Central

America are available thanks to the development of heat tolerant and BGYMV resistant cultivars (Porch et al., 2010; Rosas et al., 2018a; 2018b). High yields (>2,000 kg/ha) can be obtained with disease resistant and stress tolerant cultivars using irrigation, fertilizer and favorable crop and soil management practices.

Drought stress and low fertility soils affect more than 70% of bean production worldwide; and frequently, these stresses occur together in the tropics (Graham et al., 2003; Frahm et al., 2004; Ho et al., 2005; Henry et al., 2010). Unfortunately, most small farmers in Central America do not have the resources to implement irrigation systems and soil conservation and management practices, to reduce the effects of poor soil fertility and drought on bean productivity. Improvement of tolerance to drought and low fertility consists on identifying genotypes with superior performance under stress conditions. The importance of root architecture in plant productivity is based on root spatial distribution, which determines the plant's ability to exploit the nutrient and water available in the soil (Ho et al., 2005; Lynch, 2005; Henry et al., 2010). The presence of both limiting factors in most Central American bean production zones makes the development of stress tolerant cultivars a greater challenge for bean improvement programs.

Improvement of the bean-*Rhizobium* symbiotic nitrogen (N_2) fixation relationship is an important strategy to develop adaptation to low nitrogen soils, which is a major constraint for bean production in Central America. Advanced bean breeding lines have been developed that combine greater nodulation capacity, disease resistance, tolerance to abiotic stresses, desirable agronomic traits and commercial seed type after two cycles of recurrent selection. A method for *Rhizobium* strain characterization and techniques to evaluate the effectiveness of resident populations on farmer fields using a differential nursery were developed in collaboration with UPR researchers (Rosas et al. 2017). Several small red and black bean cultivars previously released in Central America and the Caribbean performed well in Puerto Rico under low nitrogen soils by having greater nodulation and N₂-fixation (J.S. Beaver, personal communication).

Since 2005, the BRP-Zamorano has collaborated with CIAT to develop and release bean cultivars with improved iron and zinc nutrient content as a strategy to reduce nutrient deficiency in low-income populations including women and children in Central America. Sources of germplasm with higher iron and zinc contents were used in a breeding and selection process conducted by CIAT in collaboration with Central American NARs. To guarantee farmer adoption of cultivars with higher nutrient content, other traits such as tolerance to drought and disease resistance were included as part of the development of these breeding lines. Seed samples of promising lines are sent to CIAT for analysis of nutrient content and are field tested in Central America in the Agrosalud Bean Regional Trial. Several small red and black bean cultivars with greater iron and zinc contents have been released in El Salvador, Guatemala, Honduras and Nicaragua in the last decade.

The Mesoamerican Participatory Plant Breeding Collaborative Program (PPB-MA) has been active in Central America since 2000. This program includes universities, research institutions, non-governmental (NGO) and farmer organizations of participating countries. The main PPB activities include breeding and selection for better adaptation of bean and maize cultivars to specific production systems (Rosas, 2001; Rosas, Gallardo and Jimenez, 2003; 2006; Humphries, Rosas and Gomez, 2016). Additional activities include *in situ* conservation of landrace germplasm, community seed banks, local seed production and use of agro-ecological management practices of

beans and maize, the two main food crops in the target region. The PPB approach has led to the release of numerous beans, maize and sorghum cultivars throughout Central America.

In Costa Rica, PPB cultivar release is approved by a formal system so that seed can be produced and sold nationally by small farmer organizations. In countries such as Honduras, the releases of PPB cultivars are at a community level and approved by municipal authorities. Although seed production of PPB cultivars in Honduras is not officially approved, many farmers are involved on local seed production and several PPB cultivars have been informally disseminated to other regions due to their superior agronomic performance and commercially desirable traits.

In Honduras, PPB activities are conducted by Zamorano-BRP in collaboration with two NGOs, the Foundation for Participatory Research with Farmers of Honduras (FIPAH) and the Rural Reconstruction Program (PRR). Groups of farmers are organized in Local Agricultural Research Committees (CIALs). More than 150 CIALs are active in Honduras and reaching >10,000 farmers in major bean-maize production regions including the dry corridor (Humphries et al., 2016). Honduras CIALs include both genders and emphasize youth participation through involvement of rural communities and agricultural high schools.

Collaboration with NARs from Central America and the Caribbean through the CAC-BRN and PPB-Mesoamerica networks has facilitated the development and testing of improved breeding lines and release of improved cultivars, which have benefited small farmers by increasing bean yield and stability. The opportunity to provide new and improved breeding lines every year has made it possible to maintain a continuous flow of improved bean germplasm through the SISTEVER (nurseries and trials regional system) for testing in specific bean production regions.

The valuable and continuing collaboration of scientists from CIAT, the University of Puerto Rico and Nebraska, Michigan and Penn State Universities and the USDA/ARS/TARS of Puerto Rico, has allowed research in Central America to achieve many long-term objectives. In Honduras, the collaboration of DICTA, NGOs and farmers from CIAL organizations has made possible many achievements as the result of bean research activities. Farmer collaboration is extremely important for the development of better-adapted bean cultivars and their wider adoption today and in the future.

More than 60 improved cultivars have been released in Central America and the Caribbean region by Zamorano University and regional and US collaborators mentioned above. Adoption of small red cultivars has been successful in Central America and some cultivars were released in several countries under different names (Rosas et al., 2004). 'Tio Canela 75' was originally released in Honduras (1996), and later on as 'CENTA 2000' in El Salvador, 'INTA Canela' in Nicaragua and 'Rojo Chiricano' in Panamá. 'Amadeus 77' was first released in Honduras (2003), and later on as 'CENTA San Andrés' in El Salvador, 'INTA Rojo' in Nicaragua, 'Cabecar' in Costa Rica and 'IDIAP IR-3' in Panama.

Adoption of Tio Canela, after five years of its release, reached up to 40% in the departments of El Paraiso and Olancho, where almost 50% of the beans are produced in Honduras (Mather et al., 2003). In 2004, 70% of the area planted with beans in the Brunca region of Costa Rica were the improved cultivars Cabecar, 'Bribri' and 'Telire' (Hernandez and Elizondo, 2006). A study conducted in 2010 in Central America to provide estimates of the impact of improved cultivars

develop by Zamorano-BRP, UPR, CIAT and the CAC-BRN network under the Bean/Cowpea CRSP project, suggest that the adoption rate of improved cultivars ranged from 46% in Honduras to 82% in Nicaragua (Reyes et al., 2016). For more than 10 years, CENTA in El Salvador has produced and distributed seed of improved bean cultivars that have disease resistance and greater drought tolerance and heat stress adaptation on an annually basis to more than 100,000 farmers, resulting in high levels of adoption.

In other countries in Central America, dissemination of improved bean cultivars is hindered by the lack of sustainable seed systems that offer good quality seed at the right price, time and place. Formal seed systems in some Central America and the Caribbean countries do not have the capacity to provide the seed in the quantity and quality needed to reach most bean farmers. The general lack of interest of private companies to make investments to obtain higher yields in their bean seed multiplication plots has prevented them to sell bean seed at prices that farmers can afford and still be a profitable business. Formal seed systems need to recognize that local seed production by well-trained farmer organizations can help increase the availability to small farmers of good quality seed at affordable prices.

Researchers at Zamorano-BRP are also involved in the teaching and practical training of undergraduate and graduate students. The program has assisted more than 50 Zamorano graduates and technical personnel of NARs and NGOs in Central America to pursue graduate degrees training in U.S., Europe and Latin America universities. More than 12 graduate students from U.S. universities have conducted part of their bean research in Zamorano. Additionally, the Zamorano-BRP has trained more than 60 technical personnel from NARs and NGOs of Central America and the Caribbean on diverse areas of research. Hundreds of farmers from CIALs and other organizations were trained on germplasm rescue and conservation, breeding and selection, crop and soil management practices and seed production, through short courses, workshops and inservice training at Zamorano facilities. Several training activities on breeding and selection, *in situ* conservation of landrace germplasm and community seed banks, local seed production and conditioning, using learning-by-doing approaches were conduct on farmer fields of participating communities.

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THE PRODUCTION AND GENETIC IMPROVEMENT OF BEANS IN THE CARIBBEAN

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The Caribbean has a long tradition of producing and consuming common (*Phaseolus vulgaris* L.) and lima (*Phaseolus lunatus* L.) beans. Approximately 300,000 ha of dry beans are harvested in the region each year, which is more than twice the area of beans annually harvested in Canada (Figure 1). The largest producers of beans in the Caribbean are Cuba, Haiti and the Dominican Republic (Figure 2). During the past 10 years, harvested area in Cuba ranged between 100,000 and 125,000 ha. Approximately 35,000 ha were harvested annually in the Dominican Republic. The harvested area of beans in Haiti has increased during the past decade from approximately 100,000 to 150,000 ha.



Figure 1. Dry bean area harvested from 2008 to 2017 in the USA, Canada and the Caribbean.



Figure 2. Dry bean area harvested from 2008 to 2017 in Cuba, Haiti and the Dominican Republic. In 2017, the cumulative population of the islands in the Greater Antilles was approximately 38 million (Table 1). An additional 10 million people from the Caribbean live in the U.S. and Canada. Haiti and Cuba annually produced more than 100,000 MT of dry beans or more than 10 kg per capita. The approximately 35,000 MT of dry beans produced in the Dominican Republic represents a per capita production of only 3.5 kg/ha. In 2009, Haitian farmers produced about 80% of the locally consumed beans (Anonymous, 2010). In contrast, imported beans have represented about 50 percent of local consumption in the Dominican Republic (USDA-FAS, 2017). Puerto Rico, Jamaica and other Caribbean countries import almost all their dry beans.

	Country	Population living in the	Population living in	Bean production	Bean production	
	population	U.S.	Canada	in 2017	per capita	
Country	(million)	(million)	(million)	(MT)	(kg)	
Haiti	10.7	1.0	0.2	111,398	10.4	
Dominican Rep.	10.3	1.8		35,777	3.5	
Cuba	11.1	1.7		132,174	11.9	
Jamaica	2.8	1.1	0.3			
Puerto Rico	3.2	5.1				
Total	38.1	10.7	0.5			
Sources: FAOSTAT and World Bank.						

Table 1. Po	pulations, b	bean production	and bean	production p	per capita o	of Caribbean	countries.
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Various strategies are used in the Caribbean to enhance the value of the bean crop. Beans are frequently produced in multiple cropping systems. Intensification and diversification of crop production increases income on small-scale farms and enhances food security. Common and lima beans are often consumed at the green-shelled stage of development. This allows a portion of the crop to be harvested in fewer days and sold at a higher price. Harvesting green-shelled beans also helps to avoid some of the production problems such as excessive rainfall that can occur toward the end of the growing season. Bean straw is often used in the Caribbean as a forage to feed ruminants during the dry season. Farmers produce seed types such as yellow beans in Haiti, red mottled beans in the Dominican Republic and white beans in Puerto Rico that have higher local market value. Collaborative bean research in the Caribbean supported, in part, by USAID led to the development and release of multiple virus resistant red mottled (Prophete et al., 2014), yellow (Beaver et al., 2016) and white (Beaver et al., 2018; Beaver et al., 2008) common bean cultivars.

Considering the greater use of inputs and transportation costs, the carbon footprint of an imported bean is greater than locally produced beans, especially in rural communities. Income generated from beans produced in the Caribbean circulates in the local economy. The importation of beans for consumption in major urban areas of the Caribbean is likely to increase. The importation and consumption of pinto beans, which has gained the reputation as a quick cooking bean, has increased throughout the region.

Although the Caribbean is not considered a center of genetic diversity of the common bean, germplasm from the region has proven useful for the improvement of the crop. Bean germplasm from the Caribbean should be considered as a potential source of unique combinations of genes for traits of economic importance. Many bean landraces from the Caribbean, especially those from

Haiti, and most recently released germplasm and cultivars in the region are derived from hybridization between the Middle American and Andean gene pools (Cichy, 2015; Durán et al., 2005). Examples of valuable bean germplasm from the Caribbean include the red mottled cultivar 'PC-50' released in the Dominican Republic (Saladin et al., 2000). 'PC-50' is a rust differential and was identified by researchers at the University of Nebraska to possess the Ur-9 resistance gene that mapped to Pv01 and the Ur-12 resistance gene that mapped to Pv07 (Jung et al. 1998). Park et al. (2001) reported that 'PC-50' has three OTLs for white mold resistance. 'Pompadour Checa' (G 6616), a red mottled bean landrace from the Dominican Republic, was used by CIAT breeders as a parent in the cross G 6616/SEL 54//G12727 to develop the small red bean cultivar 'Catrachita' (RAB 205). 'Catrachita' was the first cultivar release in Honduras that combined the I gene for BCMV resistance and commercial small red seed color. 'San Cristobal 83' is a Dominican red mottled bean landrace used by Terán and Singh (2002) as a source of drought tolerance. 'Indeterminate Jamaica Red' (PI 163122) is an Andean source of heat tolerance originally collected in India but having a pink striped seed type found in the Caribbean (Román-Aviles and Beaver, 2003). 'Salagnac 90A' is an Al tolerant bean line originally collected by INRA researchers from a market in Kenskoff, Haiti (Chardón-Alcázar, 2004). 'Salagnac 86' is powdery mildew resistant bean line obtained from the same market sample from Kenskoff (Messiaen et al., 1989). L-136 is a root-knot nematode resistant lima bean landrace variety from Puerto Rico (Allard, 1954). L-136 was used as a parent to develop root-knot resistant small-seeded lima bean cultivar 'Cariblanco N' (Helms et al., 2004). L-136 was used as the source of resistance in a study that identified three specific genes for resistance to the root-knot nematode (Roberts et al., 2008). Researchers at Zamorano University in Honduras recently identified a lima bean landrace from Haiti that is resistant to Bean golden yellow mosaic virus. The current number of common and lima bean accessions in the U.S. National Plant Germplasm System from the Caribbean is limited (Table 2).

Origin	Phaseolu	Phaseolus lunatus	
	Total	Cultivars and germplasm released during the past 30 years	Total
Cuba	7	0	4
Dom. Rep.	22	6	0
Haiti	18	2	1
Puerto Rico	60	19	2
Total	107	27	7

Table 2. Number of accessions from the Caribbean in the U.S. National Plant Germplasm

 System.

Zapata et al. (2004) and Miklas et al. (1999) developed and released common bacterial blight resistant bean germplasm lines which derive their resistance from *P. coccineus*. These releases represent unique and underexploited sources of resistance to this important bean disease.
The unique USDA-ARS tepary bean breeding program in Puerto Rico has already led to the release of improved germplasm (Porch et al., 2013). Interspecific crosses hold the promise of significant contributions to the improvement of both common and tepary beans.

Bean producers in the Caribbean face many challenges. Beans are planted in a diversity of soil types. Puerto Rico, for example, has eleven different soil orders that present a wide range of edaphic constraints. The mountainous terrain results in beans being produced in different temperature regimes and rainfall distribution patterns. Increasingly frequent extreme weather events including tropical storms and hurricanes can cause major disruptions in bean production and in the availability of seed. Successful bean cultivars also need to adapt to a range of levels of technology and different cropping systems used to produce the crop. Finally, bean producers in the tropics generally face greater disease and pest pressure.

A major constraint for bean producers in the Caribbean is the availability of high-quality seed of improved cultivars. Local institutions such as the National Seed Service in Haiti do not have adequate resources to produce and distribute enough common bean seed to farmers for production on thousands of hectares. The San Juan de la Maguana Farmers Association, on the other hand, is an example of a successful bean seed production system for a region that produces about 60% of the beans in Dominican Republic. Seed producers in the Caribbean would benefit from a reliable source of high-quality foundation seed produced in the Western U.S. During the summer of 2016, Basin Seeds demonstrated that it was possible to produce in Idaho seed of 'Sankara', which is a multiple virus resistant black bean cultivar released in Haiti (Beaver et al., 2014). Twelve tons of seed was produced, bagged and shipped to Haiti in time to respond to a bean seed shortage caused by hurricane Michael. The successful production of bean seed from the Western U.S. for the Caribbean requires measures that would build confidence between seed producers and potential buyers in the region.

During the past few decades, bean breeding programs in the Caribbean have focused on the development of cultivars with multiple virus resistance. The initial group of improved black cultivars released in the Caribbean such as 'Arroyo Loro Negro' had the dominant I gene for resistance to Bean common mosaic virus (BCMV) (Arnaud-Santana et al., 2000). This was followed by the development Bean golden vellow mosaic virus (BGYMV) resistant cultivars for the Caribbean utilizing breeding lines developed by CIAT/ICTA in Guatemala as sources of resistance. The University of Puerto Rico bean research program collaborated with researchers from the USDA-ARS, the University of Florida and CIAT to study the inheritance of BGYMV resistance and to develop molecular markers for resistance to BGYMV. The black bean cultivars 'Aifi Wuriti' developed at Zamorano University and 'ICTA Ligero' from Guatemala gained popularity in Haiti due to their BGYMV resistance and earlier maturity. 'Erecta 2222', a recent black bean cultivar release in Cuba, has BGYMV and BCMV resistance. The arrival of Bean common mosaic necrosis virus (BCMNV) created the need to develop cultivars for the Caribbean that combined the dominant I and recessive bc-3 resistance genes. Recent releases of cultivars that combine resistance to BCMV, BCMNV and BGYMV include the black beans 'DPC-40' in the Dominican Republic and 'Sankara' in Haiti and the red mottled cultivar PR0737-1 in Haiti.

In recent years, Central American and Caribbean breeding programs have focused on combining multiple virus resistance and other traits of economic importance. Plant pathologists in the

Caribbean have made significant contributions in the identification of pathogens and characterization of their virulence patterns and in the deployment of the most effective combinations of resistance genes (Porch et al., 2104; Rodríguez et al., 2019; Valentín Torres et al., 2016; Vega et al., 2009; Zapata, 1997). Both Middle American and Andean cultivars and bean germplasm and cultivars have been released that combine BCMV, BCMNV and BGYMV with resistance to common bacterial blight (Beaver et al., 2018; Prophete et al., 2014) and rust (Beaver et al., 2015). Recent breeding efforts in the Caribbean also include the development of beans with greater resistance to web blight (Rosas et al., 2018), root rot (Porch et al., 2014), leafhoppers (Porch et al., 2019; Beaver et al., 2016), bruchids (Kusolwa et al., 2016) and greater tolerance to low N soils (Beaver et al., 2018; Porch et al., 2014), higher temperatures (Beaver et al., 2018; Porch et al., 2012; Porch et al., 2010) and drought (Porch et al., 2012).

Finally, improved crop management practices are required to increase the productivity of beans in the Caribbean. Reduced tillage practices appropriate for small-scale farmers are needed that help prevent erosion and conserve water. The importance of timely weeding needs to be emphasized, especially in soils prone to drought or soils with low fertility. Bean producers need decision-making tools concerning soil fertility management and the appropriate use of pesticides. Greater mechanization of production is needed using technologies that are appropriate for small-scale farmers. Improved capacity to store beans on farms would improve food security, allow farmers to obtain higher market prices and increase availability of seed for the next planting season. Training of specialists and research dealing with better management practices for beans is urgently needed throughout Central America and the Caribbean.

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EMBRAPA COMMON BEAN BREEDING PROGRAM: MAIN OBJECTIVES AND OPPORTUNITIES FOR COLLABORATIONS

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Brazil is a major producer and consumer country of common bean worldwide (FAO, 2019). In 2018, around 2.25 million tons of beans were harvested on 1.50 million hectares (Feijão, 2019). Several commercial classes are grown in three distinct growing season, on the majority states of the country. The national market is divided into approximately 70% carioca beans, 20% black beans, and 10% other commercial classes.

The Embrapa common bean breeding program is one of the pioneers in Brazil, being developed for over four decades (Melo, 2009; Faria *et al.*, 2013). From 1984 to 2019, 71 new varieties were released, which currently cover over 50% of the Brazilian seed market share. The Embrapa breeding program objectives include the release of varieties adapted to different production systems and regions in Brazil, with high yield and production stability, besides commercial, culinary, nutritional and functional seed quality. In addition, the program aims to develop varieties resistant to major biotic and abiotic factors restricting production, efficiency in nutrient uptake and use, including efficiency in biological nitrogen fixation. Different breeding methods are used, such as backcrossing, recurrent selection, and modified pedigree and bulk methods. Marker-assisted selection and genetic engineering have also been used for disease resistance and seed quality traits (Melo, 2009; Faria *et al.*, 2013; Souza *et al.*, 2018; Rodrigues *et al.*, 2019).

In addition to the carioca and black commercial classes, varieties from several other market classes have also been developed, including "mulatinho", "roxo", "rosinha", "jalo", "rajado" (medium-sized cranberry-sugar bean), "vermelhinho" (medium-sized red seeded beans), white, cranberry-sugar bean, dark red kidney, light red kidney and calima types, focusing on internal gourmet or international markets.

Technical and/or financial collaborations have been established with 31 research centers and institutions, including 10 Embrapa research centers, nine Brazilian state research institutes, nine universities and three international research institutions (CIAT, Cali, Colombia; USDA, Beltsville, EUA; and NARO, Kampala, Uganda). However, there is room, opportunity and interest in establishing collaborations with the world scientific community to advance knowledge and to develop technical solutions to improve the common bean crop.

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COMMON BEAN BREEDING IN AFRICA: CURRENT STATE AND FUTURE PERSPECTIVES

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Bean breeding in Africa takes place within the fastest changing social context on the planet, with great implications for the objectives of breeding programs. Population continues to grow at a rate of 2% or higher in nearly all bean producing countries, implying the need for rapid increases in yields. Poverty as measured by a criterion of less than US\$1.90 per capita income per day continues above 60% in many countries. Urbanization will reach 50% in East Africa by 2050, with accompanying growth of markets and demands for convenience foods. Meanwhile child stunting remains above 40%, and anemia afflicts a staggering 50-60% of children under 5 years of age. The contribution of beans within this scenario is one of a source of both nutrition and income.

PABRA, the Pan-African Bean Research Alliance, coordinates bean research activities across 31 countries in East, southern and West Africa in three regional networks, with facilitation by CIAT and the active participation of CIAT scientists. National breeding programs are in varying stages of development, with at least nine programs making crosses and selecting in segregating populations, while other programs are limited to testing finished lines.

Objectives of breeding programs must respond to both current limitations and future demands. Biotic constraints of pests (especially *Ophiomyia* spp.) and diseases (anthracnose, angular leaf spot, common bacterial blight, rust, root rots) continue to be priorities. Marker assisted selection for disease resistance is practiced by outsourcing DNA analysis to laboratories outside of Africa. Among abiotic constraints, drought and soil fertility are foremost limitations, while high temperatures will be a severe problem within three decades. Support is rendered from CIAT headquarters in Cali, Colombia with introgression from tepary beans for high temperature and drought, and from scarlet runner bean for soil constraints.

In the 1980's, climbing beans for mid-altitudes were introduced from CIAT with early spectacular results; yields were tripled over bush beans. Farm families with extreme limitations on land availability and living on as little as half a hectare found relief with climbing beans. However, experience over time suggests that when farmers are not drastically limited by land availability, the cost of staking and of labor investment in climbing beans limits their adoption, and farmers prefer to continue to plant bush beans.

High levels of malnutrition and anemia in partner countries led CIAT to participate in the HarvestPlus program for the nutritional improvement of staple crops, or biofortification. CIAT seeks to increase iron concentration in bean by 44 ppm above the level normally found in local varieties, while 50% of the goal, or a 22 ppm increase, obtains the label of "biofortified". At least 6 African governments have included biofortification in their national food and nutrition security policies, and high iron beans are one of the most common components of a nutritionally improved food basket. High iron beans have been released in several countries, initiating with high iron climbing beans in Rwanda.

A significant fraction of beans are sold in local markets, but there is an increasing trend within PABRA to focus breeding on formal markets through "bean corridors". These are geographically discrete regions characterized by major commercial grain classes, from production hubs, to aggregation hubs, to consumption hubs. This is essentially a refined description of a value chain, with the identification of key actors, and quantified with regard to volumes of grain produced in defined areas, and shipped to areas of consumption. For example, sugar beans (similar to cranberry type) are produced in southern Tanzania, Zambia and Malawi, and flow to markets in Lusaka and Johannesburg, among other places. A major grain trader stands at the center of this effort, to assure an outlet for production, and to facilitate extension among producers. An assured market in turn motivates farmers to invest in improved crop management, which is a major component in increasing yields. The various actors meet on a regular basis in business platforms which are forums whereby supply and demand for beans can be linked together.

Product profiles of six commercial grain types of wide interest (navy; small red; yellow; sugar; red mottled; black) have been agreed with researchers in national programs, and serve to focus breeding efforts and define priorities. However, other locally important grain types may be addressed by national partners.

The breeder in the context of a bean corridor must create varieties that meet evolving market demands. Urban markets require varieties for a nascent food processing industry – a standard practice for mature breeding programs in the United States but a new challenge in Africa. Food processors have evaluated a range of breeding lines and released varieties for quality as "precooked beans". Both urban and rural women prefer the convenience of a fast cooking product. Cooking time is a long term request from consumers in Africa, and is now being incorporated systematically into breeding.

Grain traders require excellent quality on a regular basis to meet demand for beans. This implies the need for varieties that can stabilize production for a continual market flow. This is consistent with conventional breeding objectives of biotic and abiotic stress tolerance, but takes on new urgency in light of climate change.

In light of a rapidly changing social context and the challenges of climate change, an effort is currently underway to develop a foresight exercise with economists and with climate experts. Climate modeling includes experimentation to quantify the effects of drought and higher temperatures on elite genotypes and local materials.

STATUS AND LANDSCAPE OF SNAP BEAN BREEDING WORLDWIDE

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Snap beans are the vegetable cousin to dry beans and share the same basic biology. There are some differences; for example, nutritionally, snap beans have lower protein and carbohydrates but possess certain vitamins that dry beans lack. The do not receive the same funding support for research and their production is not tracked with the same level of scrutiny as dry beans. Snap bean genetic diversity is about as great as it is in dry beans as a whole and we have found that snap beans can be partitioned into eight gene pools (Wallace et al., 2018).

Snap beans share common origins with dry beans, and research problems

Table 1. Traits of importance for snap bean yield,productivity and quality.

Pod traits	Plant traits
Size (sieve distribution)	Pod distribution on plant
Wall fiber	Pod detachment force
Suture strings	Concentration of pod set
Wall thickness	Growth habit
Cross section shape	Lodging
Smoothness	Yield
Color hue	Seed traits
Color uniformity	Immature green seed
Interlocular cavitation	Mature white seed
Freedom from pigments	Slow development
Flavor volatiles	Shape
Texture	Germination & emergence
Resistance to sloughing	Resistant to mechanical &
	imbibitional cracking

overlap, but many aspects of snap bean breeding and genetics related to their use as a vegetable are unique to the crop. They have been selected for low fiber, stringlessness and thick succulent pods. Yield is more complex because of the need to balance with quality. Types for processing have additional requirements such as round pod cross-section, white seed and concentrated pod set (Table 1).

Globally, Phaseolus spp. snap beans are most consumed in developed temperate zone countries.



Figure 1. US snap bean consumption from 1970 to 2013 by type of processing or handling.

In the U.S. overall consumption has remained level but there have been changes in consumption patterns among different types with canned beans in decline, and increase in fresh and frozen compensating for the decrease in canned production (Figure 1). In Asian and other subtropical regions, other species (primarily *Vigna unguiculata*) may fill the snap bean niche. Some statistical agencies in various countries may not differentiate among species so it can be hard to get good statistics on snap bean production and value on a global scale (Myers & Kmiecik, 2017).

The plant breeding landscape differs substantially from that of dry beans with more private sector cultivar development. Applied research in the U.S. is addressed by about 1 ³/₄ FTE in the public

sector and even less in Europe. Three public programs (Cornell University, Oregon State University and University of Wisconsin-Madison) breed snap beans. Public breeding programs outside of the U.S are found in Bulgaria, Brazil, Colombia, Ethiopia, Greece, Kenya and Uganda. Public sector snap bean genetics research is focused on disease resistance (viruses, ALS, anthracnose, rust, BBS, CBB, HB, white mold, root rots); QTL analysis (pod traits, color); breeding methods (cultivar maintenance, selection methods, combining ability); quality (sugars, calcium, flavor volatiles); human nutrition (phenolics); and adaptation to organic systems. Public-private partnerships for snap bean research have been important. For example, the OSU breeding program has conducted industry supported research on *Fusarium* and *Aphanomyces* root rots and flavor volatiles, which has supported the training of three graduate students.

Two research tools that provide a vehicle for cooperative research are screening nurseries and diversity panels. Public programs offer yield and quality evaluation and disease screening nurseries. The second research tool is diversity panels. Two diversity panels have been developed in the U.S.: Bean CAP Snap Bean Diversity Panel (SBDP) and the Snap Bean Association Panel (SnAP), and third one has been developed in Europe. Once genotyped, the diversity panels can be phenotyped against any trait of interest, and the genetic architecture of that trait can be revealed through genome wide association studies. A number of future activities to enhance breeding efforts will include use of diversity panels to identify, characterize and map candidate genes for traits of greatest interest to the snap bean research community. Some of the needs of the snap bean industry include solving the rogue problem. Companies spend thousands of dollars each year roguing seed fields for traits such as reversion to strings and flat pods. Identifying ways to prevent these from happening would increase the efficiency and profitability of seed companies. Genetic diversity remains an understudied topic. There may be Native American beans with interesting vegetable traits and there are distinctive groups – such as the Refugee types – that bear further study and use. I would emphasize that snap beans can be a unique source of germplasm for dry beans. We have found that to be the case for white mold resistance and it may apply to other traits as well.

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CREATING GREATER AWARENESS OF PULSES TO IMPROVE FOOD SECURITY, ENVIRONMENTAL SUSTAINABILITY, RESEARCH FUNDING AND INTERNATIONAL TRADE

Cindy Brown

Global Pulse Confederation

The Global Pulse Confederation is a small organization with a BIG mission. Our mission is to create greater aware of pulses in order to improve: (1) food security; (2) environmental sustainability (3) research funding, and (4) international trade.

The Global Pulse Confederation brought greater awareness and attention to pulses via the successful 2016 UN International Year of Pulses and the recently declared UN World Pulses Day, which takes place February 10 of each year. Yet, despite important achievements resulting from these efforts, pulses remain an underutilized and underappreciated plant protein.

While often labeled a "Superfood" for their ability to address both malnutrition and overnutrition, pulses still suffer from lack of awareness, lack of research investments and lack of support at governmental levels. Funding available for pulses research is a small fraction of the funding provided to cereals crops. And other government support available to pulses such as production subsidies is almost non-existent.

Sixty years ago, one in three people of the world's 3 billion population suffered from malnutrition. Today, "only" one in eight people in the world suffer from malnutrition but a nearly equal number of people suffer from overnutrition. Obesity levels have skyrocketed in countries like the United States, Australia and Mexico as consumers have moved away from traditional healthy diets that featured pulses to eat more ultra-processed foods and meals at quick serve restaurants. Pulses are an important part of the solution to this problem.

We must work together because the people growing most of the world's food are also often the most impoverished. They are small farm holders in Africa, Asia and Latin America struggling to produce crops on degraded lands that are also subject to the extreme impacts of ever worsening climate change. To be successful, we must reach out to governments and private sector members to make them aware of the benefits of pulses for sustainability and economic development. Only by working together will government, industry and the scientific community be able to feed 820 million people who will need more and better food.



To meet UN sustainable development goal 2 of zero hunger and to bring rural farmers out of poverty requires thoughtful, proactive, and committed multi-stakeholder partnerships. Small hold farmers in developing countries lack access to the new technologies and advances that were made in agriculture in more developed countries.

Global agricultural productivity is increasing at a lower rate than the rate of population growth. In addition to climate change, many farmers still lack access to technology and seed, particularly in low income countries. Poor storage facilities and distribution systems lead to food losses and food not being available to those that need it.



While GM technologies reduce crop production risk, there continues to be great fear and emotion surrounding GMO foods, particularly in many of the world's least developed countries. Looking forward, new technologies such as gene editing offer great possibilities. We could see the acceleration of CRSPR and other relatively low-cost gene editing tools make advanced seed more readily available to poor farmers in Asia, Africa and elsewhere.

Governments and the agricultural industry could have done a much better job educating consumers about GMO foods over the last 20 years. Unfortunately, consumers today don't understand the difference between gene editing and gene modification. Science and technology hold the key, but public opinion is paramount to acceptance. Moving forward, it's **critical** that we work together to communicate with consumers and get their feedback.

Working together we can encourage greater research investment about the environmental benefits of pulses for small farm holders; we can help develop new markets and products that create pull through demand for pulses. Working collaboratively, we can facilitate projects that provide improved pulse varieties and economic prospects to farmers. Pulses are the future of food, for the health of the global population and the health of the planet and by working together we can change the world.

IDENTIFICATION AND INTROGRESSION OF DROUGHT AND HEAT ADAPTATION FROM TEPARY BEANS TO IMPROVE ELITE COMMON BEAN BACKGROUNDS

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INTRODUCTION: The production of several crops are negatively impacted by climate change (Beebe et al., 2011). Heat and drought, two of the leading constraints of climate change, significantly limit grain yield in common bean (*Phaseolus vulgaris* L.), one of the most consumed legumes around the world (Beebe et al., 2011). Solutions for these problems can be found through introgressions from the tepary bean (*Phaseolus acutifolius*) into the common bean. The tepary bean is a related species that shows a unique array of genetic traits including high heat tolerance (Porch and Hall, 2013) and water use efficiency (Markhart, 1985; Rao et al., 2013). This research included the first multi-environment selection for drought and heat tolerance in tepary beans to identify prospective tepary parents for improving heat and drought tolerance in common beans through interspecific crosses.

MATERIALS AND METHODS: We identified potential drought-tolerant tepary parents for interspecific crossings by evaluating 22 tepary beans under drought and well-irrigated conditions at three locations: a humid tropical environment at CIAT, Colombia; a semi-arid environment at the University of California, Davis, CA; and a semi-arid climate at the University of Nebraska, Scottsbluff, NE. Field experiments were planted in a randomized complete block design with 2 to 3 replications. Drought treatments involved reducing crop water requirements by 70% between flowering and harvest. We identified potential heat-tolerant tepary parents in trials planted in two hot and humid tropical locations in Colombia (Alvarado, Tolima, and Caribia, Magdalena) and one desert climate in California (Extension Center, El Centro, CA). Average night temperatures were 23C°, 25°C, and 27°C in Alvarado, Caribia, and El Centro, respectively. Yield data were analyzed using SAS and R programs with values reported as BLUPs (best linear unbiased predicted). Data were spatially adjusted in the R program following the methodology published by Rodríguez-Álvarez et al. (2018).

RESULTS AND DISCUSSION

Drought Trials: Drought stress reduced tepary bean yield (kg/ha) at all three locations. Yield reduction was greatest at Palmira (44%) followed by Davis (12%), and Scottsbluff (1%). Yield was lower than expected at Scottsbluff and did not differ significantly between irrigated and drought treatments. Average yield of tepary bean was greatest at Davis under both drought (1,811 kg/ha) and irrigated (2,054 kg/ha) conditions. Yield under the two treatments was correlated at all locations (i.e. genotypes that performed well under irrigated conditions also performed well under drought conditions). TARS-Tep 22, TARS-Tep 23, G40068 and G40173A had the greatest yield under all three drought environments.

Heat trials: Although high temperatures greatly reduced the yield in tepary beans, five genotypes (TARS-Tep 22, TARS-Tep 23, G40019, G40119, and G40036) had yields >750 kg/ha in the

experiments conducted in Colombia. Tep 23 performed exceptionally well under extreme heat in El Centro, CA with a yield of 1,552 kg/ha. DOR 390, a common bean check, did not tolerate the high temperatures as it went from yielding 3,247 kg/ha under optimal conditions to 0 kg/ha under heat stress. SEF 60, an interspecific tepary-common bean, showed some heat tolerance when temperatures did not exceed the 24 °C. However, it yielded 0 kg/ha under extreme heat (27°C).



Figure 1. Evaluation of the tepary bean yield (kg/ha) under drought stress yield vs heat stress

In Figure 1, we identify 3 groups of tepary genotypes: a drought and heat susceptible group (G40111, G40168, and G40284), which was not used in the interspecific crossing program, an intermediate drought and heat tolerant group, which included the majority of the tepary genotypes evaluated, and one exceptional genotype (Tep 23), which was highly drought and heat tolerant. The intermediate drought and heat tolerant genotypes and Tep 23 were included in our interspecific crossing program. These results confirm the capacity of tepary beans to tolerate heat and drought stress and the importance of using them for improving the common bean.

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EFFECTS OF HIGH NIGHT TEMPERATURE STRESS ON POLLEN RELEASE IN LIMA BEAN (*Phaseolus lunatus*) AND THE DISTRIBUTION OF THIS EFFECT IN THE GENE POOL

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INTRODUCTION

Heat stress reduces yields of lima bean (*Phaseolus lunatus*) in the Mid-Atlantic Region of the US. High night temperatures during flowering and seed development reduce or delay pod set, resulting in delayed harvest, split pod sets and lower yield. Breeding heat tolerant small and large seeded lima beans is a goal of the University of Delaware lima bean breeding program. Past greenhouse experiments with a few genotypes indicated that a higher amount of pollen shed onto the stigma and style under heat stress is correlated with higher yield under heat stress, and that there is genotypic variation for this trait. Greenhouse and field experiments were used to screen a diversity panel for amount pollen shed under heat stress to determine if this phenomenon is widespread within the gene pool.

MATERIALS AND METHODS

A set of 131 diverse lima bean genotypes was screened under field and greenhouse conditions. Yield under heat stress was determined in two greenhouse experiments planted on 6 Jul. 2016 and 13 Jul. 2018. Night temperature in the greenhouse was maintained at 27 °C. Vining and bush genotypes were separately arranged in a randomized complete block design with 3 replications in 2016 and 2 replications in 2018. The number of pods and seeds and weight of seeds produced by each plant was determined.

Pollen release under heat stress was determined for the 131 genotypes in a field trial. Bush and vining genotypes were planted in separate but adjacent areas with each type planted in a randomized complete block design with three replications. Plots were 60 cm long with 8 seeds per plot for small seeded genotypes and 5 seeds per plot for large seeded genotypes. Ten flowers per plot were visually evaluated for the quantity of pollen on the stigma and style using a three-level rating scale: no pollen, some pollen, or abundant pollen. Bush genotypes were rated on 17 Aug. 2018 (56 DAP) and vining genotypes on 20 and 21 Aug. 2018 (56 and 57 DAP). Average daytime highs and lows in the 10 days prior to rating date were 32 and 21°C for the bush genotypes and 31 and 21°C for the vining genotypes. Bush genotypes in the 2018 greenhouse heat screen were rated for pollen quantity and keel deformity on 27 Aug. 2018 (50 DAP).

Pollen quantity rating data were analyzed nonparametrically according to the methods described by Shah and Madden (2004) using Proc Mixed (SAS Version 9.4; SAS Institute, Cary, NC). Relative treatment effects (REpollen) and confidence intervals were calculated using a SAS macro, LD_CI. Yield under heat stress was analyzed and least squares means obtained for each genotype using Proc GLM. Regression analysis for the model yield = REpollen was performed using Proc Reg.

RESULTS

Most of the genotypes (93%) were of U.S. origin because much of the germplasm available from outside the U.S. is photoperiod sensitive and does not flower under summer conditions in Delaware. Germplasm was classified as either Andean or Mesoamerican background based on

genotype data. The relative effect for genotype on pollen quantity rating ranged from 0.084 to 0.735 with statistically significant differences between genotypes. Genotypes also produced significantly different yields under heat stress (p<0.0001). Based on linear regression the relative effect for pollen quantity rating (REpollen) under heat stress could be used to predict yield under heat stress; yield = 46.0 x REpollen – 5.7, R2=0.539 (Figure 1).

Genotypes derived from the Andean gene pool generally produced lower yields under heat stress, with many genotypes producing little or no yield. Two Andean genotypes (PI 549493 and PI 549497) produced moderate yields under heat stress and had moderate pollen ratings (Figure 1). Two Andean genotypes had pollen ratings that were in the range of that of the best yielding Mesoamerican genotypes, but they did not produce equivalent yields (PI 549461 and PI 249040). This indicates that factors beyond the quantity of pollen released, such as pollen germination rate, pistil function, or photosynthate partitioning also play a role in determining yield under heat stress. Some Andean genotypes did have higher yield and pollen ratings under heat stress and this germplasm may be source of useful heat tolerance traits.

Among the Mesoamerican genotypes, the three accessions with the highest yield and pollen ratings were PI 347829, PI 347786 and PI 347787. These three lines were collected in California and Arizona and suggests that some germplasm from the western U.S. may be useful in improving heat tolerance in baby lima types. Other heat tolerant genotypes originated from India, Haiti and other U.S. regions. Certain Mesoamerican genotypes were not heat tolerant. Some of these were classified as Mesoamerican based on genotype, but have flower, pod and seed characteristics that indicate they result from crossing between the Andean and Mesoamerican gene pools. Other Mesoamerican accessions with low yield under heat stress have no indication of outcross with Andean germplasm, suggesting that heat susceptibility is not absent from the Mesoamerican gene pool.



Figure 1 Yield in grams per plant versus relative effect for rating of pollen present on the style and stigma. Triangle markers indicate vining genotypes, circles indicate bush genotypes. Green markers are Mesoamerican genotypes and orange markers are Andean genotypes.

REDEFINING IRON NUTRITION FROM THE COMMON BEAN: EVIDENCE FOR MOVING FROM BIOFORTIFICATION TO BIODELIVERY

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The common bean (*Phaseolus vulgaris*) has been formally targeted for biofortification for more than 15 years as beans are widely consumed, and relatively high in Fe content with sufficient



Figure 1: Iron concentrations of genotypes grown in nine locations across three agro-ecological zones in Uganda, field season 2015 (squares) and 2016 (circles).

indicate that utilizing the approach of identifying varieties with high Fe bioavailability maybe a more sustainable approach (Figure 2). Such studies are feasible to accomplish via the use of the established Caco-2 cell bioassay for Fe bioavailability.

Recent reports also indicate that the basic assumptions of biofortification via breeding for high Fe are not met as the average bean Fe

variability to suggest that high Fe can be a tractable trait. However, recent studies indicate that the primary biofortification approach of breeding for high Fe concentration (ie. 85-90 μ g/g) may not be sustainable as Fe content is profoundly influenced by environment and genotype by environment interaction. As illustrated in Figure 1, Fe concentration can be highly variable in Uganda. However, studies of Fe bioavailability from the same samples

Uganda GxE Study



Figure 2: Iron bioavailability of genotypes grown in nine locations across three agro-ecological zones in Uganda, field season 2015 (squares) and 2016 (circles).

concentration of non-biofortified varieties in markets such as east Africa is approximately 70 μ g/g, significantly higher than the assumed average of 50-55 μ g/g and essentially identical to most



varieties that have been released as biofortified (Figure 3). High Fe bean varieties are also known to have higher levels of polyphenolics and phytate that can lower fractional Fe bioavailability and negate the nutritional benefit. Moreover, bioavailability estimates that have guided the high Fe approach are potentially flawed due to the caveats of extrinsic isotopic labeling.

"Biodelivery" may be the best term to describe an alternative approach to improve Fe nutrition from staple food crops that focuses on factors that

enhance Fe bioavailability. Factors such as processing to disrupt the cotyledon cell wall releasing

intracellular Fe, influence of other components in the diet or meal, and traits such as seed coat polyphenolic profiles that enhance Fe uptake rather than inhibit are part of the biodelivery approach. Biodelivery utilizes models such as the Caco-2 cell bioassay coupled with a poultry feeding model to identify, confirm and monitor nutritional gains.



A significant example of utilizing the biodelivery approach for improving Fe nutrition from beans is the recent identification of the enhanced Fe bioavailability from slow darkening pinto bean varieties grown in North Dakota (Figure 4). All varieties in this experiment were relatively average in Fe concentration (approximately 60-65 $\mu g/g$); however, the slow darkening pinto varieties delivered significantly

more bioavailable Fe. These results are significant as they identify varieties with enhanced Fe nutrition that are already in agricultural production with agronomic and appearance traits desired by farmers and consumers. Changes in the seed coat polyphenolic profile associated with the slow darkening trait are likely to explain the enhanced Fe bioavailability. Initial studies indicate that more Fe uptake promoting polyphenols and less inhibitory compounds are present in the slow darkening versus the normal pinto varieties. In vivo studies are planned for confirmation of the above in vitro observations.

Additional examples of the application of the biodelivery approach can also be found in recent literature (see references below), documenting a link between fast-cooking bean varieties and improved Fe bioavailability.

Overall, in contrast to the high Fe biofortification strategy, the biodelivery approach is not based on assumptions. Biodelivery simply utilizes proven screening techniques that identify crop varieties, meal conditions and diet plans that provide more absorbable Fe.

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ON-FARM EVALUATION FOR IRON CONCENTRATION AND IRON BIOAVAILABILITY OF THE FAST COOKING MANTECA YELLOW BEAN (*Phaseolus vulgaris* L.) IN UGANDA

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OBJECTIVES: Yellow and red mottled beans (*Phaseolus vulgaris* L.) are rich in iron and are two important market class sold in East Africa, where farmers select varieties based on yield and enduse quality traits such as seed color and cooking time. Recent evidence shows the fast cooking properties of the Manteca yellow bean are highly hereditable [1], but its iron and iron bioavailability have never been evaluated across multiple production environments. A genotype by environment (GxE) study was conducted in Uganda to evaluate the iron concentration and bioavailability of yellow beans with fast and slow cooking properties.

METHODS: This study compared two fast cooking Manteca genotypes (Ervilha, Cebo) to eight other white, yellow and red mottled genotypes, which included farmer local check varieties NABE 4, NABE15 and Masindi yellow (Table 1). Genotypes were produced as a group across nine on-farm locations in Uganda over two field seasons. Cooking time was standardized with a Mattson cooking device and iron bioavailability was determined with a Caco-2 bioassay, which measures ferritin formation (ng ferritin/mg cell protein) relative to a navy bean reference control (cv. Merlin) as an indicator of iron uptake.

RESULTS: Figure 1A shows that iron concentrations of the cooked beans were highly variable across Uganda with low board sense heritability (plot basis 0.40). Iron concentrations in cooked seed ranged from 41 to 97 µg/g, with a mean of 67 µg/g across the nine production environments. The results in Figure 1B show that iron bioavailability ranged from 8 to 116% of navy bean control and was highly heritable (plot basis 0.80) among this subset of white, yellow and red beans. The fast cooking white (Blanco Fanesquero) and two Manteca yellow beans consistently had the highest iron bioavailability (64 – 116% of control) across all locations in Uganda. There was no significant relationship between iron concentrations and iron bioavailability (r = -0.091, p = 0.23). Instead, iron bioavailability more associated with seed coat color and cooking time (r = -0.438, $p \le 0.05$) of the white, yellow and red beans produced in Uganda.

CONCLUSIONS: This study demonstrates the high iron bioavailability trait of the two fast cooking Manteca yellow beans are stable across different production environments in Uganda, and that the fast cooking Manteca yellow bean is worthy of germplasm enhancement through the added benefit of improved iron quality after cooking. Low heritability of iron concentrations indicates this trait is sensitive to the different production environments. This study presents further evidence that breeding for fast cooking times in yellow beans is not only a valuable end-use quality trait beneficial to smallholder farmers in Uganda, but could also be a sustainable approach for delivering more bioavailable iron to consumers in East Africa [2].

Genotypes Grow	n in Uganda	a				
Genotype	Gene pool	Origins	Cultivation	Seed type	(m)	Al Mars
INIAP425	Andean	Ecuador	Variety	White	Y LAY	a ler la
Ervilha	Andean	Angola	Landrace	Yellow (Manteca)	White	
Cebo	Andean	Angola	Landrace	Yellow (Manteca)	white	Red Mottle
PI527538	Andean	Burundi	Landrace	Yellow (Njano)		
Amarelo	MA	Angola	Landrace	Yellow (Amarillo)	0000	MO.
Chijar	MA	Puerto Rico	Landrace	Red mottled	Sova Niano	Amarillo
Maalasa	Andean	Tanzania	Landrace	Red mottled		
PR0737-1	Admix	Puerto Rico	Variety	Red mottled		
Rozi Koko	Andean	Tanzania	Landrace	Red mottled	Morr	
NABE 04			Variety	Red mottled	Mantana	Conomi
NABE 15	LOCAL	CHECKS	Variety	Red mottled	Manteca	Canary
Masindi Yellow			Landrace	Yellow (Canario)		

 Table 1. Description, Gene pool, Origins and Culivation of the Ten

 Genotypes Grown in Uganda



Figure 2. Scatter plot showing the iron concentrations (**A**) and iron bioavailability (**B**) of genotypes grown in nine locations across three agro-ecological zones in Uganda. Each value represents the mean of two field replicates per genotype from each location for field season 2015 (squares) and 2016 (circles; n = 18). Local checks Masindi yellow and NABE 4 are distinguished from NABE 15 by their respective yellow and pink colors. Blue bars indicate mean iron concentration for each genotype. Blue line indicates the overall mean for all ten genotypes across the 2015 and 2016 field seasons (n = 179).

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WHITE AND YELLOW BEANS (*Phaseolus vulgaris* L.) PROCESSED INTO HEAT TREATED FLOUR ENHANCES THE IRON BIOAVAILABILITY OF BEAN-BASED SPAGHETTI

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INTRODUCTION: Dry beans (*Phaseolus vulgaris* L.) are primarily sold as uncooked or canned whole seed products, but food manufactures are seeking to expand the use of beans in food products by processing them into flour. Processing techniques can disrupt the cotyledon cell walls of beans, allowing digestive enzymes greater access to their intercellular stores of micronutrients such as iron during digestion. Polyphenols responsible for the seed coat color of beans are also important factors that change the appearance and nutritional quality of bean flour after processing. This study evaluated the iron bioavailability of seven bean varieties with different seed coat colors (white, yellow, cranberry, red, black) after processing into spaghetti pastas, which were formulated with heat treated bean flour as the major ingredient. To evaluate the nutritional value of bean flour ingredients, this study compared bean-based pastas (broken cell walls) to their boiled bean counterparts (intact cell walls), and to a durum wheat spaghetti control, which was formulated under the same conditions as the bean-based pastas.

METHODS: Commercially produced dry bean varieties with contrasting seed coat colors (Figure 1) were processed into bean flour for the formulation of bean-based spaghettis at a locally owned pasta making facility (Eataliana Homemade Pasta, Shelby Township, MI, USA). Pasta prototypes were formulated with heat treated bean flour (90%), tapioca starch (9%) and xanthan gum (1%) before extruding into spaghetti (Pama Roma, Model P/30, Italy). Iron concentrations were measured with ICP-AES and iron bioavailability was determined with a Caco-2 bioassay, which measures ferritin formation (ng ferritin/mg cell protein) as an indicator of iron uptake.

RESULTS: Table 1 shows the iron concentrations varied significantly ($p \le 0.05$) between the bean-based spaghettis, and reflected the genotypic concentrations of their whole bean counterparts. Overall, iron concentrations were significantly ($p \le 0.05$) affected after processing the seven bean varieties into spaghetti, and the small losses of iron can be mostly attributed to the bean flour ingredient being 90% bean. The results in Figure 2 show that after processing into spaghetti, the iron bioavailability of white and yellow bean varieties (Snowdon, Alpena, Samurai, SVS 0863) increased significantly ($p \le 0.05$), with values 3 – 4 times higher as compared to their boiled whole bean counterparts. The iron bioavailability of white and yellow bean-based pastas (16 – 32 ng ferritin/mg protein) was also significantly ($p \le 0.05$) higher as compared to a durum wheat spaghetti (5.6 ng ferritin/mg protein). No improvements in iron bioavailability were observed after the cranberry (Etna), red kidney (Red Hawk) and black (Zenith) bean varieties were processed into spaghetti pasta (Figure 2B).

CONCLUSIONS: Spaghettis formulated with flour from all seven dry bean varieties tested in this study were rich in minerals including iron, but the benefits of improved iron bioavailability after processing appears to be limited only to the market classes with white or yellow seed coat colors. Iron bioavailability of bean-based pastas was not dependent on their iron concentrations, but rather appears to be associated with the breaking of the cotyledon cell walls after processing. Low iron bioavailability of the dark colored bean varieties and their bean-based pastas suggests that seed

coat color compounds may have a negative impact on the absorption of iron from foods prepared with these products.

Figure 1. Photographs depicting the different sizes and seed colors of the seven dry bean varieties used to generate heat treated flour for bean-based spaghetti.



Table 1. Iron Concentrations of Whole Beans and Bean-Based Spaghettis After Cooking.¹

	Iron (µg/g)				
Variety (market class)	Whole Bean	Spaghetti			
Snowdon (white kidney)	66 ^{cd}	66 ^{cd}			
Alpena (navy)	72 ^{ab}	69 ^{bc}			
Samurai (Otebo)	64 ^{de}	60 ^e			
SVS 0863 (Mayocoba)	74^{a}	72^{ab}			
Etna (<i>cranberry</i>)	66 ^{cd}	61 ^e			
Red Hawk (red kidney)	67^{cd}	64 ^{de}			
Zenith (black)	66 ^{cd}	63 ^{de}			
Wheat (durum)		36 ^f			

¹Values are means of four replicate samples per variety. Means sharing the same letter are not significantly different at $p \le 0.05$. Total iron concentration measured as micrograms per gram of cooked lyophilized/milled whole seed or cooked lyophilized/milled bean spaghetti (dry weight).



Figure 2. Iron bioavailability expressed as Caco-2 cell ferritin formation (ng ferritin/mg cell protein) of the three white bean varieties and their corresponding bean-based spaghettis (**A**), as well as the four colored bean varieties and their corresponding bean-based spaghettis (**B**). Values are the means (\pm Standard Deviation) of six measurements from each variety. Blue hyphenated line indicates the iron bioavailability of a non-fortified durum wheat pasta control extruded, cooked and processed in the same manner as the bean-based spaghettis. * Significantly ($p \le 0.05$) higher Caco-2 cell ferritin formation when compared to their whole bean counterparts after cooking. ** Significantly ($p \le 0.05$) lower Caco-2 cell ferritin formation in the Red Hawk pasta when compared to whole bean after cooking.

EXPLORING COMMON BEAN ROOT RESPONSE TO FUSARIUM ROOT ROT IN A MIDDLE AMERICAN X ANDEAN POPULATION

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INTRODUCTION

Middle American genotypes tend to have shorter taproots, fewer basal roots, but more adventitious roots than the Andean counterparts¹. Root architecture has been used in many species, including dry beans, for ideotype based breeding². There may also be a root ideotype that supports resilience to root rots. There has been ample evidence over the last 20 years that indicates root architecture may influence root rot in multiple legume species with various root pathogens. This includes co-localization of loci regulating root traits and root rot resistance or strong negative correlations between root rot scores and root traits^{3–6}. We had previously screened an Andean by Middle American recombinant inbred line (RIL) population for FRR resistance⁷. Susceptible lines were leggy and less robust than their resistant counterparts. A QTL for root biomass and FRR resistance co-localized on chromosome two. For these reasons, this population is a reliable resource for studying the influence of root architecture on FRR resistance.

MATERIALS AND METHODS

A subset of the most consistently resistant and susceptible lines from the population and planted them in three research farms at Michigan State University. One of the fields also had Fusarium inoculated-sorghum tilled into the soil to increase disease pressure. We dug out the plants using the established "shovelomics" techniques at the three time-points (growth stages) recommended by CIAT for FRR evaluation^{8,9}. Traits measured include disease severity, fibrosity, lateral root density, stem width, hypocotyl width, taproot width, basal root width, basal root number, adventitious root number, and distribution of adventitious roots. Spearman correlations of calculated root related phenotypes to disease severity scores.

Plants were grown in the greenhouse for one week in vermiculite and either treated with *Fusarium brasiliense* (same as in the field) or mock-treated (water). One week after inoculation, roots were washed and scanned on an Epson V550 flatbed scanner. Root traits were measured using ImageJ.

RESULTS AND DISCUSSION

Root traits were measured at each growth stage for each field location, and then compared to disease severity scores to identify traits that correspond to low disease scores. In 8 out of 9 experimental combinations, the resistant lines had lower disease scores than the susceptible. Plants at the V1 stage shows frequent negative correlations while R7 has more frequent positive. Scores at the R7 stage likely represents disease progression. At this stage, stem and hypocotyl width were noticeably swollen if infected and lateral root density was also reduced. Significant correlations relate to resistance as early as the V1 stage. Basal root number, taproot width, and adventitious root distribution were all significantly correlated at the V1 stage. These phenotypes may represent potential breeding targets. For this reason, two correlation networks were created using root traits

for the resistant lines and for the susceptible lines to test for fundamental differences between a resistant and susceptible root system at the V1 stage. The root matrices were significantly different from one another (p<0.001). Root phenotype relationships that differed significantly between resistant and susceptible matrices occurred most frequently between basal roots, including the traits fibrosity, lateral root density, and stem width, basal root width and adventitious root distribution.

The resistant and susceptible RILs were evaluated in the greenhouse to better assess basal root traits at the V1 stage. A number of root phenotypes were evaluated, including the length of apical unbranched zone (LAUZ) which drives root system growth. Shorter LAUZs and faster growth rates are related to FRR resistance. Resistant lines were found to have an even distribution at the root crown; susceptible lines have uneven spacing between roots.

In short, root architecture can be a complementary breeding target for improving root rot tolerance. Root growth in early season (V1) is the time for screening root architecture traits against root rot symptoms. Basal root number and distribution are breeding targets. Shorter unbranched zones may prove an easy-to-screen phenotype for resistance.

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IDENTIFICATION OF RESISTANCE GENES OF COMMON BEAN LINE MAIII -16.153 TO Pseudocercospora griseola

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INTRODUCTION

The angular leaf spot, caused by the fungus *Pseudocercospora griseola*, is one of the main diseases of common bean (Nay et al., 2019). The most effective strategy for control is the use of genetic resistance (Nelson et al., 2018). Therefore, it is important to identify resistance genes for use in breeding programs. An alternative to accumulate several resistant alleles to *P. griseola* including those of small effect is by use of recurrent selection. The line MAIII-16.156, obtained from the recurrent selection program for ALS conducted by the Universidade Federal de Lavras in partnership with Embrapa, has shown high resistance to *P.griseola* strains (Amaro et al., 2007; Arantes et al., 2010; Nay et al., 2019). During the Common Bean Disease Workshop on Angular Leaf Spot and Root Rot, held in Skukuza, South Africa, in 2015, this strain was indicated to compose the new international set of differentiating cultivars for ALS (Souza et al., 2016). Despite the high level of resistance presented by this line, there is no information about the resistance genes that it possesses. Therefore, the aim of this work was to identify the number of resistance genes in this line through the evaluation of a F₂ segregating population.

MATERIALS AND METHODS

The BRS Horizonte line (susceptible) was crossed with MAIII-16156 line (resistant) and the F1 and F₂ generations were obtained. The F₂ plants were assessed in four experiments. Two P. griseola strains, race 63-63 and 63-23, were inoculated in plants in V2 and V3 stages. 109 seeds were used for each experiment. The F₂ seeds were sown in trays and 12 days after planting, the seedlings were inoculated with a conidial suspension. The conidial suspension was obtained from colony mycelium discs of P. griseola strains, which were replicated into test tubes containing PDA (potato-dextrose-agar) medium and maintained at 24 ° C in an incubator (BOD) for a period of 12 days. Subsequently, the mycelium was scraped with the addition of 5-10 mL of sterile distilled water in each tube. The suspension obtained was filtered through a layer of cheesecloth. The inoculum was composed of a suspension of conidia at a concentration of 2.0 x 10⁴ conidia mL⁻¹ and the inoculation was performed with manual spray. 12 days after planting, the seedlings were inoculated with a conidial suspension at the V2 stage. After 25 days, the seedlings were inoculated in the V3 stage. Disease severity was assessed 15 days after inoculation using a diagrammatic scale (1 to 9 scores). For the V2 stage, the diagrammatic scale proposed by Librelon et al., (2015) and for V3 stage the 9 level scale proposed by Pastor-Corrales and Jara (1995) (Vital et al., 2006) was used. The seedlings were classified according to their resistance or susceptibility reaction to P. griseola. Seedlings with scores of ALS severity from 1 to 3 were considered resistant and seedlings with scores above 3 were classified as susceptible.

RESULTS AND DISCUSSION

The segregation observed for plants inoculated with isolates of the race 63-63 and 63-23 in the V2 stage were 73:36 and 85:24, approximating the ratio 3R: 1S (Table 1). The segregation observed for plants inoculated with isolates of the race 63-63 and 63-23 in the V3 stage were 78:32 and 85:23, approximating the ratio 3R: 1S (Table2).

Segregation in the F2 revealed a gene of major effect, and that dominant allele is responsible for resistance in the line MAIII -16.153.

Table 1. Segregation observed for plants inoculated with isolates of the race 63-63 and 63-23 in V2 stage

	F ₂ Generation										
Race	V2 Stage										
	O	bs.	Expec.F	req.	\mathbf{v}^2	P-value					
	Fr	eq.			Λ						
	R	S	R	S	-						
63-63	73	36	3:1		3.64	0.062					
63-23	85	24	3:1		0.51	0.472					

Table 2. Segregation observed for plants inoculated with isolates of the race 63-63 and 63-23 in V3 stage

	F ₂ Generation											
Race		V3 Stage										
	Obs.	Freq.	Expec.Freq.	\mathbf{v}^2	Р-							
	R	S	R S	- Λ	value							
63-63	78	32	3:1	0.98	0.321							
63-23	85	23	3:1	0.18	0.668							

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GENETIC IMPROVEMENT OF DRY BEAN FOR RESISTANCE TO WHITE MOLD USING A MAGIC POPULATION

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INTRODUCTION: White mold (*Sclerotinia scleriotorum* Lib. De Bary) is considered one of the most important diseases for dry bean in U.S.A. When susceptible varieties are planted in historically infected fields, seed yield losses can be up to 100%. The use of resistant varieties is the most effective strategy to fight field diseases. For white mold, this has been difficult to incorporate because of the quantitative nature of genetic resistance. This trait is complexly inherited and highly influenced by the environment. The objectives of this research were to identify resistant lines from a Multi-parent Advanced Generation Inter-Cross (MAGIC) population with good agronomic performance and the identification of genetic factors involved.

MATERIALS AND METHODS: The phenotypic data obtained from screening the first set of lines (n=500) from the WM-MAGIC population (n=1070) in 2018 was used for association mapping. A total of 52k SNPs were developed using genotyping-by-sequencing (Schröder et al., 2016). Besides the 1-9 quantitative disease rating scale (Arkwazee and Myers, 2017), the phenotypic data was transformed into two additional classes: binomial and polynomial distributions. GEMMA software (Zhou, 2016) was used to perform the association mapping. Potential candidate genes associated with white mold reaction were searched in a window of \pm 50 Kb upstream and downstream from the significant peak SNP.

RESULTS AND DISCUSSION: Greenhouse screening allowed the identification of 20 lines with high levels of resistance to white mold. GWAS results detected a total of 30 genomic regions associated with the reaction to white mold. New genotype-phenotype associations were detected for all three phenotypic distributions on chromosomes Pv11 (25.67 Mb). Gene model Phvul.011G123500 was located as a potential candidate gene and is an *HSL1* gene, a leucine-rich-repeat receptor kinase (LRR-RK) (Jinn et al., 2000) in Arabidopsis (www.uniprot.org). A second shared region was also found on Pv11: 17.24 Mb using the binomial and polynomial distributions with the same peak SNP. Gene model Phvul.011G117400, annotated as ankyrin repeat protein family, was located at 8 Kb downstream from the most significant SNP. This protein family has been well studied in different crops confirming its involvement in plant resistance against pathogens. Here we are proposing naming Pv11: 17.24 Mb and Pv11: 25.67 Mb regions as WM11.2 and WM11.3 QTL, respectively. Besides the identification of new novel regions, this research also validated QTL WM8.3 reported previously (Miklas and Delorme, 2003; Maxwell et al., 2007; Soule et al., 2011).



Figure 1. Manhattan plots of A) quantitative data B) polynomial data and C) binomial data Mixed Model for WM-MAGIC population resistance to white mold.

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INTERACTION OF *BC-U* GENE WITH RECESSIVE RESISTANCE GENES IN DIFFERENT GENETIC BACKGROUNDS FOR CONTROL OF *BEAN COMMON MOSAIC VIRUS* AND *BEAN COMMON MOSAIC NECROSIS VIRUS*

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INTRODUCTION

Bean common mosaic virus (BCMV) and Bean common mosaic necrosis virus (BCMNV) are two related but distinct seed-borne potyviruses transmitted by aphid vectors. Infection causes severe yield loss in common bean worldwide. Four recessive loci bc-1, bc-2, bc-3, and bc-u, and associated alleles $bc-1^2$ and $bc-2^2$, and the dominant I gene have been associated with BCMV and BCMNV resistance (Drijfhout 1978; Melotto et al. 1996; Miklas et al. 2000; Naderpour et al. 2010). Our objective was to further characterize interactions of bc-u with the other resistance genes.

MATERIALS AND METHODS

The Durango Diversity Panel (DDP) and Snap Bean Association Panel (SnAP) consisting of 180 and 378 accessions, respectively, were screened in the greenhouse for separate reactions to NL-3 (Pathogroup VIa) and NL-8 (PG III) strains of BCMNV. DDP subsets were also tested with US-6 (PG-VII) strain of BCMV. Whole genome sequencing (WGS) and Genotype by Sequencing (GBS) were used to identify SNPs in the DDP (687,782 SNPs) and SnAP (19,888 SNPs), respectively. Other materials: Andean Diversity Panel (ADP), Othello/VAX1 and Othello/VAX3 RIL populations, and $F_{2:3}$ families from multiple crosses among DDP lines, were used to validate gene interactions. SNP markers, tagging each recessive gene, were developed and screened by Tm-shift assay.

RESULTS AND DISCUSSION

GWAS and linkage mapping revealed the location of bc-u and bc-2 recessive genes on Pv05 and Pv11, respectively (Fig. 1 & Fig.2), and narrowed the position of bc-1 on Pv03 (Table 1) and I gene on Pv02. Many interesting and important interactions were observed: I and bc-u showed delayed top necrosis (TN) to NL-8; I and bc-1 exhibited TN to NL-3 and vein necrosis (VN) to NL-8; and I, bc-u, and bc-1 had VN to both NL-3 and NL-8. No alleles at the bc-1 locus were observed. Two different bc-u loci, 3 Mb apart, interacted with bc-2. The bc-u(a) combined with bc-2 exhibited resistance to BCMV US-6 strain but was susceptible to both BCMNV strains. The bc-u(b) with bc-2 was resistant to BCMNV strains but was susceptible to US-6. This latter combination with I gene had a local lesion response to NL-3. No alleles at the bc-2 locus were observed. 83% of Andean lines and most snap beans possess bc-1 for resistance to the NL-8 strain. The breeding goal for these backgrounds should be to incorporate the bc-u(b) gene.



Fig. 1 & Fig. 2. Manhattan plots produced by GWAS analysis with genome association and prediction integrated tool (GAPIT) for resistance to BCMV and BCMNV. Left - bc-u gene positioned on Pv05. Right - bc-2 gene positioned on Pv11.

Table 1. Nine candidate genes defining to the bc-1 gene located between 4.0 to 4.2 Mb on chromosome Pv03 of the *Phaseolus vulgaris* v1.0 reference genome (G19833).

Phaseolus vulgaris	Glycine max	Gene
genes ID	homologues ID	Annotation
Phvul.003G038300		18S Pre-Ribosomal assembly protein GAR2-related protein
Phvul.003G038400	Glyma.02 g122500	ACT domain repeat 5
Phvul.003G038500	Glyma.02 g122400	Putative unknown protein
Phvul.003G038600	Glyma.02G122200	Chaperone DnaJ-domain superfamily protein
Phvul.003G038700	Glyma.02G122000	LRR (Malectin-like) protein kinase family protein
Phvul.003G038800	Glyma.02G121900	LRR (Malectin-like) protein kinase family protein
Phvul.003G038900	Glyma.02G121800	Adenine nucleotide alpha hydrolases-like superfamily
		protein
Phvul.003G039000		pre-mRNA-splicing factor CWC22
Phvul.003G039100	Glyma.02G121700	RING/U-box Zinc finger, C3HC4 type protein

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GENE MAPPING AND MARKER DEVELOPMENT USING YOUR BREEDING PROGRAM: A CASE STUDY ON COMMON BACTERIAL BLIGHT

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INTRODUCTION: One of the biggest challenges a breeding program faces is the allocation of resources. Customer-driven demand puts an emphasis on cultivar development and release in the shortest time span possible. However, basic upstream research to identify underlying genetics and gene function provides invaluable knowledge that can be leveraged during cultivar development. Traditionally, these two avenues of research required different types of populations. Utilizing the same population for both cultivar development and functional genomics would allow consolidation of limited resources.

Common bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) is a world-wide disease that is complexly inherited with both major and minor QTL (reviewed in Miklas et al., 2006). Several major QTL have been identified and are actively selected for in breeding programs using marker-assisted breeding (MAB) including QTL on Pv10 (SAP6), Pv08 (SU91) and Pv06 (BC420). This research looked at the suitability of using the population of genotypes in the advanced and preliminary yield trial stages of NDSU's dry bean breeding program for gene mapping and marker development focusing on CBB.

MATERIALS AND METHODS: The test population consisted of 823 genotypes undergoing yield trials within the NDSU dry bean breeding program. The population included genotypes from both the Andean and Middle American gene pools. Plants were infected in the greenhouse with *Xap* and rated following the protocols and rating scale described on the Bean Improvement Cooperative website (http://www.bic.uprm.edu/?page_id=79). The population was sequenced using the method described by Schroeder et al. (2016). SNPs were identified using the unified genotyper function in GATK 3.6 and filtered for quality and minor allele frequency. For sequence analysis and genome-wide association studies (GWAS), the genotypes were divided by gene pool, Andean (130 genotypes) or Middle American (MA; 693 genotypes).

RESULTS AND DISCUSSION: Over 30,000 SNP were used in GWAS to identify genomic intervals associated with resistance to CBB (Figure 1). Both populations allowed the mapping of CBB resistance. However, the most significant interval on chromosome Pv10, a region known to harbor CBB resistance (SAP6), was considerably smaller in the MA population than the Andean population, (0.18 vs. 1.11 Mb). The smaller interval consisted primarily of a family of *lipoxgenase* genes known to be involved in disease resistance allowing the identification of candidate genes suitable for future testing.

Further analysis of the most significant SNP in the small interval, Pv10:41.66-41.84, within the MA population indicated one SNP allele could be used to reduce the number of CBB susceptible genotypes in the program by 55% with 96.8% efficiency (Table 1). Another significant SNP from an interval on Pv07 could be used to remove 14% of the genotypes with 100% of the genotypes removed exhibiting a susceptible CBB reaction. The SNP on Pv07 is more likely to be adapted for MAB since more genotypes would remain in the breeding program for further testing of other important traits.

CONCLUSIONS: Breeding populations for cultivar development can be used for gene mapping and marker development.



Figure 1. GWAS identified multiple regions significantly associated with the CBB disease reaction within the MA population. The intervals on Pv10 are located in a similar physical location as the MAB marker, SAP6, and the interval on Pv08 is similar to SU91.

Table 1. Utility of select	significant SN	IP identified	through GV	WAS for use in	n MAB for the
NDSU dry bean breeding	program.				

SNP		Dopulation	Disease Rating		Number of		SNP Efficiency in	
		Population			Genotypes		MAB for CBB	
		Occurrence	Unifoliate	Trifoliate	Res.	Sus.	Retain	Remove
S07_28,772,508	CC	84.7%	4.2	4.4	91	463	16.4%	83.6%
	TT	14.1%	5.9	5.1	0	92	0%	100%
S10_41,784,824	AA	33.9%	3.5	3.9	70	152	31.5%	68.5%
	GG	56.7%	5.1	4.8	12	359	3.2%	96.8%

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MINING THE COMMON BEAN MIDDLE AMERICAN DIVERSITY PANEL TO DISCOVER GENETIC FACTORS FOR RESISTANCE TO THE MOST PREVALENT Uromyces appendiculatus RACES IN NORTH DAKOTA

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INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of the most important grain legumes globally. Bean rust, caused by *Uromyces appendiculatus* (Pers:Pers), can result in complete yield loss under favorable conditions. North Dakota produces approximately 35% of US common beans, most notably, pinto, black, great northern and navy market classes which belong to the Middle American gene pool. The appearance of new races of *U. appendiculatus* which overcome the long-deployed *Ur-3* gene is threatening common bean production in North Dakota, and elsewhere. The objectives of this study were to detect novel genomic regions associated with race-specific resistance to *U. appendiculatus* and verify previously reported genomic regions.

MATERIAL AND METHODS

To identify potential sources of genetic resistance, greenhouse screening with races 20-3 and 27-7 (two prevalent races found in North Dakota) was carried out on 290 lines from Middle American Diversity Panel (MDP). *U. appendiculatus* inoculations were performed as previously described (Monclova-Santana et al. 2019). Rust reactions were scored 14 days post-inoculation by measuring ten arbitrarily chosen pustules on primary leaflets of each plant using a 6× Pocket Comparator (Edmund Optics Inc., Cat. #30-585). A genome wide association study was conducted using pustule diameter (reactions where no pustules developed were given a value of 0.1) and MA HapMap consisting of 205,293 SNPs (Oladzad et al., 2019) to detect the presence of genomic regions associated with resistance to *U. appendiculatus* races 20-3 and 27-2 and markers significantly associated with resistance using JMP-Genomics 9 (SAS Institute Inc.).

RESULTS AND DISCUSSION

Approximately 21 and 26% of accessions were resistant to races 20-3 and 27-7, respectively. Five significant genomic regions associated with resistance to race 20-3 were identified on Pv01, Pv04, Pv06, Pv08 and Pv11 (Figure 1a). Four significant genomic regions associated with resistance to race 27-7 were identified on Pv04, Pv06, Pv10 and Pv11 (Figure 1b). Two genomic regions on Pv06 and Pv11 were detected in the same genomic interval for both races. The genomic regions on Pv06 and Pv10 identified during this study are novel. The current research confirms or refines the position of previously identified rust genes on Pv04 and Pv11 (Hurtado-Gonzales et al. 2017; Valentini et al., 2017). The peak observed in our study on Pv04 was located in the 524,536 -554,762 bp interval. Multiple U. appendiculatus resistant genes including Ur-5 and Ur-14 have been mapped on chromosome Pv04 (Miklas et al., 2006). High resolution mapping located Ur-14 at 1,230,785bp on Pv04 (Valentini et al., 2017) and Ur-5 is overcome by race 27-7. Therefore, we conclude that the significant SNPs identified in this study may be novel. Clusters of NLR genes were present on both Pv04 and Pv11 in close proximity to the peak SNPs. The Ur11 confirmed on Pv11 may be highly valuable for developing bean varieties with resistance to multiple U. appendiculatus races, and molecular markers identified in this study will greatly assist in moving this gene into different bean market classes. The identified resistant genotypes and genetic factors

in this study are valuable resources to provide a higher level of resistance to races present in North Dakota and elsewhere.



Figure 1. Manhattan plots from genome wide association analysis of Middle American diversity Panel (MDP) including Mesoamerican and Durango races showing significant marker trait associations for pustule diameter for resistance to *Uromyces appendiculatus* race 20-3 (a), and 27-7 (b). *Phaseolus vulgaris* chromosomes (1-11) are represented on x axis, a –log 10 (p) values are shown on y axis. The red line indicates threshold at significance value of –log10(p)=3 and the purple line indicates threshold at correction of pFDR=0.01.

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REACTION OF TEPARY BEANS TO RACES OF THE BEAN RUST PATHOGEN THAT OVERCOME ALL COMMON BEAN RUST RESISTANCE GENES

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INTRODUCTION

Bean rust, caused by *Uromyces appendiculatus*, is a major disease of dry and snap bean worldwide (Stavely, 1984). Host resistance is an important component of rust management (Mmbaga et al., 1996). However, populations of the rust pathogen comprise an extensive and shifting virulence diversity that could render susceptible all known rust resistance genes in common bean. Conversely, it has been suggested that certain tepary bean (*Phaseolus acutifolius*) accessions are broadly resistant to *U. appendiculatus*. We report here the reactions of 34 tepary beans to eight races of the rust pathogen. Some accessions were highly resistant to all races.

MATERIALS AND METHODS

To test the hypothesis that some tepary beans are highly resistant to the rust pathogen, we selected 34 domesticated, wild, and interspecific accessions of tepary bean for inoculation with six Mesoamerican [13-2 (43), 15-3 (47), 22-6 (49), 31-1 (53), 31-22 (67), and 22-52 (108),] and two Andean [21-0 (72), 37-1 (84)] races of *U. appendiculatus*. Several common bean control cultivars were included with known single rust resistance genes (*Ur-3*, *Ur-4*, *Ur-6*, *Ur-11*). The susceptible common bean Pinto 114 was included in this assay conducted at the USDA-ARS Bean Project in Beltsville, MD. The inoculum of each of the eight races was prepared by suspending uredinospores in a water-Tween 20 solution. The primary leaves were inoculated with each of the races seven days after planting. The inoculated plants were placed in a mist chamber at 100% RH and at about 19° C for 16 hours. Then, the plants were placed in a greenhouse. The reaction of each accession and control was scored 12 days after inoculation. The reaction grades were recorded using the standard bean rust scale of Stavely et al. (1983).

RESULTS AND DISCUSSION

All control common bean cultivars were susceptible to one more of the races of U. appendiculatus used in this study. Conversely, four domesticated tepary bean accessions (G40142, G40148, G40161, and G40237A) and two improved lines (TARS-Tep 22 and TARS-Tep 23) were immune to all eight races (Table 1). The immune reaction with no visible symptoms exhibited by six tepary beans is not known to occur in common bean. Moreover, two domesticated tepary bean accessions (G40274 and G40279) and one wild P. parvifolius (G40264) accession, were also resistant to all eight races; but these accessions exhibited either hypersensitive reactions (HR) with necrotic spots without sporulation, tiny sporulating pustules (TP), and one accession (G40279) also exhibited some immune reactions. The HR and TP reactions do occur on common beans. In addition, one domesticated tepary bean (G40019), one common bean (SEF 10), and five interspecific lines (INB 834, INB 841, VAP 1, VAP 2, VAP 3), were all resistant to the same five races but were susceptible to races 31-22 and 22-52 (Table 1). The other accessions were susceptible to several races. These results show the presence of new and unique rust resistance reactions in tepary beans that maybe conferred by different resistance genes. In summary, nine tepary bean accessions lines (26.4%) were resistant to all eight races of the rust pathogen. Identifying and transferring the rust resistance present in P. acutifolius to P. vulgaris, via bridging parents, is an important future objective.

Genotype	Species			Uron	nyces appe	ndiculatus	Races ^ª		
Genotype	Opecies	13-3	15-3	22-6	31-1	31-22	22-52	21-0	37-1
Pinto 114	P. vulgaris	4,5	5,6	4,5	5,6	4,5	4,5	5,6	5,6
Aurora (Ur-3)	P. vulgaris	4,5	5,4	5,4	2	4,5	2	2	2
Early Gallatin (Ur-4)	P. vulgaris	4,5	4,5	2,2++	4	4	2, 2++	4,5	5,6
Golden Gate Wax (Ur-6)	P. vulgaris	2,2+, 2++	2,2++	4	4,5	4,5	4,5	4,5	2, 2++
PI 181996 (Ur-11)	P. vulgaris	f2	f2	f2	f2	f2, 3	5,4	f2	f2
G 40142	P. acutifolius	1	1	1	1*	1	1	1	1
G 40148	P. acutifolius	1	1	1	1	1*	1*	1	1
G 40161	P. acutifolius	1	1	1	1	1	1	1	1
G 40237A	P. acutifolius	1	1	1	1	1	1	1	1
TEP 22	P. acutifolius	1	1	1	1	1	1	1	1
TEP 23	P. acutifolius	1	1	1	1	1	1	1	1
G 40264	P. parvifolius	2	3	3	2+	2	3	3	2++
G 40274	P. acutifolius	2++	2++	2++	2+	f2, 3	2++	2++	2++
G 40279	P. acutifolius	f2	f2	f2	f2	1	1	f2	f2
G 40019	P. acutifolius	3	3	f2; 3	3	4	4,5	f2	2+
INB 834	interspecific	3, f2	3, f2	3, f2	3	4,5	5,4	f2	f2, 3
INB 841	interspecific	3, f2	3, f2	3, f2	3	5,4	5,4	f2	f2
SEF 10	P. vulgaris	3, f2	3, f2	3, f2	3, f2	5,4	6,5,4	f2	f2, 3
VAP 1	interspecific	3,f2	3,f2	3,f2	3	5,6	5,6	f2	f2, 3
VAP 2	interspecific	3,f2	3,f2	3,f2	3	4,5	4,5	f2	f2
VAP 3	interspecific	3,f2	3,f2	3,f2	3	4,5	4,5	f2	f2, 3
G 40084	P. acutifolius	4,5	4,5	4,5	2	3, f2	3, f2	2	2++
G 40001	P. acutifolius	4,5	4,5	4,5	4,5	f2, 3	f2, 3	f2	3
SMR 139	P. vulgaris	4	4	3,f2	3, f2	4,5	4,5	3,f2	3, f2
G 40036	P. acutifolius	4	4,5	4,5	3, f2	4	4,5	2,2+	f2, 3
G 40119	P. acutifolius	4,5	4,5	4,5	3, f2	4	4	2	2
G 40200	P. acutifolius	4,5	4,5	4,5	4,5	3, f2	4	f2	3
G 40284	P. acutifolius	4,5	4,5	4,5	3	4	4	f2	3
SMR 155	P. vulgaris	4,5	4,5	3,f2	4	4,5	4,5	3,f2	3
G 40022	P. acutifolius	4,5	4,5	4,5	5,4	4,5	4,5	3	3
G 40068	P. acutifolius	4,5	4,5	4	4,5	4,5	4,5	2	2
TEP 29	P. acutifolius	4	4	4	4	4,5	5	f2	2
SMC 214	P. vulgaris	5,4	4,5	4	4,5	5,4	5,4	f2	3, f2
SEN 118	P. vulgaris	4,5	4,5	4,5	4,5	5,4	4,5	3	3, f2
G 40111	P. acutifolius	4,5	4,5	4,5	5,6	4,5	4,5	2	4
G 40173A	P. acutifolius	4,5	4,5	4,5	5,4	4,5	4,5	3	4
TEP 32	P. acutifolius	4,5	4,5	4,3	4,5	5,4	5,4	4,3	3
G 40056	P. acutifolius	NA	NA	NA	NA	4	4	NA	NA
G 40287	P. acutifolius	NA	NA	NA	NA	f2, 3	f2, 3	NA	NA

Table 1. Reaction of tepary and common bean genotypes to Mesoamerican and Andean races of U. appendiculatus

^aRace name: Current (Original): 13-3 (43), 15-3 (47), 22-6 (49), 31-1 (53), 31-22 (race 67), 22-52 (108), 21-0 (72), and 37-1 (84). The first sis races (from 13-3 to 22-520 are are considered Mesoamerican. races 21-0 and 37-1 are considered Andean.

* Most plants were immune but 2-3 plants were susceptible; NA: no evaluated

Evaluation Scale: 1 = Inmune with no visible symptoms. 2, 2+, 2++ = Hypersensitive reaction (HR), necrotic spots (NS) without sporulation; 3 = Tiny sporulating pustules (less than 0.3mm in diameter); 4 = Large sporulating pustules, uredinia 0.3-0.5 mm in diameter; 5 = Large s[porulating pustules, Uredinia 0.5-0.8 mm in diameter; 6 = Very large sporulating pustules with uredinia larger than 0.8mm in diameter. Plants with reaction grades 1, 2, and 3 (types of reactions) are considered resistant; plants with large pustules (grades 4,5, and 6) are considered susceptible.

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TOOLS FOR VISUALIZING AND ANALYZING GENOTYPE, GENETIC, AND GENOMIC INFORMATION FOR *Phaseolus*

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A growing collection of online resources has been developed to facilitate the increasingly dataintensive research in plant breeding and biology. Here, we describe resources associated with the Legume Information System (LIS; https://legumeinfo.org). LIS is a collaborative project between the USDA-ARS and the National Center for Genome Resources (NCGR).

As of early 2020, LIS maintains genome browsers for three common bean assemblies: for Andean accession G19833 (assembly versions 1 and 2) and for Mesoamerican accession UI111. A feature of these browsers is the synteny tracks between these assemblies and other legume species, e.g. soybean, pigeonpea, *Lotus, Medicago*, and cowpea. The synteny comparisons generally show extensive conservation of chromosomal structure among species in this group of legumes - which enables comparison and transfer of research results among these species.

A strength of LIS is the suite of comparative tools that enable traversal between species, across related features – such as homologous genes, genomic regions, and even trait associations. Comparisons can be carried out quite straightforwardly through homology among the "beans" (species in the Phaseoleae), now including six species represented in LIS. Tools that facilitate these linkages include browsers, BLAST targets, gene trees, and other viewers and search tools.

Gene families and trees display explicit relationships among all of the legume species in LIS. For example, the "flowering locus T" gene, which controls the vegetative-to-floral transition, has functional and genomic orthologs many species. The gene identified in Arabidopsis is called *Tfl1* (terminal flower 1); in bean the primary ortholog is called *Fin*; and in soybean the primary ortholog is called *Dt1*. These orthologous genes are shown in legume family L_18BH5B https://legumeinfo.org/chado_phylotree/legfed_v1_0.L_18BH5B (Fig. 1).

The phylogenetic trees are interactive. One can remove or add species (to focus on particular species groups), look at the protein alignment, search and add user-supplied sequences (through the "Annotate your sequences" button on the LIS home page), or link out to various resources from any sequence in a tree (by clicking on a colored dot adjacent to the sequence ID).

An important resource available from the nodes in each tree is the "Genomic Context Viewer" (Fig. 2). The Context Viewer provides views of microsynteny, spanning a number of genes upstream and downstream of the query/focal gene, in all species held within LIS. The basic procedure involves a search of genomes for regions that have similar "genic contexts" as determined by sequence similarity and order of local groups of homologous genes. The genes in candidate regions are then aligned and displayed. In Fig. 2, the columns of colored triangles are homologous genes from regions from legume species, represented by the horizontal lines of triangle symbols representing genes.

Another important feature of LIS is the Data Store. This holds data collections such as genome assemblies, annotations (gene model coordinates and sequences), expression data sets, and variant data. For common bean, the assemblies and annotations of G19833 and UI111 are available, as is the genotype (SNP variant) data from the U.S. bean CAP project.

Germplasm resources for bean are available in several forms - from static data sets in the Data Store, to interactive tools including the germplasm Geographic Information System (GIS) tool for
viewing locations of accessions from the U.S. National Plant Germplasm Inventory System (Fig. 3), and the GCViT tool (Genotype Chromosome Visualization Tool) for comparing variants between selected accessions (Fig. 4). The GCViT tool displays differences between several accessions and a reference accession. GCViT can display any selected set of accessions from the bean CAP SNP variant data.

Two additional multi-purpose tools at LIS are the BeanMine and ZBrowse. BeanMine, an instance of the InterMine system, provides an interface for constructing queries primarily involving lists and regions. For example, the BeanMine can be used to easily find all genes within a genomic region around a GWAS peak, and further filter these by expression. ZBrowse is a specialized genome browser that is configured to display GWAS data from multiple experiments, and to allow comparisons between species, with the aid of synteny information.



Fig. 1 (upper left): part of gene tree for family 18BH5B.

Fig. 2 (upper right): part of Genome Context Viewer around a gene selected from family above. Fig. 3 (lower right): bean accessions in the U.S. NPGS, colored by the photoperiod trait (green insensitive; red sensitive).

Fig. 4 (lower left): haplotype view of accessions: Andean Redhawk (SNP density, right, gray) and differences vs. Andean accessions Redrover, Black Wonder (red, yellow) and vs. Mesoamerican accessions Orca, OAC_Rex (light and dark green).

COMMON BEAN ANTHRACNOSE RESISTANCE CLUSTERS CONTAINING NB-LRR AND RLK PROTEIN DOMAINS ON CHROMOSOMES Pv01, Pv04 AND Pv11

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INTRODUCTION: Bean anthracnose (ANT), caused by *Colletotrichum lindemuthianum*, is responsible for severe yield losses and low quality of common bean grains (Singh and Schwartz, 2010). The most effective strategy to manage this disease is the use of resistant cultivars. There are currently more than 20 ANT resistance genes identified and mapped in common bean chromosomes (Meziadi et al., 2016). Identification of candidate genes for independent ANT resistance genes of different cultivars provides a better understanding about the ANT resistance response. In this study, we investigated candidate genes to each cultivar mapped on clusters 1.1 (Pv01), 4.1 (Pv04) and 11.1 (Pv11) chromosomes.

MATERIALS AND METHODS: To identify the physical position of ANT resistance loci in the reference genome (version 2.1) we performed a BLASTn search in Phytozome using the sequence of the molecular marker (linked to the ANT resistance gene) described in the literature. We also checked for candidate genes flanked by the molecular markers.

RESULTS AND DISCUSSION: Clusters of anthracnose resistance genes on Pv01, Pv04, and Pv11 are shown in Figure 1 and 2. Cluster 1.1 located at the end of Pv01 contains resistance genes of the Andean cultivars AND 277, Hongyundou, Xana, Jalo EEP558, Paloma, Amendoim Cavalo and California Dark Red Kidney (Gonçalves-Vidigal et al., 2011; Campa et al., 2014; Richard et al., 2014; Chen et al., 2017; Lima Castro et al. 2017; Gilio et al., 2017; Gonçalves-Vidigal et al., 2019). Candidate genes encoding kinase proteins, two LRR and proteins with other domains are present in this cluster.



Fig 1. Cluster 1.1 chromosome representation with specific candidate genes within and close to the genomic region where anthracnose resistance genes were mapped. Molecular markers linked to the resistance genes are illustrated in green. Candidate genes that encode kinases are represented in red, NL in dark blue and candidate genes with other domains in black.

Cluster 4.1 at the beginning of Pv04 contains genes of the Mexico 222, BAT 93, Ouro Negro, Crioulo 159, Michigan Dark Red Kidney, and Xana cultivars, which contain various candidate genes encoding NB-LRR and/or other domains (Rodríguez-Suárez et al., 2008; Campa et al., 2009; Gonçalves-Vidigal et al., 2013; Campa et al., 2014; Coimbra-Gonçalves et al., 2016; Murube et

al., 2019). Cluster 11.1 is located at the end of Pv11 containing the resistance gene *Co-2* present in Cornel 49-242 cultivar (Geffroy et al., 1998; Campa et al., 2014) and resistance gene in AB 136 (Campa et al., 2017). The largest amount of typical resistance proteins close to resistance loci were found in this chromosome, mainly NB-LRR. ANT independent resistance genes of different cultivars are encoded by different candidate genes, which encode diverse proteins. These candidate genes may be useful for further studies to validate their function in ANT resistance response, and to understand how they interact with metabolic pathways.



Fig 2. Cluster 4.1 and cluster 11.1 chromosome representations with specific candidate genes within and close to the genomic region where anthracnose resistance genes were mapped. Molecular markers linked to the resistance genes are illustrated in green. Candidate genes that encode kinases are represented in red, NL in dark blue and candidate genes with other domains in black.

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DEVELOPMENT AND VALIDATION OF A MARKER LINKED TO THE *Ur-4* RUST RESISTANCE GENE IN COMMON BEAN

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INTRODUCTION

Bean rust is a major disease of common bean in the world, especially in the Americas and Africa. Molecular markers tightly-linked to disease resistance genes are vital tools for marker-assisted selection (MAS); they accelerate the development of disease resistant cultivars and enable the combination of disease resistance genes (gene pyramiding) needed to attain durable resistance. Most of the published molecular markers tagging common bean rust resistance genes were developed over twenty years ago, without the benefit of genomic knowledge and technologies. Many of these markers were not closely linked to the targeted gene. Thus, there is a need to develop molecular markers that are closely linked and accurately tag the disease resistance genes of interest. The objective of this study was to develop molecular markers closely linked to Ur-4 rust resistance gene of Andean origin. Ur-4 confers resistance to 53 (60%) of 88 races maintained in the Beltsville Laboratory. When Ur-4 is used in combination with other rust resistance genes, such as Ur-5 of Mesoamerican origin, the spectrum of resistance increases significantly up to 90%. Because the Ur-4 gene is of Andean origin, it also provides resistance to rust races of Mesoamerican origin.

MATERIALS AND METHODS

The fine mapping approach included phenotyping with specific races of the rust pathogen, inheritance of resistance studies, and bulk segregant analysis (BSA) combined with highthroughput genotyping using the SNP chip BARCBEAN6K 3. Fine mapping also included haplotype identification from rust phenotyped whole genome re-sequenced bean lines, and customized SNP marker development using the KASP technology. About 400 segregating F₂ plants from the Early Gallatin x Mexico 309 cross were inoculated with races 6-15 (73) and 22-52 (108). Early Gallatin, a snap bean of Andean origin was the resistant parent carrying Ur-4, while Mexico 309, a black-seeded dry bean of Mesoamerican, was the susceptible parent. The following cultivars with known rust resistance genes were included in the inoculation as internal controls of successful rust inoculation: Aurora (Ur-3), Golden Gate Wax (Ur-6), Great Northern 1140 (Ur-7), and PI 181996 (Ur-11), and the susceptible control Pinto 114. All rust phenotype evaluations were made according to Stavely and Pastor Corrales (1989). Segregating F₂ and F_{2:3} populations, the resistant and susceptible parents, and cultivars used as controls were also inoculated with the same races. The BSA was performed using new trifoliate leaves of the resistant and susceptible parents and of F₂ or F_{2:3} populations. Each bulk was generated with DNA from at least eight F₂ plants. These were screened with 5,398 single nucleotide polymorphism (SNP) markers in the BARCBEAN6K 3 Illumina BeadChip. Allele calls from the BeadChip were visually inspected for errors. Additional methodologies were used for developing KASPs markers, fine mapping, statistical analysis, linkage and recombination analysis and haplotype analysis were described in Hurtado-Gonzales et al. (2017). A total of 238 bean cultivars were genotyped with candidate KASP markers closely linked to Ur-4 gene. Bean lines that were not previously known to carry

Ur-4 but resulted positive based on the KASP SNP marker, were further phenotyped with races 49 (22-6), 53 (31-1), 73 (6-15), and 108 (22-52).

RESULTS AND DISCUSSION

Fine mapping determined the precise physical location of the Andean Ur-4 rust resistance gene on chromosome Pv06. A segregation ratio of 3:1 confirmed the presence of Ur-4 as a single dominant gene (p=0.33). BSA analysis identified SNPs positively associated with Ur-4 on a 1.69 Mb genomic region between SNPs at 24,229,643 bp and 25,913,633 bp on chromosome Pv06. Further evaluation of recombinant F2:3 families (570 plants) permitted to narrow the position of the Ur-4 locus to a 200-kb region. Haplotype analysis of this region using 33 whole genome sequenced bean lines including Early Gallatin and Lark (also has the Ur-4 locus), allowed the identification of SNPs highly associated with the Ur-4 locus. Several Ur-4-linked SNP were detected and converted into KASP markers (SS208, SS209, SS210, and SS240). These markers were used to genotype 238 bean cultivars that included Andean and Mesoamerican dry and snap beans with and without the Ur-4 locus, with Ur-4 alone and in combination with other rust resistance loci. This validation revealed that KASP marker SS240, unlike a previously identified molecular marker tagging Ur-4, was highly accurate for the identification of the Ur-4 locus and without false positive or false negative results. Another advantage of this new Ur-4 molecular marker is that it is accurate regardless of the origin of the breeding material (Andean or Mesoamerican), a limitation previously encountered in an older Ur-4 molecular marker (Miklas et al., 1993). Bean line PC50 (Pompadour Checa 50) was also showed to carry Ur-4 based on SS240 (Table 1). Studies are ongoing to learn more about PC50. The molecular marker reported here will be most useful in MAS to develop bean cultivars combining two or more disease resistance genes. This marker will also significantly reduce the time and labor associated with current phenotypic detection of these rust disease resistance genes.

Tabl	e 1. F	Reactions	of sev	ven com	mon bean	cultivars	, used as	s controls	s to fou	r specific	races of the
bean	rust	pathogen	n and	results	of KASE) marker	SS240	tagging	<i>Ur-4</i> .	BB=Ur-4	, AA= <i>ur-4</i> .
HR=1	iypei	rsensitive	respo	nse; S=s	susceptibl	e, R=resis	stance, r	oustules <	< 300 µ	m in diam	eter.

Differential Cultivar	Ur Gene	SS240	22-6	31-1	6-15	22-52
Early Gallatin	Ur-4	BB	2+ (HR)	4,5 (S)	2+(HR)	2+(HR)
Pompadour Checa 50	Ur-9	BB	2+ (HR)	4,5 (S)	2+(HR)	2+(HR)
Golden Gate Wax	Ur-6	AA	4,5 (S)	4,5 (S)	2+(HR)	4,5 (S)
Great Northern 1140	Ur-7	AA	3,f2 (R)	4,5 (S)	4,5 (S)	3,f2 (R)
Aurora	Ur-3	AA	4,5 (S)	2+ (HR)	4,5 (S)	2+ (HR)
Mexico 309	Ur-5	AA	4,5 (S)	3,f2 (R)	4,5 (S)	4,5 (S)
PI 181996	Ur-11	AA	3,f2 (R)	3,f2 (R)	3,f2 (R)	4,5 (S)

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PHENOTYPING IMPROVEMENTS AND QTL MAPPING OF COLOR RETENTION IN CANNED BLACK BEANS

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INTRODUCTION AND METHODS

When black beans are hydrothermally processed prior to consumption, water-soluble anthocyanins are released from the seed coat, resulting in a faded brown color in the cooked product that is undesirable to consumers. Traditional methods of phenotyping for color retention and other quality characteristics of canned beans are time and labor intensive, necessitating improvements.

Two half-sibling black bean recombinant inbred line (RIL) populations segregating for postprocessing color retention were developed from Michigan-adapted black bean lines exhibiting extreme phenotypes for this trait (Bornowski et al., 2017). The commercial variety 'Zenith' (Kelly et al., 2015) and breeding line B12724 maintain a dark black color, while breeding line B14311 becomes light brown once canned and was the common female parent to both RIL populations. The BARCBean6k 3 BeadChip microarray (Song et al., 2015) was used to genotype the RIL populations. The mapping populations were canned using a modified version of the canning protocol developed by Hosfield and Uebersax (1980) and evaluated for color retention and other quality traits. Canned samples were rated by trained panelists using a 1-5 scale for color retention, where a score of 1 was given to samples with light brown seed coats and a score of 5 was given to samples with dark black seed coats. In addition to panelist ratings, the canned samples were weighed, imaged with a digital camera and spectrophotometer, and compressed with a texture analyzer. The digital images were processed in ImageJ (Schneider et al., 2012) using a custom script to measure CIELAB color components (CIE, 2008). Color retention measurements from the trained panel, spectrophotometer, and digital images were compared to each other and mapped as separate traits during QTL (quantitative trait loci) analysis.

RESULTS AND CONCLUSIONS

RILs from both mapping populations exhibited a range of color retention, with minimal segregation distortion. CIELAB color components from the digital images were more strongly correlated to panelist color scores than the color components from the spectrophotometer. Whereas previous studies have used the color component L^* as an indicator of canned black bean color (Wright and Kelly, 2011; Cichy et al., 2014), the color component b^* was generally more highly correlated with panelist color scores regardless of measurement instrument (**Fig. 1**). These results show that CIELAB color components from digital images can accurately estimate the color of canned black bean and that b^* may be more useful at approximating perceived canned black bean color than L^* . All eleven *P. vulgaris* pseudomolecules were constructed during linkage mapping, though marker number and distribution varied substantially across chromosomes between the mapping populations. QTL were identified for color retention and other traits. Color retention QTL

on Pv08 and Pv11 were consistently detected across phenotyping methods, populations, and years, and the Pv11 QTL mapped to a tight region near 52.5 Mb (**Fig. 2**). The QTL identified should be useful for breeders looking to meet consumer demands by improving canned black bean color.



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INVESTIGATION OF THE EFFECTS OF THE SEED COAT NON-DARKENING TRAIT ON AGRONOMIC TRAITS IN PINTO BEAN POPULATIONS

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INTRODUCTION: The beige seed coat background in conventional pinto beans turns brown with age, due to autooxidation of proanthocyanidins. This process is known as afterdarkening or postharvest darkening (PHD) (Basset, 2007; Duwadi et al., 2018). Afterdarkening is conditioned by the J gene in common bean (Phaseolus vulgaris L.) and jj genotypes never darken with age (Basset, 2007). Beans that can retain the beige colour during aging are appealing to the consumer and command a higher price in the market (Marles et al., 2008). To reduce the risk of PHD in pinto beans, the genotype jj has been introduced from 'Wit-rood boontje' (PI439540, USDA National Centre for Genetic Resources Preservation at Ft. Collins, CO) into the genetic background of pinto beans in the bean breeding program at University of Guelph (Erfatpour et al. 2018). Introduction of a gene from a donor parent to improve a cultivar for a specific trait invariably affects other traits due to pleiotropy or linkage drag. In population improvement programs, several traits are usually considered simultaneously. Therefore, it is important to maintain all traits at optimum levels when selecting crops for a trait of interest. There is lack of information about the association between the seed coat ND phenotype and agronomic traits in pinto beans. An understanding of the genetic and phenotypic correlations between the seed coat ND phenotype and agronomic traits, as well as the heritability of traits, provides guidelines for adopting breeding strategies for development of ND pinto bean varieties. The objectives of this study were to: (i) determine variance components and the broad sense heritabilities of the traits under study and (ii) estimate the genetic and phenotypic correlations between the seed coat ND phenotype and important agronomic traits.

MATERIALS AND METHODS: Two populations consisting of 337 F₂-derived F₇ lines were developed from two different crosses. Population 1, which included 110 RD and 66 ND lines was made from a cross between 16-NDP1, a ND genotype, and Stampede, a RD variety. Population 2, which included 117 RD and 48 ND lines was generated from a cross between P13HR088, a ND genotype, and La Paz, a RD variety. 16-NDP1 and P13HR088 were developed at University of Guelph. Field trials were conducted using an alpha-lattice in four environments each with two replicates in 2016 at Elora and Woodstock, Ontario, Canada. F₂-derived F₇ lines were phenotyped for agronomic traits including: days to 50% flowering (DTF), days to maturity (DTM), harvestability (HAR), and yield (YLD), as well as 100 seed weight (100SW) and hydration capacity (HC). Seed coat colour was qualitatively evaluated by visual observation for ANOVA and quantitatively evaluated using a Minolta colorimeter for correlation analysis. The data was analyzed using the MIXED procedure in SAS with line, seed coat phenotype, and pedigree as fixed effects, and rep and location as random effects. Genotypic and phenotypic correlations between traits and the heritability of the traits were estimated using Proc Mixed in SAS (Holland, 2006).

RESULTS AND DISCUSSION: Among the three colour parameters (L*, a*, and b*), the a*, value, which is related to the redness of seed coats, was selected as the main discriminative parameter between the darkening and non-darkening seed coat phenotypes (Fig. 1). There was no significant difference between the RD lines and ND lines for DTF, DTM, and HAR in both populations (ANOVA not presented). RD genotypes showed higher yield and 100SW than ND genotypes, but the differences were only significant in population 2 and population 1, respectively. In both populations, ND individuals displayed higher hydration capacity than the RD individuals. The a* parameter and hydration capacity were significantly negatively corelated. The values for genetic and phenotypic correlations in population 1 were -0.19 and -0.42, respectively, and in

population 2 they were -0.25 and -0.40, respectively (Table 1 and 2). There was a negative but non-significant genetic correlation (-0.15) and a negative, significant, and low magnitude phenotypic correlation (-0.19) between the a* parameter and DTM in population 1. No correlation was found between the a* parameter and other agronomic traits. Estimates of heritability were from 45% to 83% for DTF, 68% to 86% for DTM, 81% to 83% for 100-seed weight, and 59% to 72% for hydration capacity, across two populations. For harvestability and yield, the estimates of heritability ranged from 24% to 53% and 36% to 62%, respectively. Water absorption is negatively correlated with cooking time and can be used as an indirect selection method for cooking time in dry beans (Elia et al., 1997). The higher HC observed in ND genotypes suggests that they may cook faster than regular darkening lines. The presence of high broad-sense heritability values for important traits such as DTF, DTM, 100SW, and HC implies that there are strong contributions of genetic factors on the traits, providing an opportunity to improve these traits in common bean through selection of the best performing lines in the early F₂-F₄ generations. We suggest that additional crosses are required for further investigation of the possible linkages between the gene responsible for the seed coat non-darkening trait and genes that control maturity, yield, and seed size.



Fig. 1 The biplot of observations and variables obtained from colorimeter readings of the seed surface of 176 F₂-derived F₇ lines of 16-NDP1 \times Stampede cross. The observations bordered by blue circle are ND lines, the observations with brown border are RD lines, PC1: the first principal component, and PC2: the second principal component.

Table 1. Genetic (r_g) and phenotypic (r_p) correlations between seed traits and agronomic traits in 16-NDP1 × Stampede population.

valiable a	a*	DTF	DTM	HAR	YLD	SW	HC
$a^* r_p$		0.053	-0.15	0.09	0.08	-0.02	-0.19*
rg		0.02	-0.19*	-0.10	0.14	-0.003	-0.42*
DTF r_p			0.40**	-0.07	0.10	0.08	0.05
rg			0.59**	-0.16*	0.40**	0.11	0.14
DTM r_p				-0.12	0.16*	0.32**	-0.08
r _g				-0.26**	0.31**	0.41**	-0.17*
HAR r_p					-0.03	-0.02	0.01
r _g					-0.03	-0.02	-0.01
YLD r_p						0.22**	-0.13
rg						0.31**	-0.32**
SW r _p							-0.20**
rg							-0.24**

Table 2. Genetic (r_g) and phenotypic (r_p) correlations between seed traits and agronomic traits in P13HR088 × La Paz population.

				emiceee	2414		irem
Variable	a*	DTF	DTM	HAR	YLD	SW	HC
$a^* r_p$		0.09	-0.07	-0.04	0.12	-0.11	-0.25**
rg		-0.039	-0.08	-0.013	0.19*	-0.12	-0.40**
DTF r_p			0.77**	0.18*	0.42**	0.15	-0.19*
rg			0.88**	0.27*	058**	0.16*	-0.22**
DTM r_p				0.27**	0.47**	0.21**	-0.22**
rg				0.39**	0.60**	0.22**	-0.28**
HAR r_p					0.10	0.11	0.06
rg					0.09	0.21**	-0.16*
YLD r_p						0.02	-0.28**
rg						0.007	-0.42**
SW rp							-0.12
rg							-0.08

REFERENCES: Basset 2007; Duwadi et al. 2018; Elia et al. 1997; Erfatpour et al. 2018; Holland 2006; Marles et al. 2008.

A MULTIDRUG AND TOXIN EXTRUSION (MATE), PvMATE8, IS A VACUOLAR TRANSPORTER OF PROANTHOCYANIDIN MONOMERS AND INVOLVED IN SEED COAT DARKENING IN COMMON BEAN (*Phaseolus vulgaris*)

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INTRODUCTION

In common bean (*Phaseolus vulgaris*), proanthocyanidins are responsible for the postharvest seed coat darkening. Proanthocyanidins are a polymer of catechin and epicatechin which is synthesized in the cytoplasm. However, the polymerization occurs in the vacuole, indicating the involvement of vacuolar transporters in the pathway. In *Arabidopsis thaliana* and *Medicago truncatula*, a multidrug and toxin extrusion (MATE) like transporter named *TRANSPARENT TESTA 12 (TT12)* and *MtMATE1*, respectively, have been identified to transport epicatechin 3'-O-glucoside (E3'G) to the vacuole [1, 2]. Here, we report the identification of a vacuolar transporter, PvMATE8, by comparing global gene expression profiles of two pinto bean cultivars, CDC Pintium (regular darkening) and 1533-15 (slow darkening). PvMATE8 shows higher expression in CDC Pintium as compared to 1533-15, is localized in the vacuole and able to rescue the *tt12* mutant phenotype in *Arabidopsis thaliana*. Characterization of vacuolar uptake of E3'G by PvMATE8 is in progress.

MATERIALS AND METHODS

RNA sequencing (RNA-Seq) data of pinto bean cultivars, CDC Pintium and 1533-15 were analyzed for differentially expressed MATEs [3]. Each of the candidate MATEs was cloned into pEarleyGate101 vector for subcellular localization and pEarleyGate100 for Arabidopsis transformation by Gateway cloning technology. Subcellular localization was performed by infiltrating the MATE-vector constructs into Nicotiana benthamiana plants and imaging was done by confocal laser microscopy. Arabidopsis transformation was performed on *tt12* plants by flower dip infiltration method and seeds were screened on Murashige and Skoog's plates containing BASTA. Positive plants were grown and seeds were stained with pdimethylaminocinnamaldehyde (DMACA) for confirming PA accumulation.

RESULTS AND DISCUSSION

Five MATE candidates were identified from differential expression analysis in the seed coat tissues of pinto bean, CDC Pintium and 1533-15. PvMATE1, PvMATE7 and PvMATE8 localize in the vacuolar membranes which was determined by co-infiltrating with vacuolar localization marker (Figure 1). Arabidopsis transformation revealed that only PvMATE8 was able to restore *tt12* to the wild type (Ws-2) phenotype in T2 seeds (Figure 2).

CONCLUSIONS

PvMATE8 was confirmed as an orthologue of Arabidopsis TT12. Further experiments are in progress to identify the biochemical substrates of PvMATE8.



Figure 1: Vacuolar localization of three PvMATEs (Not scale adjusted). Small bubble like structures indicate developing vacuoles.



Figure 2: A. Unstained and DMACA-stained seeds of Arabidopsis wild type (WT) and *tt12*. B. Phenotype restoration in T2 seeds of the *PvMATE8* complemented lines after DMACA staining as compared to *PvMATE1* and *PvMATE7*.

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NITROGEN FIXATION IN COMMON BEAN AFFECTED BY NITROGEN FERTILIZER

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INTRODUCTION

Legumes are able to fix atmospheric nitrogen. Although a legume, common bean (*Phaseolus vulgaris* L.) in usually considered to be a poor nitrogen fixer (Bliss, 1993). Unlike soybeans, bean production does not rely on nitrogen fixing capability. To achieve high yields, bean crops are routinely fertilized with nitrogen. However, the addition of nitrogen fertilizer inhibits nitrogen fixation. It was demonstrated that a small amount of nitrate (<5 mM) promotes nodule formation, while the higher amounts of nitrate inhibit nodule formation (Jiang, unpublished). In addition, plants cannot use all the nitrogen fertilizer that is applied to the soil, which leads to groundwater contamination and air pollution. The objective of this work was to test a selected set of bean genotypes for their capacity to fix atmospheric nitrogen under different nitrogen regimes.

MATERIAL AND METHODS

Replicated trials were conducted on nitrogen-poor soils at the Elora research station (ERS, University of Guelph) in three years (2016, 2017 and 2018). Twenty-two bean genotypes (17 from a Mist x Sanilac mapping population) were evaluated in split-split-plot design with two levels of nitrogen (0 and 100 kg ha⁻¹) and rhizobia (present and absent), added at planting (Farid, 2015). Genotypes were evaluated for nitrogen fixation [measured as nitrogen derived from atmosphere (%Ndfa) in the seed], yield and a number of yield-related traits [flowering, maturity, plant height, harvestability, seed weight and leaf chlorophyll content (SPAD)]. Data were analyzed using the generalized linear mixed models (Glimmix) procedure in SAS (Statistical Analysis System) v.9.4 software (SAS Institute, 2013). The relationships among traits (were analyzed by Pearson's correlation and multivariate (principal component, PCA) analysis. Soil physico-chemical properties (0-30 cm) at the experimental sites and the weather data for the bean growing season (May-October) at ERS were also collected. In addition, Unmanned Aerial Vehicle (UAV) based remote sensing data were collected for the trial in 2018. Drone imagery from six weekly flights were used to calculate various vegetation indices.

RESULTS AND DISCUSSION

Significant differences among genotypes were identified for all analyzed traits in all three years and the level of nitrogen significantly affected most of the traits, including %Ndfa and yield. In contrast, application of rhizobia significantly affected only few traits [%Ndfa, flowering, harvestability and leaf chlorophyll content (SPAD)] and the effect was inconsistent among the years. Significant interactions indicated that bean genotypes responded to nitrogen sources in different ways. Common (**Figure 1**) as well as year- and/or treatment-specific relationships were identified among analyzed traits. SPAD readings can be used as an indication of quality/effectiveness of nitrogen fixation as indicated by significant correlations between SPAD and %Ndfa (Volmann et al., 2011). On the other hand, inconsistent correlations between yield and %Ndfa indicated that breeding for high nitrogen fixation would not necessarily result in high yield. The RN/R ratio for the %Ndfa data, indicated that nitrogen application reduced symbiotic nitrogen fixation (SNF) to different degrees in different bean genotypes. Nitrogen fixation in lines with a larger RN/R %Ndfa ratio was less inhibited by nitrogen fertilizer. This variation suggests that SNF in common bean can be improved through breeding and selection for the ability of bean lines to fix nitrogen in the presence of reduced fertilizer levels. There is potential to use UAV-based vegetation indices to predict yield and indicated by significant positive correlations between the traits (**Figure 2**). In addition, the results of this work indicated that there is a potential to simplify and accelerate breeding for good nitrogen fixing and high yielding bean cultivars indirectly based on SPAD leaf readings, RN/R %Ndfa seed ratios and/or UAV-based vegetation indices. Bean varieties with good nitrogen fixing capacity would be a major advance in profitability for the common bean industry and would significantly improve the ecological footprint of the crop.



Figure 1. Principal component analysis (PCA) of selected bean genotypes - three-year average of treatments. First two PCs explained 62.2% of the variability for the analyzed traits.

Figure 2. Relationships (preliminary - one year data) between yield and various vegetation indices derived from a drone flight at (25.08.2018) day 235 (Julian).

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NITROGEN FIXATION OF DRY BEANS BRED FOR HILLSIDE AND MARGINAL LAND PRODUCTION IN HONDURAS

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INTRODUCTION: Dry beans (*Phaseolus vulgaris* L.) have a beneficial nutritional profile and are an affordable source of protein making them a dietary staple in developing economies. Beans are an important crop in Honduras, however yields are low averaging 717 kg ha⁻¹ (Reyes et al, 2016). Seventy percent of Honduran farmers are subsistence, farming steep hillside plots (15-30°) with nutrient-poor soils, and with limited access to crop inputs including N fertilizers. Dry beans form symbiotic associations with *Rhizobium* spp. which convert atmospheric nitrogen into useable plant forms, a process termed symbiotic nitrogen fixation (SNF). The capacity for SNF could be exploited to ensure yields in production systems where alternative sources of N are not accessible. In the early 1990s, farmer participatory research was initiated with farmer groups (CIALs) who identified dry beans as a crop to improve through participatory plant breeding (PPB). In this study, we collaborated with the Fundación para la Investigación Participativa con Agricultores de Honduras (FIPAH), an NGO which works with a large group of CIALs on PPB. Our objectives were to evaluate a panel of bean genotypes for SNF capacity, to determine genetic relatedness among the panel, and to identify promising genotypes for use in breeding to enhance SNF performance.

MATERIALS AND METHODS: The Honduran panel consisted of dry bean genotypes belonging to four breeding history groupings: a) Honduran conventional varieties (n = 7)developed by the bean breeding program at Zamorano, b) landraces, locally termed 'criollo' (n =33), traditionally grown by subsistence farmers in marginal production regions of Honduras, c) PPB varieties (n = 31) developed through collaboration between Zamorano and FIPAH, and d) North American market class check genotypes (n = 6). The Honduran panel was grown in an alpha-lattice design (two replicates/location) in three low-nitrogen, inoculated field trials in Ontario (2014, 2015) and Honduras (2014-2015). Various agronomic and SNF-related parameters were measured in the field and post-harvest. Isotope analyses were performed on ground seed samples and $\delta^{15}N$ values were used to quantify nitrogen-fixing capacity using the natural abundance method (Shearer and Kohl, 1986). The non-nodulating dry bean mutant R99 (Park and Buttery, 1988) was used as the non-fixing genotype in percent nitrogen derived from the atmosphere (%Ndfa) calculations. Analysis of variance and multi-variate analyses were performed in SAS 9.3 (SAS Institute). Single nucleotide polymorphism (SNP) genotyping was performed on 72 genotypes of the Honduran panel at the Québec Innovation Center with the Illumina BARCBean6K 3 BeadChip (Hyten et al., 2010).

RESULTS: The phylogenetic tree (Figure 1) indicates the evolutionary relatedness of the genotypes in the Honduran panel. The landraces group together in the lower branches of the tree, while PPB varieties group together in the upper branches. 'OAC Rosito' (HON63), is more closely related to the PPB varieties than other check genotypes, and 'Dorado' (HON56) groups with the landraces unlike other Honduran conventional genotypes. A STRUCTURE analysis of genetic composition and a PCA analysis returned similar population structure results.

Nitrogen fixation ranged from 14 to 67 %Ndfa A significant genotype x across all trials. environment interaction was observed, and significant differences were found between genotypes and between breeding categories at each trial location. A majority of the landraces had above-average %Ndfa performance in each trial. In breeding category means comparisons, the landrace group consistently had higher %Ndfa performance than the PPB group. Correlations of agronomic traits with %Ndfa were not consistent across locations. For example, yield was significantly correlated with %Ndfa at Elora 2014 (r = 0.38), and while this positive trend was also observed at Yorito, the trend between these traits was negative at Elora 2015. At Yorito, 14 genotypes had above average yield combined with above average %Ndfa; 6 were PPB varieties, 5 were landraces. and 3 were Honduran conventional varieties. Landraces are a promising source of nitrogen fixing capacity and their continued use in participatory breeding could increase yields in nutrient-poor farming systems.

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Figure 1 Phylogenetic tree showing genetic structure and relatedness of 72 genotypes of the Honduran panel. Improved (PPB)•, Landrace (CRI)•, North American check (CHK)•, and Honduran conventional (CNV)•.



A DESCRIPTIVE SENSORY EVALUATION AND VOLATILE QUANTIFICATION OF A DIVERSE GREEN BEAN PANEL

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INTRODUCTION: Green beans (*Phaseolus vulgaris* L.) are a valuable source of micronutrients not found in dry beans, including carotenoids and vitamin C. In the United States, green bean consumption is quite significant at approximately 3kg per person annually. From the consumer's point of view, consumption of healthy foods, such as green beans, is modulated by perceived flavor and other quality traits. Plant breeders have previously shunned complex traits, such as flavor, for traits with a more immediate impact on profitability for the farmer. This is changing though with a renewed interest in industry and seed production on flavor. Previous research has established the importance of 1-octen-3-ol, 3-hexen-1-ol, and linalool to the flavor of green beans, but correlating sensory experience to volatile concentration has not been rigorously established. To this end, descriptive sensory evaluation and Gas Chromatography – Mass Spectrometry (GC-MS) were utilized in parallel to investigate flavor in green beans.

MATERIALS AND METHODS: Two hundred and five bean varieties were selected for sensory evaluation. These included the BeanCAP snap bean diversity panel and 58 landraces from an uncatalogued collection of beans from China that was assembled by Michael Dickson (Emeritus, Cornell Univ., Ithaca, NY). One hundred ninety-four non-wax snap beans were evaluated by eleven volunteers who individually tasted all the bean varieties over 16 sessions spread out over four weeks. Eleven wax beans were also broken out from the collection and evaluated separately, but the statistical power was low, and few significant correlations were found. A resolvable incomplete block design generated using PROC OPTEX in SAS was used to maximize the number of single pairwise occurrences in each session. Eight descriptors were included on the ballot given to panelists: floral, sweet, fruity, bitter, sour, nutty, green, and beany. Linear mixed-effect models were used to determine panel consistency and differences between sensory attributes. The data was normalized on an individual panelist basis by subtracting the mean from an observation and dividing by the standard deviation. Correlations between sensory descriptors and linalool and 1octen-3-ol were performed using the Spearman's rank correlation coefficient. GC-MS was done on a Shimadzu GCMS-QP2010 Ultra Instrument with a Stabilwax column. GC-MS data was analyzed using Shimadzu GCMSsolutions Postrun Analysis software. Mass spectrometry fragment patterns were identified with a NIST/EPA/NIH Mass Spectral Database (NIST 11).

RESULTS AND DISCUSSION: The arithmetic mean concentration of linalool was 6091.3 μ g/L, and the arithmetic mean for 1-octen-3-ol was 127.2 μ g/L. A threshold for human detection of linalool in water is 5.3 μ g/L, and 98% of samples had a concentration higher than 5.3 μ g of linalool. The threshold for 1-octen-3-ol in water is 2x10-3 μ g/L, and 100% of samples exceeded this threshold. Linear mixed-effects models analysis showed interactions between panelist and session in every case, except for the "nutty" descriptor. Interactions between sample and session were seen for sour and beany. Interactions between sample and panelist were seen for sour and green. Interactions with session were also seen for fruity and sour. There was no three-way interaction between session, panelist and sample. The correlation of sweet with fruity and floral (correlation coefficients of 0.77 to 0.39, respectively) is a clear example of congruency between commonly associated tastes and flavors as fruits are often sweet with floral fragrances and would naturally

create this mental association. The correlation between bitter and sour (correlation coefficient of 0.32) is a common confusion in sensory panels and has been identified in numerous other sensory studies. Bitter was positively correlated to beany (correlation coefficient of 0.20) but was negatively correlated to floral, fruity, and sweet (correlation coefficients of -0.14, -0.42, and -0.52, respectively). Linalool was correlated to the floral descriptor and 1-octen-3-ol was correlated to the nutty descriptor (correlation coefficients of 0.16 and 0.21, respectively). The correlation between 1-octen-3-ol and nutty has not been reported before but is consistent with the known presence of this compound in hazelnuts, chestnuts, and almonds. Linalool and 1-octen-3-ol were negatively correlated with each other (correlation coefficient of -0.39). This negative correlation is consistent with early research by Dr. James Baggett and Dr. Tex Frazier at Oregon State University, which showed higher levels of linalool but lower levels of 1-octen-3-ol in 'Tendercrop' snap beans but the reverse ratio in 'Blue Lake' snap beans.



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GENOMIC SHIFTS IN DIFFERENT AGRICULTURAL MANAGEMENT SYSTEMS

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INTRODUCTION: A question that confronts breeders is whether to breed in a target environment or whether selection in a non-target environment will allow sufficient adaptation and productivity in the target environment. Such is the debate among those who breed for conventional and organic environments. Will cultivars bred in conventional environments perform well in organic production systems? Conventional and organic production systems differ in several aspects but primarily in soil fertility and microbiome, and weed and pest control. Conventional systems are more highly managed than organic systems. As such, cultivars grown organically need better genetic against environmental buffering perturbations. The objective of this research was to better understand how might snap beans adapt to conventional or organic environments during the inbreeding process.

METHODS: recombinant Two inbred bean populations, snap OR5630 x Black Valentine (ORBV) and Hystyle x Provider (HYPR), were split after the F₁ generation and grown in parallel organic and conventional systems from the F_2 through F_6 generations. resulting four in populations, two in each system (Table 1). Detailed information about each of the parents can be found in King (2019). Systems treatments (organic and conventional) differed for seed

Year	Gen.	Location	Systems	Generation advance method
2015	F_1	VF	С	Bulk
2016	F_2	LBF	O & C	Single seed descent
2017	F ₃	GH	O & C	Single pod descent
2017	F4	LBF	O & C	Single plant selection
2018	F5	GH & CA	C only	Bulk
2018	F ₆	LBF	O & C	Bulk

Abbreviations: C = conventionally managed; O = organically managed; VF = OSU Vegetable Research Farm; LBF = OSU Lewis Brown Research Farm; GH = OSU greenhouse; CA = Southern California winter nursery.



Figure 2. Segregation distortion patterns in the F_5 generation of OR5630 x Black Valentine (ORBV) and Hystyle x Provider (HYPR) crosses following natural selection in organic and conventional management systems. Blue: regions uniquely distorted in organic, red: in conventional, and yellow: shared regions of distortion. Black regions are either monomorphic or follow expected Mendelian ratios. Chromosome length based on all SNPs on BARCbean6k_3 beadchip. X's indicate midpoint of centromeric region (based on Supplementary Table 6 in Schmutz et al., 2014).

fungicide treatment, fertilizers, herbicides, and pesticides. We genotyped 94 families/population/system) in the F₅ generation with the Illumina SNP BARCbean6k 3

Beadchip. Linkage maps were assembled for all four populations, and segregation distortion patterns and QTLs for important phenotypic traits were analyzed in each.

RESULTS: We hypothesized that selection in the systems would result in genomic shifts. Figure 1 shows the differences in segregation distortion in the F_5 generation of the two populations following natural selection in organic and conventional management systems. Table 2 summarizes QTLs found for five phenotypic traits evaluated in the ORBV population. Interestingly, there was no overlap in SNPs underlying the traits from different production systems.

Trait	Conventional	Organic	Shared between systems	Shared among traits
	No. of QTLs	C	2	
DtFlr	3	5	0	1
DtMat	3	2	0	2
Biomass	2	3	0	2
Yield	3	1	0	1
Seed/plt	1	1	0	0

Table 2. QTL in the ORBV	RI population under natural
selection in conventional &	organic environments

CONCLUSIONS: Maps, QTLS, and segregation distortion showed similarities in populations from the same cross, but also many unique regions for each, most notably in segregation distortion. In the ORBV cross, large differences in maps and distortion between systems were observed, with strong skewing in the organic population in a number of regions towards Black Valentine alleles. In the HYPR cross, there were more similar maps and spread of distortion throughout the genome; both had unique and shared regions of distortion. The parents used for the HYPR cross were more closely related (Wallace et al., 2018) and this resulted in fewer polymorphic markers and smaller maps, which may explain in part the greater similarity between maps from different systems. For both crosses, different traits within population/systems shared the same QTLs but there were no shared QTL for the same cross between systems. Many QTLs were associated with regions of segregation distortion. There were observed differences in map size and recombination, and unique patterns of segregation distortion and QTLs produced from natural selection in each system. These differences support the use of direct selection (selection within the target environment) to capture the desired alleles and traits for each system.

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COSMETIC STAY-GREEN IN SNAP BEAN: UNDERSTANDING DELETERIOUS EFFECTS ON GERMINATION AND EMERGENCE

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INTRODUCTION

The persistent color (pc) trait in snap bean is a member of the cosmetic stay-green gene family and is desired for its positive influence on pod quality. The trait provides a highly uniform dark green color to pods and expresses a light green color in seeds. Currently, approximately 40% of the total acreage in the US is of pc snap bean cultivars (Myers et al., 2018).

Cultivars with this trait have lower rates of germination of unprotected seed compared to whiteseeded and colored-seeded genotypes. Fungicide treatment of pc lines increases the germination and emergence levels to that equivalent to fungicide-treated white-seeded genotypes (Al-Jadi et al., 2016). From this, we hypothesize that soil-borne pathogens are the cause of poor germination and establishment of pc seeds. Why pathogens may locate and colonize pc bean seeds may be related to a higher level of solutes in the surrounding rhizosphere of the seeds. As to why higher levels of solutes may be present, pc seeds may be more fragile, and crack more easily thereby releasing seed solutes into the soil. Researching on why the trait has deleterious effects in germination will help us understand why, which can help in devising solutions to overcoming these effects and thereby increase production of pc cultivars.

MATERIALS AND METHODS

Different seed types including pc, white- and colored-seeded genotypes were compared by germination tests in both the laboratory and the field. The lab germination test was conducted with 39 cultivars, using 25 untreated seeds for each. The field germination was performed with three untreated lines using 30 seeds for each. After planting and every three days three meters from each line was dug and the seed or seedlings evaluated over a period of two weeks.

The rate of water uptake was analyzed using 40 seeds in four replicates of three pairs of isogenic snap bean lines. Two pairs were nearly identical except at the *pc* locus (OSU6523-*pc* and OSU6523-wh and Ulysses [white-seeded] and Spartacus [*pc*], sister lines from Seminis. The third pair comprised a comparison of white- and colored-seed using OR91G-*p* and OR91G- p^{gri} . The p^{gri} locus allows some expression of underlying color genes normally suppressed by *p*. The seeds were situated in beakers in distilled water, then weighted at 90 min. intervals. After imbibition, seeds were dried and examined for cracking.

The same isogenic pairs were compared by electrical conductivity to evaluate solute leaking. Onehundred seeds, divided into two reps, were soaked in 250 ml distilled water for 24 hours at 22°C, after which readings taken by a Thermo Scientific benchtop conductivity meter. Conductivity readings were formulated as micro-Siemens per centimeter or μ S/c to gram of seed weight. The method was modified according ISTA (2005).

RESULTS

The germination tests from the lab with no fungicide treatment and no soil borne pathogens present showed no significant differences among seed types. However, in the field a significantly higher rate of infected seeds (34.8 %) were observed for *pc* genotypes, while the other seed types (white-and colored-seeded) did not show any pathogenic symptoms on seeds or seedlings. Samples from lesions on hypocotyl and cotyledon sections were confirmed to be *Fusarium* and *Rhizoctonia* spp. by the OSU Plant Disease Diagnostic Clinic.

The rate of water uptake of paired snap bean lines did not exhibit significant differences among white and colored-seeded cultivars. However, there was a higher rate of imbibition in *pc* genotypes compared to their white seeded isolines. After imbibition, *pc* types exhibited approximately twice as many cracked seeds as their white-seeded counterparts (Spartacus: 70% vs. Ulysses: 37.5%; OSU6523-*pc*: 17.6% vs. OSU6523-wh: 10%).

The electrical conductivity analysis revealed significantly higher electrical conductivity from pc seeds (Figure 1). Among each pair, the pc genotype always had higher electrical conductivity.

We also found more rapid water uptake and a higher rate of imbibitional cracking in *pc* seeds. The increased rate of cracking may cause the increased electrical conductivity that was observed and indicating that seeds would leak more solutes into the soil which would serve as an attractant to soil borne pathogens who colonize the seed before significant



seedling development. We previously found that pc seeds had a thinner seed coat (Cirak et al., 2019) which may explain the more rapid water uptake and increased seed cracking. Taken together, these results suggest that pc seeds require special handling during harvest and seed conditioning.

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SNP MARKERS ASSOCIATED WITH SLOW DARKENING TRAIT IN CARIOCA COMMON BEAN

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INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is a crop of great economic relevance in Brazil and it is consumed across the entire country. Currently, carioca market class represents about 70% of the Brazilian consumption. A significant challenge for Carioca bean producers and seed dealers is to deal with the darker bean color, which often occurs during storage of the product. In addition, dark Carioca cultivars are an indication of old beans, consequently, they are often rejected for consumption. A single recessive gene expressed in the seed coat through the maternal tissue, regulates genetic control for slow darkening (Silva et al., 2008). For that reason, evaluation of segregating populations for this trait is usually time-consuming. In this sense, identification of molecular markers might improve selection of genotypes for the slow darkening trait. Based on this knowledge, we investigated single nucleotide polymorphism (SNPs) markers associated with slow darkening in the carioca bean line LP97-28.

MATERIAL AND METHODS

A total of 146 $F_{2:6}$ recombinant inbred lines derived from the cross Iapar81 × LP97-28 were developed at the Núcleo de Pesquisa Aplicada à Agricultura (Nupagri) at the Universidade Estadual de Maringá (UEM) with collaboration of the Instituto Agronômico of Paraná (Iapar). LP97-28 is a Carioca bean line characterized by slow darkening (SD) of seed coat, whereas Iapar81 is cultivar with intense seed coat darkening after harvesting (characterized as regular darkening - RD). $F_{2:8}$ recombinant inbred lines (RILS) derived from the cross between Iapar 81 × LP97-28 were cultivated in the field during 2017 year crop season, and seeds were stored in a cold room for 12 months (temperature $10 \pm 2^{\circ}$ C and relative humidity of 48%). After this period, the lines were categorized into two groups:SD lines and RD lines.

SNP genotyping of each RIL was performed with BARCBEAN6K_3 BeadChip at the Soybean Genomics and Improvement Laboratory, USDA-ARS, Beltsville. After filtering process, a total of 773 polymorphic SNP markers for the RIL population were selected for association analysis. To identify polymorphic SNPs associated with slow darkening trait in LP97-28, we carried out a Genome wide association study (GWAS) using TASSEL 5.0 MLM model (Bradbury et al., 2007). Chi-square test (χ^2) was applied to verify the significance of the deviations for the RIL population using the Genes software (Cruz, 2013).

RESULTS AND DISCUSSION

The slow and regular darkening Carioca phenotypes were clearly distinguished after 12 months of storage. The segregation for the 146 families derived the from the cross between Iapar 81×LP97-28 was 83RD:63SD, which fitted to an expected segregation ratio of 1RD:1SD ($\chi^2=2.74$, P-value = 0.0979) for a single gene in a RIL population (Table 1). The 63 families with SD phenotype

were identical to the parent LP97-28, while families exhibiting a RD phenotype were identical to the Iapar81 cultivar.

]	Fabl	e 1.	Segrega	tion	of seed	coat	darkening	of RIL	population	derived	from	the	cross	Iapar81
(RD)	×L	.P97-28 ((SD)	after 12	mon	ths storage	in a co	ld room					

Cross	Observed Plants	Expected Plants	χ^2	P value
C1055	RD:SD	RD:SD	_	
Iapar81 × LP97-28	83:63	1:1	2.74	0.0979

Association analysis of the LP97-28 cultivar revealed that SNPs ss715643259 and ss715640487 were associated with SD at the positions 32,675,523bp and 39,295,293 bp on linkage group Pv07 (Figure 1). *Loci* for SD located on Pv07 were described for the Carioca market class (Alvares et al., 2014) and for Pinto bean cultivars (Felicetti et al., 2012). Based on this knowledge, the use of molecular markers is a promising strategy to improve selection of Carioca genotypes exhibiting the SD trait, as phenotypic selection for SD is a complex process that involves recessive inheritance and expression in maternal tissue.



Figure 1– Manhattan and Q-Q plotshow SNPs associated with slow darkening trait inRILs population from the cross Iapar81 \times LP97-28.

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GENETIC CONTROL OF SLOW SEED COAT DARKENING IN CARIOCA BEAN

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INTRODUCTION

Common bean is one of the most complete and accessible staple foods of tropical and subtropical populations. Among commercial bean groups, carioca is the one that rapidly loses its market value, because during grain storage, chemical changes after harvesting occur and they affect seed coat color and quality (Elsadr et al., 2011). Therefore, seed coat post-harvest darkening is a problem of great concern for bean producers (Araújo et al., 2012). For that reason, genetic control of seed coat post-harvest darkening is an essential approach for breeding programs with the goal to design cultivars with slow grain darkening during storage (Silva et al., 2014). The objective of this research was to study the genetic control of seed coat post-harvest darkening of common bean line LP 97-28.

MATERIALS AND METHODS

Seeds from F_1 and F_2 generations of cross carioca type LP 97-28 (SD - slow darkening of seed coat) × cultivar Pérola (RD - regular darkening of the seed coat) were increased by the common bean Breeding Program at the Instituto Agronômico of Paraná (Iapar), Brazil. Segregating populations in the $F_{2:3}$ and $F_{2:4}$ generations were generated by the SSD method (Single Seed Descent) in a greenhouse (Núcleo de Pesquisa Aplicada à Agricultura- Nupagri, Universidade Estadual de Maringá, Brazil) in the years of 2012 and 2013, respectively.



Figure 1- Post harvest darkening phenotypes: Pérola; LP 97-28; $F_{2:3}$ and $F_{2:4}$ generations for lines 302 and 393.

After harvesting, seeds of the $F_{2:3}$ and $F_{2:4}$ families were maintained in a cold room at 48% relative humidity and at a temperature of $10 \pm 2^{\circ}$ C. We evaluated the seed coat darkening trait of 193 $F_{2:3}$ and 180 $F_{2:4}$ progenies in the year 2019 (Figure 1). To verify genetic control of seed darkening, segregation was tested to define a genetic control model that would better fit the observed frequencies. Chi-square test (χ^2) were applied at 5% level of probability to define goodness of fit for the observed to expected phenotypic ratios for segregation in the $F_{2:3}$ population. Statistical analyses were performed with the software Genes (Cruz, 2013).

RESULTS AND DISCUSSION

We observed a monogenic segregation of 3RD:1SD (140RD:53SD) for the seed darkening trait in populations of cross Pérola × LP 97-28 (Table 1). These results suggest that a dominant allele of the gene responsible for regular seed darkening (RD) controls this trait. On the other hand, slow darkening of seed coat is controlled by a recessive single gene, characterized by LP 97-28 line (SD). According to Felicetti et al. (2012) and Silva et al. (2014), SD is trait expressed by the maternal tissue, thus, determination of F₂ population phenotype from a segregating population demands phenotyping from selfed seed of the F_{2:3} family. Interestingly, SD phenotype of 100% F_{2:3} families remained with the same as in the F_{2:4}. In addition, 14 % (10 out 140) of F_{2:3} families with the RD phenotype in the previous generation. Studies conducted with BRSMG Majestoso (Silva et al., 2008) and BRSMG Madrepérola (Araújo et al., 2012) cultivars revealed that seed coat slow darkening inheritance is controlled by a unique recessive gene. Therefore, future efforts of common bean breeding programs of Nupagri-UEM will focus on the transferring of the slow darkening gene of LP 97-28 line into elite cultivars that do not exhibit this trait.

Table 1- Phenotypic segregation for the slow darkening seed trait in $F_{2:3}$ generation derived from cross between Pérola (RD) × LP 97-28 (SD)

Pérola × LP 97-28	_	Segregation ratio	Obser Rati	rved o	Expected ratio	$-\chi^2$	<i>P</i> value
	Progenies	F ₂	RD	SD	RD:SD	ĸ	
F _{2:3} Generation	193	3RD:1SD	140	53	3:1	0.62	0.43

RD: regular darkening; SD: slow darkening.

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SCREENING ONTARIO-ADAPTED DRY BEAN GERMPLASM FOR REACTIONS TO BACTERIAL DISEASES

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INTRODUCTION: Bacterial blights of common bean include Common Bacterial Blight [CBB; caused by Xanthomonas axonopodis pv. phaseoli (Xap) and Xanthomonas fuscans pv. fuscans (Xff)], Halo Blight [HB, caused by Pseudomonas syringae pv. phaseolicola (PSP)] and Bacterial Brown Spot [BBS, caused by P. syringae pv. syringae (PSS)]. These bacterial diseases favour humid conditions for development, with CBB preferring higher temperature for infection while HB and BBS prefer moderate to cool temperatures for disease development (Bailey et al. 2003). All three of the diseases can be problematic under favourable environments, resulting in yield losses in excess of 40% (Singh and Schwartz 2010). In Ontario, breeding for tolerance to CBB has been a primary focus of dry bean breeding programs. Recent disease surveys and field trial observations have indicated that bacterial disease symptoms have been showing up in cultivars that were considered to be tolerant or moderately tolerant to CBB. Using differential selective media, disease surveys of Ontario dry bean fields indicate BBS is the primary bacterial pathogen found on dry beans, other than CBB. Halo blight is not commonly found in Ontario dry bean disease surveys, however symptoms of the disease are similar to CBB and BBS and therefore testing for this disease was included to gain knowledge about bacterial blight diseases in Ontarioadapted dry beans. The objective of this research project is to gain knowledge about CBB, BBS and HB reactions in Ontario-adapted white, large-seeded coloured and small-seeded coloured dry bean market classes.

MATERIALS AND METHODS: The experimental design was the same for CBB, BBS and HB nurseries and was a randomized complete block with four replications. Each variety was planted as a 'hill plot' of seven seeds with 75 cm between plots within a row and 61 cm between rows. The CBB nursery was planted at Harrow, ON which is typically a warmer location than London, ON where the BBS and HB nurseries were located. Artificial inoculation was carried out twice in each nursery, two weeks apart. CBB inoculum was prepared by mixing equal amounts of Ontario collected isolates; two fuscans isolates 12 and 118, and two non-fuscans isolates 18 and 98. BBS inoculum was prepared in a similar manner mixing two isolates, 39 and 40. HB inoculum was prepared using isolate 41 (PSP race 2) which was provided by S. Chatterton (AAFC-Lethbridge). The final inoculum concentrations for CBB, BBS and HB was 10⁸ CFU/ml. The plots were mechanically inoculated when the plants were approximately 30-40 cm tall using a high-pressure sprayer at a constant pressure of 200 psi. The disease nurseries were irrigated immediately afterwards and were regularly irrigated to encourage disease infection before ratings were completed. Three ratings were made, 10, 17 and 24 days after inoculation (DAI), for CBB and BBS. HB was slower to develop and therefore four ratings were made at 16, 23, 34 and 40 DAI. A 0-5 scale was used for disease severity ratings based on a visual estimate of disease symptoms on total leaf area, where 0 = no symptoms and 5 = more than 80% of inoculated areas showing symptoms. Ratings below two were considered resistant. Dataset was selected for analysis based on largest data variance and analysed using PROC MIXED and PROC CORR procedures in SAS (ver 9.2).

RESULTS AND DISCUSSION: CBB and BBS nurseries provided good symptoms 17 DAI, however the HB nursery was considerably slower to develop symptoms and by 34 DAI more variation in symptoms were visible, but not at high levels relative to the CBB and BBS nurseries. Analysis of disease nursery data indicated that significant correlations exist between bacterial diseases, indicating the possibility of broad resistance to bacterial diseases in white beans (Fig. 1). Small seeded coloured beans showed similar trends to white beans, however, generally the small seeded coloured beans were susceptible to the bacterial diseases (Fig. 1). Large seeded coloured beans showed no significant trends between ratings across different bacterial blight pathogens. Significant variation for disease resistance exists within the white bean market class for all of the bacterial diseases tested. However, large seeded coloured and small seeded coloured bean market classes showed significant variation for BBS only. Large seeded coloured bean varieties are known to be susceptible to CBB, however, several had BBS ratings below 2 which indicates resistance (Fig. 1). Further testing will be completed over the next three years to confirm these results, but this could prove to be important in understanding bacterial resistance in large seeded dry beans. Furthermore, due to the potential difference in environmental range based on optimal temperatures for these pathogens, a BBS resistant large seeded dry bean could be targeted to the cooler regions, where BBS tends to be more prevalent than CBB, providing growers seed production and crop quality benefits.



Figure. 1. Graph of Common Bacterial Blight, Bacterial Brown Spot and Halo Blight data plotted against each other to examine disease resistance relationships within market classes. All disease ratings are based on a scale of 0-5. Each data point is the mean rating for a dry bean variety within the market class.

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EFFECTIVENESS OF RECURRENT SELECTION FOR ANTHRACNOSE RESISTANCE IN COMMON BEAN

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INTRODUCTION: *Colletotrichum lindemuthianum*, the causal agent of anthracnose in common bean, presents great evolutionary potential to overcome genetic resistance (Padder et al., 2017). Although a large number of genes have been identified in common bean conferring *C. lindemuthianum* race-specific-resistance (Meziadi et al., 2016), the development of varieties with durable resistance to anthracnose is still a challenge in breeding programs. Quantitative Resistance Loci (QRL) have also been reported (González et al., 2015), suggesting that resistance to anthracnose may involve more genetic complexity. Recurrent selection has been successfully used for accumulate favorable alleles of different traits in common bean, including resistance to diseases (Rezende et al., 2014; Leite et al., 2016). The objective of this study was to develop and evaluate the effectiveness of a recurrent selection program as an alternative to obtain common bean varieties resistant to different isolates of *C. lindemuthianum*.

MATERIALS AND METHODS: A mixture of 45 F₂ populations (S₀ population), derived from the diallel cross of ten common bean lines, with variability for the reaction to different isolates of *C. lindemuthianum*, was used to form the base population (Cycle 0). From Cycle 0, five cycles of evaluation, selection and recombination were carried out. In each cycle, S₀ plants were selected for resistance to races 65, 73 and 89 of *C. lindemuthianum*. About 40 S_{0:2} progenies from each selective cycle were obtained. The progenies of the five cycles were evaluated for the reaction to isolates of races 65, 73 and 89 of *C. lindemuthianum* in a randomized complete blocks design with 3 replicates, being 9 seedlings per plot. Anthracnose severity was assessed according to the descriptive scale from 1 to 9 (Schoonhoven and Pastor-Corrales, 1987). The mean of anthracnose severity scores of the progenies of each cycle, for each isolate separately, were used to obtain the linear regression equations. The genetic progress (GP) for resistance to each isolate of *C. lindemuthianum* was estimated as follows: GP(%) = $\left(\frac{b_1}{\overline{X}_{Cl}}\right) * 100$, were b₁ is the linear regression coefficient and \overline{X}_{Cl} is the mean of anthracnose severity scores of S_{0:1} progenies of Cycle I.

RESULTS AND DISCUSSION: The mean of anthracnose severity scores of the $S_{0:2}$ progenies decreased over the five recurrent selection cycles (Figure 1). Considering the $S_{0:2}$ progenies of the first cycle, the estimates of selection gain, per cycle, was 10.7%, 9.0% and 8.0% for the isolates of race 65, 73 and 89 of *C. lindemuthianum*, respectively. It was observed that there was a progressive increase in the number of resistant progenies to a greater number of isolates of *C. lindemuthianum* from the first to the last selective cycle. In the first cycle, 14 S_{0:2} progenies were susceptible to the three isolates used. However, in the fifth cycle most of the progenies were resistant to these three isolates (Figure 2). Besides that, it is important to highlight that new sources of genetic resistance can be added in any cycle, so that the host can respond dynamically to the genetic variability of the pathogen. Therefore, the recurrent selection can be an effective breeding method to obtain common bean cultivars with broad spectrum of anthracnose resistance.



Figure 1. Linear regression for the mean of anthracnose severity scores of the $S_{0:2}$ progenies from selective cycles I to V of recurrent selection.



Figure 2. Frequency of progenies with favorable alleles (anthracnose severity scores 1.0 - 3.0) to none, one, two and three isolates of *C. lindemuthianum* in the first and fifth cycles of recurrent selection cycles aiming anthracnose resistance.

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ASSOCIATION MAPPING REVEALS REGIONS ON CHROMOSOMES Pv03 AND Pv05 RELATED TO ANTHRACNOSE RESISTANCE IN COMMON BEAN

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INTRODUCTION: Common bean anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara, is a serious disease that, under disease-promoting conditions, can result in yield losses up to 100 percent. Currently, resistance to *C. lindemuthianum* is attributable to one or more of 20 anthracnose resistance loci identified by the symbol *Co.* They map on eight chromosomes: Pv01, Pv02, Pv03, Pv04, Pv07, Pv08, Pv09, and Pv11 (Meziadi et al. 2016). Genome-wide association study (GWAS) is a tool to perform high-resolution mapping using germplasm collections that have minimal genetic structure. GWAS takes advantage of cumulative recombination generating natural variation in populations. The objective of this study was to perform GWAS analyses on 89 common bean accessions from Brazil inoculated with race 1545 of *C. lindemuthianum*.

MATERIALS AND METHODS: Plant material used in this study is part of the gene bank collection from the Núcleo de Pesquisa Aplicada a Agricultura (Nupagri), Universidade Estadual de Maringá (UEM). Phenotypic evaluation was conducted on six seedlings per accession. When seedlings reached the first trifoliolate leaf stage, they were inoculated with race 1545 at a concentration of 1.2×10^6 conidia mL⁻¹. After inoculation, plants were placed in a mist chamber for 72 hours at $20 \pm 2^{\circ}$ C, with controlled lighting of 12 h of illumination at 680 lux, 12 h darkness, and relative humidity over 95%. Ten days after inoculation, visual evaluations were performed using a scale from 1 to 9. Scores from 1 to 3 indicate a resistant plant, whereas scores from 4 to 9 indicate a susceptible plant (Pastor-Corrales, 1991). A GBS assay was performed on 89 accessions (29 Andean accessions and 60 Mesoamerican accessions), with library preparation performed with CviAII digestions (Ariani et al. 2016). Sequence tags were aligned to the reference Andean common bean genome v1.0 (G19833; Schmutz et al. 2014). In the filtering process, we included for downstream analysis only SNPs that showed: minor allele frequency (MAF) > 0.05; minimum quality >10; and a mean read depth, across all lines, ranging from 5 to 1000 (-maf 0.05-minQ 10 min-meanDP 5 -max-meanDP 1000). Thus, a total of 28,822 SNPs were obtained. GWAS was carried out using mixed linear model (MLM) in TASSEL software version 5.2.50. The population structure matrix (Q) was obtained by principal component analysis (PCA), where the first two components explained 92% of the variation. Gene models within 100Kb upstream and downstream of significant SNPs, in common bean reference genome v1.0, were taken into account for candidate gene search on NCBI and phytozome.org.

RESULTS AND DISCUSSION: Association mapping of 89 accessions with race 1545 detected resistance on chromosomes Pv03 and Pv05 (Figure 1). One SNP, S03_13038972, was significantly associated with race 1545 on Pv03 with p-value < 0.001, and explained 15.16% of the phenotypic variation. In the reference genome v1.0 this SNP is located in the position 13,038,972 where ten gene models are found. Among them, Phvul.003G080900 encodes a protein kinase that is known to act as a pattern-recognition receptor in PAMP-triggered immunity by recognizing pathogen-associated molecular patterns (PAMPs) (Zipfel, 2014). Five SNPs (S05_706152, S05_713832, S05_739138, S05_747744, S05_75558) were significantly associated with race 1545 resistance in Pv05. Each SNP explained 14.87% of the phenotypic variation. These SNPs are located in a region of 49,406bp (from 706,152bp to 755,558bp) of the reference genome where 25 gene models

are found. Out of them, three genes might act in the resistance response. Phvul.005G008100 encodes a PPR (Pentatricopeptide) repeat that mediates gene expression. Phvul.005G008500 encodes an F-box and leucine-rich repeat protein 2/20. F-box proteins regulate transcriptional regulation and signal transduction. LRR are involved in protein-protein interaction. Lastly, Phvul.005G009000 encodes a protein kinase. Currently, there are two resistance genes mapped on chromosome Pv03. The first gene mapped was Co-13, reported in the Andean Jalo Listras Pretas landrace and linked to marker OV20⁶⁸⁰ (Lacanallo and Gonçalves-Vidigal 2015). Remarkably, the Co-13 gene also confers resistance to race 1545 (Castro et al. 2017). The second gene is Co-17, described in the Mesoamerican SEL 1308 cultivar (Trabanco et al. 2015). Previous studies have reported resistance to anthracnose on Pv03 and Pv05 chromosomes, similar to those reported in the current study. Perseguini et al. (2016) performed a GWAS in common bean accessions inoculated with race 4 of C. lindemuthianum. The authors not only identified six markers associated to resistance on Pv03 and two markers associated to resistance on Pv05, but they also reported resistance-associated markers on chromosomes Pv01, Pv02, Pv04, Pv06, Pv07, Pv08, and Pv11. GWAS for anthracnose resistance against race 81 also identified one marker on Pv05 (Wu et al., 2017). Additionally, associations with race 81 resistance were found on Pv01, Pv02, Pv04, Pv06, Pv10 and Pv11. Resistance to race 1545 has been reported on chromosomes Pv01 and Pv04 in Andean cultivars MDRK and Kaboon (Campa et al., 2011). On the other hand, Mesoamerican resistance sources to race 1545 have been described on chromosomes Pv04 (Corinthiano, Co-15) and Pv08 (SEL1308, $Co-4^2$). The current study revealed new sources of anthracnose resistance in Andean and Mesoamerican beans from Brazil detected on Pv03 and Pv05, which could be useful for future breeding programs.



Fig 1. Manhattan plots showing SNPs associated with resistance against race 1545 of *C. lindemuthianum* in a set of 89 common bean accessions from Brazil.

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SOURCES OF RESISTANCE TO Collectotrichum lindemuthianum AND Pseudocercospora griseola IN COMMON BEAN FROM BRAZIL

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INTRODUCTION

Anthracnose (ANT) and angular leaf spot (ALS) are seed-borne diseases of common bean (*Phaseolus vulgaris* L.) caused by the fungus *Colletotrichum lindemuthianum* and *Pseudocercospora griseola*, respectively. Finding new sources of resistance against these devastating diseases are top priorities in common bean breeding programs as highly virulent races of these fungi often emerge. Therefore, the objective of this study was to identify new resistance sources of ANT and ALS using a set of Mesoamerican and Andean traditional common bean Brazilian accessions.

MATERIAL AND METHODS

Pathogenicity of physiologically distinct races of C. lindemuthianum (9, 65, 73, 2047, and 3481) and P. griseola (31-23 and 63-39) were evaluated on MA traditional common bean accessions representing different Brazilian market classes. Seeds of 115 traditional common bean accessions (57 Andean and 58 Mesoamerican) from the Germplasm Bank at Nupagri (Núcleo de Pesquisa Aplicada à Agricultura, Universidade Estadual de Maringá, Paraná, Brazil) were used in this study. The accessions were obtained from farmers of Paraná, Santa Catarina, and Mato Grosso states. To obtain seedlings, seeds of each accession were sowed separately in plastic trays containing a sterilized substrate MecPlant. After germination, seedlings were maintained in a greenhouse until the first trifoliate was fully developed at stage V₃ (Gepts, 1988). Monosporic cultures of C. lindemuthianum (9, 65, 73, 2047 and 3481) and of P. griseola (31-23 and 63-39) races were prepared according to the methodologies of Mathur et al. (1950) and Sanglard et al. (2009), respectively. Inocula of ANT races were produced on young green common bean pod (Cárdenas et al. 1964) incubated at 22°C for 14 days. ALS inocula were grown in tomato medium maintained in a BOD incubator at 24°C for 15 days (Sanglard et al. 2009). The spore suspensions were prepared by scraping the surface of the fungal colonies and adjusting to 1.2×104 conidia mL⁻¹. Fifteen-day old seedlings (12 plants per accession) were spray-inoculated on the underside of the leaf with each race separately. Seedlings were inoculated and maintained under a controlled environment for 72 h and seven days after inoculation ANT and ALS symptoms were evaluated using the disease severity scales (1 to 9) proposed by Pastor-Corrales et al. (1995) and Inglis et al. (1988), respectively. Plants with disease reaction scores between 1 and 3 were considered resistant, whereas plants with scores from 4 to 9 were considered susceptible.

RESULTS AND DISCUSSION

In this study one Mesoamerican (BGF 44) and six Andean common bean accessions (BGF 41, BGF 82, BGF 89, BGF 114, BGF 174, BGF 188) were identified with resistance to all of *C. lindemuthianum* and *P. griseola* races used (IR= 100%) (Table 1). Furthermore, four Mesoamerican (BGF 44, BGF 85, BGF 112, BGF 117) and eight Andean (BGF 6, BGF 11, BGF 41, BGF 82, BGF 89, BGF 114, BGF 174, BGF 188) accessions showed resistance to all (IR= 100%) races of *C. lindemuthianum* evaluated (Table 1). On the other hand, 13 (22.41%) of

Mesoamerican and 30 (52,63%) of the Andean accessions were found to be resistant to both P. *griseola* (31-23 and 63-39) races used.

Accessions	C. lindemuthianum races					DI (0/)	P. griseola races	
	9	65	73	2047	3481	KI (%)	63-39	31-23
MESOAMERICAN ACESSIONS								
BGF 44	R	R	R	R	R	100	R	R
BGF85	R	R	R	R	R	100	S	R
BGF112	R	R	R	R	R	100	S	S
BGF117	R	R	R	R	R	100	R	S
ANDEAN ACESSIONS								
BGF6	R	R	R	R	R	100	S	R
BGF11	R	R	R	R	R	100	S	R
BGF41	R	R	R	R	R	100	R	R
BGF82	R	R	R	R	R	100	R	R
BGF89	R	R	R	R	R	100	R	R
BGF114	R	R	R	R	R	100	R	R
BGF174	R	R	R	R	R	100	R	R
BGF188	R	R	R	R	R	100	R	R

Table 1. Reaction of Mesoamerican and Andean traditional accessions of *Phaseolus vulgaris* andResistance Index (%) to *C. lindemuthianum* and *P. griseola* Mesoamerican races

The results show that both Andean and Mesoamerican traditional common bean accessions evaluated in this study represent high genetic variability in response to races of *C. lindemuthianum* and of *P. griseola* pathogens evaluated. Some of these accessions will be valuable sources of resistance to ANT and ALS in future bean breeding efforts.

ACKNOWLEDGEMENTS

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GENETIC MAPPING OF THE ANTHRACNOSE AND ANGULAR LEAF SPOT DISEASE-RESISTANCE GENES IN THE COMMON BEAN CULTIVAR CALIFORNIA DARK RED KIDNEY

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INTRODUCTION: Common bean (*Phaseolus vulgaris* L.) is the *Phaseolus* species most consumed worldwide and it is an important primary source of protein in several countries, particularly in those falling below the poverty line (Broughton et al., 2003). For instance, anthracnose (ANT), caused by *Colletotrichum lindemuthianum* (Sacc. and Magnus) Briosi and Cavara, and angular leaf spot (ALS), caused by *Pseudocercospora griseola* (Sacc.) Crous and Braun, are the most widespread, recurrent, and devastating diseases of the common bean in Latin America and Africa (Pastor-Corrales et al., 1994; Aggarwal et al., 2004). The use of resistant cultivars is the most cost-effective strategy to manage these diseases; however, highly virulent isolates of these pathogens often emerge, threatening current resistance sources. Our study revealed that the Andean California Dark Red Kidney (CDRK) cultivar is resistant to many *C. lindemuthianum* races including race 2047. Interestingly, CDRK is also resistant to *P. griseola*, making this cultivar an excellent choice to test the hypothesis that resistance against ANT and ALS is co-localized in the bean genome using the currently available RIL population from a cross between CDRK and Yolano (CY; Johnson and Gepts, 1999).

MATERIALS AND METHODS: The genetic basis of disease resistance in the genotype CDRK was study using 110 RILs derived from the cross between CDKR and Yolano (CY population). Seedlings with the first trifoliate fully expanded were inoculated with races 73, 2047 and 3481 of *C. lindemuthianum* and race 63-39 of *P. griseola* separately to determine inheritance and co-segregation of the disease reaction. Inocula of ANT races were produced on young green common bean pod medium (Cárdenas et al., 1964) incubated at 22°C for 14 days. The inoculum of race 63-39 of ALS was multiplied in Petri dishes containing 2 mL tomato medium (Sanglard et al., 2009) and maintained in a BOD incubator at 24°C for 18 days. ANT and ALS symptoms were evaluated using the disease severity scales (1 to 9) proposed by Pastor-Corrales et al. (1995) and Inglis et al. (1988), respectively. Plants with disease reaction scores between 1 and 3 were considered resistant, whereas plants with scores from 4 to 9 were considered susceptible.

Total genomic DNA was isolated using the DNeasy Plant Mini Kit (Qiagen, CA, USA) following the manufacturer's instructions. The DNA samples were screened with 5,398 SNP DNA markers on the BARCBean6K_3 Illumina BeadChip by following the Infinium HD Assay Ultra Protocol. The BeadChip was imaged using the Illumina BeadArray Reader to measure fluorescence intensity. Automatic allele calling for each locus was performed with the GenomeStudio Genotyping Module v1.8.4 software (Illumina, San Diego, CA, USA) and all allele calls were visually inspected and any errors in allele calling due to improper cluster identification were corrected, resulting in 4,633 high quality SNPs. A genetic linkage map was created using the software MapChart (Voorrips et al., 2002). A fine linkage map was developed by adding four new markers.

RESULTS: Inheritance resistance data indicate that CDRK carries a dominant locus that confers resistance to races 73, 2047, and 3481 of *C. lindemuthianum* and 63-39 of *P. griseola*, and co-

segregation analysis further revealed that the ANT and ALS resistance loci in CDRK were tightly linked at a distance of 0.0 cM. Genetic mapping of the F_{10} RILs population placed the *Co*-*CDRK/Phg-CDRK* locus in a 245 Kbp genomic region on the lower arm of chromosome Pv01.

In summary, the co-segregation analysis of the ANT and ALS resistance genes, respectively, in the RILs CY from the CDRK \times Yolano showed identical phenotypes for both diseases reactions in 110 RILs. A total of 54 RILs that were resistant to the ANT pathogen were also resistant to race of the ALS pathogen; and, 56 RILs that were susceptible (S) to the ANT pathogen were also susceptible to the ALS pathogen. Co-segregation analysis revealed that *Co-CDRK* and *Phg-CDRK* were inherited together, conferring resistance to races 73, 2047 and 3481 of ANT and race 63-39 of ALS. The *Co-CDRK* and *Phg-CDRK* genes were co-segregated and were tightly linked at a distance of 0.0 cM on chromosome Pv01. These results will be very useful for breeding programs aimed at developing bean cultivars with ANT and ALS resistance using marker assisted selection.



Fig. 1 Genetic map of common bean linkage group Pv01 containing the anthracnose and angular leaf spot resistance loci and linked single nucleotide polymorphism (SNPs) markers used to genotype the F_{10} population California Dark Red Kidney × Yolano. Physical map showing the locations of the CDRK resistance loci and SNP markers. Recombination distances are indicated on the left side of the linkage group in centiMorgans (cM), and the marker names are shown on the right side. The *CoPv01^{CDRK}/PhgPv01^{CDRK}* or resistance loci were flanked by SNP markers ss715645251 and ss715645248 in F_{10} mapping population. The map was drawn with MapChart (Voorrips, 2002).

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GENOME WIDE ASSOCIATION ANALYSIS REVEALS MARKERS TAGGING ANHTRACNOSE AND ANGULAR LEAF SPOT RESISTANCE GENES IN COMMON BEANS FROM BRAZIL

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INTRODUCTION

Common bean production can be severely affected by anthracnose (ANT) and angular leaf spot (ALS) diseases. Genome-wide association studies (GWAS) aim to identify genomic regions associated with the traits of interest. The objective of this study was to use GWAS for to identify resistance in common bean to races 9, 65, 73, 2047, and 3481 of *Colletotrichum lindemuthianum*, the ANT pathogen, and races 31-23 and 63-39 of *Pseudocercospora griseola*, the ALS pathogen.

MATERIALS AND METHODS

Common bean accessions (115) from the Andean and Mesoamerican gene pools were genotyped using the BARCBean6K_3 IlluminaBeadChip. A total of 4,633 polymorphic SNPs were identified. Phenotyping was performed by inoculating 12 seedlings of each of the 115 accessions with 1.2×10^6 and 1.2×10^4 conidia mL⁻¹ of *C. lindemuthianum* and *P. griseola*, respectively. Inoculated seedlings first were maintained under a controlled environment during 48 hrs and then placed in a greenhouse. These seedlings were evaluated for their reactions to the races of the ANT and ALS pathogens seven days after inoculation using a standard evaluation scale (Pastor-Corrales et al. (1995) and Inglis et al. (1988). GWAS was performed using the Mixed Linear Model (MLM) in Tassel software (Bradbury et al., 2007). The Bonferroni correction was used to define the significance threshold ($\alpha = 0.05 / 4,633$ SNPs), with *p* value = 1.0 x 10⁻⁵. Candidate gene for each significant SNPs were searched in the reference genome (G19833 version 1.0; Schmutz et al. 2014) at NCBI.

RESULTS AND DISCUSSION

Resistance to races 9 and 73 of the ANT and ALS pathogens (Figure 1) reside on chromosome Pv04, resistance to race 65 on Pv01, Pv04 and Pv08, resistance to race 2047 on Pv10, and resistance to race 3481 on Pv05. Furthermore, quantitative resistance loci (QRL) associated with race 31-23 of *P. griseola* was mapped on chromosomes Pv02 and Pv06 (Figure 1), whereas resistance to race 63-39 was mapped to Pv03, Pv06 and Pv08 (Table 1). Thus, the current study revealed common bean genomic regions associated with ANT and ALS resistance. These findings will guide bean breeding programs with the effective transferring disease resistance genes to commercial cultivars.
Table 1. Associations between SNPs and races 9, 65, 73, 2047, and 3481 of *Colletotrichum lindemuthianum* and 31-23 and 63-39 of *Pseudocercospora griseola*. Resistance by mixed linear models (MLM)

Races	SNP ¹	Chrom	SNP ^a position (Mb)	P-value ^b	Candidate gene
ANT 9	715649432	4	532254	3.12E-06	Phvul.004G006800
ANT 65	715640804	1	37661626	4.40E-06	Phvul.001G133400
ANT 65	715639700	8	3020556	5.82E-06	Phvul.008G036300
ANT 65	715646248	4	2142286	1.04E-05	Phvul.004G020900
ANT 73	715646896	4	1,224,240	6.83E-06	Phvul.004G012600
ANT 3481	715645319	5	39,020,188	2.50E-07	Phvul.005G165000
ANT 3481	715645320	5	39,027,362	6.40E-07	Phvul.005G165100
ANT 3481	715645321	5	39,244,615	6.40E-07	Phvul.005G164700
ANT 2047	715649489	10	7,151,050	7.90E-06	Phvul.010G046600
ALS 31-23	715647653	2	17,119,067	8.17E-06	Phvul.002G095000
ALS 31-23	715647656	2	17,382,085	8.17E-06	Phvul.002G095500
ALS 31-23	715649419	6	20,742,104	2.10E-06	Phvul. 006G088800
ALS 31-23	715649420	6	20765897	2.45E-06	Phvul.006G089000
ALS 63-39	715644759	3	31387497	6.23E-04	Phvul.003G128200
ALS 63-39	715647108	6	27,063,782	2.34E-04	Phvul.006G157900
ALS 63-39	715649499	8	44,526,137	3.34E-04	Phvul.008G170700



Figure 1. Manhattan Plot showing SNPs associated with the resistance to races 73 of *Colletotrichum lindemuthianum* and race 31-23 of *Pseudocercospora griseola* race.

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QTL FOR RESISTANCE TO ANGULAR LEAF SPOT AND RUST IN TANZANIA VS SOUTH AFRICA FOR THE ANDEAN PANEL & ROJO/CAL 143 RIL POPULATION

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INTRODUCTION: Angular leaf spot (ALS) caused by *Pseudocercospero griseola* and bean rust caused by *Uromyces appendiculatus* are common bean diseases of global importance. We sought to identify resistance genes in the Andean gene pool effective against ALS and rust in TZ & SA.

MATERIALS AND METHODS: The Andean Diversity Panel (ADP) was grown in Cedara, South Africa (373 accessions) and in Arusha and Mbeya, Tanzania (174 accessions) in single row plots with 2 to 3 replications. The 147 RILs from Rojo (Andean)/CAL 143 (Andean) (RC pop) were similarly tested. Diseases occurred naturally and were rated from 1=no disease symptoms to 9 = completely susceptible during mid pod filling growth stages. Least-square means for disease score were used in the GWAS and QTL analyses. The ADP was genotyped with 9,639 (ARS) and 124,264 (NDSU) SNPs (after filtering) obtained by GBS from the different labs. The RC RILs were genotyped with SNPs from the BARC 6K and 12K Illumina Assays, and by GBS. FarmCPU and mrMLM models were used for GWAS of the ADP, and MapDisto and rQTL were used to generate a linkage map and conduct QTL analysis for the RC population.

RESULTS AND DISCUSSION: The new 12K SNP assay which combines the old 5398 SNP assay with 6K new SNPs purposely selected for polymorphism among Andean beans, greatly enhanced the map coverage for the RC (Andean x Andean) population (Fig. 1).



Fig 1. Comparison of SNP sources – GBS (blue) vs original 5398 BARC (red) vs Andean 6K (gold) BARC Illumina assay for generating the RC RIL population linkage map (147 lines).

GWAS: (Table 1) different QTL for ALS resistance were detected in South Africa (SA) vs Tanzania (TZ): ALS-SA = Pv03, Pv04, Pv08, Pv11 vs ALS-TZ = Pv01, Pv02, Pv09. There were common Pv01 and Pv04 and uncommon QTL detected for rust resistance across locations: rust-

TZ = Pv02, Pv07, Pv08, Pv09 vs rust-SA = Pv03, Pv08, Pv09, Pv11. Many GWAS peaks were associated with known genes (ALS: *Phg-1, Phg-2, Phg-4*; rust: *Ur-5, Ur-11, Ur-12, Ur-13, Ur-14*) and previously identified QTL intervals (Nay et al., 2019).

Table 1. GWAS summary for significant SNP peaks (QTL) associated with ALS & rust resistance - FarmCPU.									
For named loci see review by Nay et al. (2019). LD heat maps shown for Phg-1 and Ua3.1 loci.									
Trait	SNP	Chr.	Position	P.value	maf	effect	Corresponding gene/QTL		
Arusha_ALS	S01_44808951	1	44,808,951	6.4E-13	0.29	0.45			
Mbeya_ALS	S01_44845701	1	44,845,701	1.26E-09	0.33	0.42			
Mbeya_ALS	S01_51175014	1	51,175,014	1.10E-07	0.13	-0.45	Phg-1		
Arusha_ALS	S02_46447110	2	46,447,110	1.6E-09	0.10	-0.62	QTL near Co-u/I gene		
Mbeya_ALS	S03_29473134	3	29,473,134	3.4E-12	0.45	0.45	ALS2.1 ^{UC} ?		
Cedara_ALS	S04_45773222	4	45,773,222	5.92E-12	0.35	0.36	Phg-4		
Cedara_ALS	S05_2599095	5	2,599,095	1.1E-08	0.14	0.32	ALS5.2 ^{UC} ?		
Cedara_ALS	S08_61825657	8	61,825,657	1.71E-14	0.11	0.58	Phg-2		
Mbeya_ALS	S09_8904746	9	8,904,746	6.7E-15	0.50	-0.51	Mahuku et al. 2009 QTL		
Cedara_ALS	S11_39102895	11	39,102,895	3.4E-08	0.07	-0.49			
Cedara_ALS	S11_48002083	11	48,002,083	8.1E-08	0.26	-0.27	Bassi et al. 2017 QTL		
Cedara_Rust	S01_3451961	1	3,451,961	1.54E-06	0.38	0.26			
Arusha_Rust	S01_4168904	1	4,168,904	2.3E-07	0.25	0.36			
Mbeya_Rust	S02_22271049	2	22,271,049	2.9E-09	0.06	-1.37			
Cedara_Rust	SO3_39441194	3	39,441,194	1.67E-09	0.33	0.44	Ua3.1		
Cedara_Rust	S04_1576956	4	1,576,956	1.18E-07	0.09	0.57	Ur-5		
Mbeya_Rust	S04_2990488	4	2,990,488	8.5E-11	0.10	-0.81	Ur-14		
Mbeya_Rust	S07_80695	7	80,695	1.23E-08	0.16	-0.68	Ur-12		
Cedara_Rust	S08_16644679	8	16,644,679	2.57E-06	0.43	0.26			
Mbeya_Rust	S08_61950262	8	61,950,262	7.6E-09	0.18	0.73	Ur-13		
Mbeya_Rust	S09_1279285	9	1,279,285	9.0E-08	0.44	-0.86			
Cedara_Rust	S09_17350557	9	17,350,557	3.7E-08	0.48	1.14			
Cedara_Rust	S11_51522580	11	51,522,580	4.38E-10	0.06	0.88	Ur-11		

Table 2. QTL identified in the RC RIL population conferring resistance to ALS and Rust in Arusha and Mbeya, Tanzania and Cedara, South Africa.											
						CAL 143					
						allele					
Trait	Chromosome	LOD	R2 (%)	Prob	(F)	effect	Start position	Peak SNP	Peak position	End position	Gene/QTL
Mbeya_ALS 2015	3	9.23	26.03	1.04E-10	***	-0.43	6,291,829	Chr03_36758898	36,758,898	38,206,863	ALS2.1 or ALS3.1
Arusha_ALS 2017	8	6.35	18.72	8.43E-08	***	-0.44	60,778,618	ss715646121	61,915,670	62,410,979	Phg-2
Cedera_ALS 2015	8	17.3	43.16	0	***	-1.59	60,778,618	Chr08_61034267	61,034,267	62,191,464	Phg-2
Mbeya_Rust 2015	3	12.01	32.44	1.71E-13	***	-0.51	30,293,469	Chr03_33490314	33,490,314	37,126,594	New - Ua3.1
Cedera_Rust 2015	3	11.96	27.46	2.41E-12	***	-0.68	29,957,612	Chr03_35695104	35,695,104	38,206,863	New - Ua3.1
Cedera_Rust 2015	4	7.36	15.62	7.06E-08	***	-0.45	42,600	ss715646916	1,901,167	2,274,277	~Ur-5 - Ua4.1

RC RIL population: (Table 2) common (*Phg-2*, Ua3.1) and uncommon QTL for ALS-Mbeya (Pv03) and rust Cedara (Pv04) were observed across SA and TZ locations. *Phg-2* (MA locus) is effective against Andean races and is more important in SA than in TZ. Oblessuc et al. (2012) never observed *Phg-2* in CAL 143 using MA isolates from Brazil. *Phg-1* (Andean locus) was more important in TZ than SA based on GWAS with the ADP. MA loci for rust resistance *Ur-5* and *Ur-11* were detected in SA whereas Andean loci *Ur-12* and *Ur-13* were detected in TZ. Ua3.1, a newly detected QTL in CAL 143 for rust resistance has large effect in SA and TZ. East Africa is known to have a mix of A and MA pathogen races for ALS (Chilagane et al., 2016) and perhaps the same is true for rust, whereas South Africa has predominately Andean races.

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IDENTIFYING RESISTANCE IN ANDEAN COMMON BEANS TO THE RUST PATHOGEN Uromyces appendiculatus RACES 20-3, 27-7, AND 29-3

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INTRODUCTION

Rust, caused by *Uromyces appendiculatus* (Pers:Pers), is an economically important disease of common beans (*Phaseolus vulgaris* L.) worldwide. Common beans of Andean origin are preferred in Eastern and Southern Africa and in Europe. In the US, approximately 10% of common beans produced are of Andean background; however, they are of higher value than beans of Middle American origin. Few US breeding programs focus on the development of Andean cultivars and the genetic base of this population is much smaller than Middle American beans, making genetic improvement more challenging. To aid in breeding for resistance, a sub-set of accessions from the Andean Diversity Panel (ADP; Cichy et al., 2015) and NDSU advanced breeding lines of Andean decent were challenged with *U. appendiculatus* under greenhouse conditions.

MATERIALS AND METHODS

Forty-nine accessions from Andean Diversity Panel (ADP) including cranberry, white kidney, light (LRK) and dark red kidney (DRK), yellow, red mottled and mayocoba were screened in the greenhouse for resistance to U. appendiculatus using race 20-3 and 27-7 (Monclova-Santana, 2019; Pastor-Corrales and Steadman, 2015). Fifty advanced LRK and DRK breeding lines from the North Dakota State University dry bean breeding program were evaluated against races 20-3, 27-7, and 29-3. Race 20-3 is most frequent in North Dakota and is virulent on resistance genes Ur-3, Ur-6, Ur-7 and the Montcalm gene. Race 27-7 is virulent on Ur-3, Ur-4, Ur-5, Ur-6, Ur-7, Ur-9, Ur-12 and Ur-13. Race 29-3 is virulent on Ur-3, Ur-4, Ur-6, Ur-7, Ur-9, Ur-12 and the Montcalm gene. To evaluate bean genotypes against the three pathogen races three plants (replicates) of each genotype were planted in a randomized complete block design and inoculated 10 to 14 days later with urediniospores in soltrol (Monclova-Santana, 2019). Pinto PI14 (UI 114) and industry standard varieties were included as controls. Disease was scored 14 days postinoculation by measuring ten arbitrarily chosen pustules on primary leaflets of each plant using a 6× Pocket Comparator. Pustule diameter (mm) was averaged across 10 plants and converted to a reaction type 1 to 6 (Stavely et al. 1984; Mmbaga et al. 1996). A reaction type ≥4,3 was considered susceptible.

RESULTS AND DISCUSSION

Reaction of lines, accessions and varieties included in this study to screen were consistent with resistant genes previously reported, where available (Pastor-Corrales and Steadman, 2015). Five ADP accessions (light red kidney cultivars Foxfire, Red Kanner and Red Kloud; Mayocoba cultivar Myasi, and yellow line Jalo-EEP558) were resistant to both races 20-3 and 27-7 (Table 1). An additional 14 ADP accessions were resistant to race 20-3 only (Table 1). Five DRK NDSU advanced breeding lines were resistant to all three races, thirteen were resistant to 20-3, 14 to race 27-7 and six to race 29-3 (Table 2). Four NDSU LRK advanced breeding lines were resistant to all three races, six were resistant to 20-3, seven to race 27-7 and four to race 29-3. Further research is needed to postulate the identity of the gene(s) conferring resistance in these lines. The identification of resistant germplasm of Andean origin provides valuable resources in the effort of developing varieties with resistance to *U. appendiculatus*. In addition, the incorporation of

resistance genes of Andean origin into Middle American beans can provide resistance to a broader range of *U. appendiculatus* races.

Table 1. Number of accessions from the Andean Diversity Panel subset (n = 49) resistant to *Uromyces appendiculatus* races 20-3 and 27-7.

	U. ap	pendicı	ılatus
Market class		race	
	20-3	27-7	Both
LRK (n = 17)	8	3	3
DRK (n = 10)	3	0	0
Cranberry (n = 14)	3	0	0
White kidney (n = 3)	2	0	0
Yellow (n = 1)	1	1	1
Mayocoba (n = 1)	1	1	1
Red mottled (n = 1)	1	0	0
Total	19	5	5

Table 2. Number of NDSU advanced breeding lines (n = 50) resistant to *Uromyces appendiculatus* races 20-3, 27-7, and 29-3.

	U. appendiculatus race						
Market class	20-3	27-7	29-3	All			
DRK (n = 30)	13	14	6	5			
LRK (n = 20)	6	7	4	4			
Total	19	21	10	9			

DRK = dark red kidney LRK = light red kidney

DRK = dark red kidney

LRK = light red kidney

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GENOME-WIDE ASSOCIATION AND FINE MAPPING OF *BGM-1* GENE AND OTHER QTLS FOR RESISTANCE TO *BEAN GOLDEN YELLOW MOSAIC VIRUS* IN DRY BEANS

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INTRODUCTION

Bean Golden Yellow Mosaic Virus (BGYMV) (family *Geminiviridae*) is a begomovirus vectored by *Bemisia tabaci* (Gennadius) whitefly that causes severe yield losses (40 to 100%) in common bean. The most effective control of BGYMV is to combine genetic resistances in the host. Two SCAR markers developed 25 years ago have been used for marker-assisted selection (MAS) programs for BGYMV resistance. The marker SR2 is linked to the *bgm-1* gene on chromosome Pv03 and the SW12 marker with a quantitative trait locus (QTL) on Pv04. Our objective was to improve MAS for BGYMV.

MATERIALS AND METHODS

QTL analysis was applied in two biparental recombinant inbred populations, DOR364/XAN176 and DOR476/SEL1309, and genome-wide association studies (GWAS) were conducted on a panel of 415 breeding lines developed by the International Center for Tropical Agriculture (CIAT) and a panel of 120 select lines/cultivars developed for abiotic stress evaluations (BASE 120 panel). Plants were evaluated for chlorosis using a 1–9 scale where 1 was equivalent to very resistant and 9 to very susceptible.

RESULTS AND DISCUSSION

Linkage mapping in the RIL populations revealed *bgm-1* on Pv03 and three QTL for BGYMV resistance on chromosomes Pv04, Pv07 and Pv08 explaining between 10 to 33 percent of phenotypic variation. GWAS revealed significant SNPs associated with *bgm-1* and the same QTL on Pv04, Pv07, and Pv08, and a novel QTL on Pv09 (Fig. 1). Two candidate genes for *bgm-1* were identified, both related to Geminivirus resistance. An indel marker was developed from one candidate gene and evaluated on multiple bean genotypes using the melting temperature (Tm)-shift method (Fig. 2). This marker was completely correlated with BGYMV resistance across more than 700 genotypes. These results enhance our understanding of the genetic mechanisms of resistance to BGYMV and provide improved MAS for resistance to BGYMV in common bean breeding programs.



Figure 1. a. Manhattan plot of BASE 120 panel identifying regions associated with resistance to BGYMV on Pv03, Pv04 and Pv07; b. In CIAT breeding population, lines with only *bgm-1* gene were used for GWAS analysis to identify epistatic QTL like BGY8.1.



Figure 2. Melting curve analysis and gel electrophoresis of indel marker developed to detect the *bgm-1* gene on Pv03.

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Pythium SPP. ASSOCIATED WITH COMMON BEAN IN NORTH DAKOTA AND MINNESOTA

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INTRODUCTION: Common bean (*Phaseolus vulgaris* L.) production worldwide is greatly impacted by pre- and/or post-emergence damping-off in seedlings and root rot caused by soilborne pathogens which can result in yield losses as high as 100%. Limited work has been done to directly assess the pathogenicity of *Pythium* spp. on common bean (Li et al 2014; Rossman et al 2017). A survey conducted nearly a decade ago established *Fusarium* and *Rhizoctonia* as the main causal agents of root rot in North Dakota, but *Pythium* was not targeted during this survey (Goswami et al., 2010). The objectives of this research were to identify *Pythium* spp. found on common bean in North Dakota and Minnesota and determine the pathogenicity and aggressiveness of *Pythium* spp. identified.

Table 1. <i>Pythium</i> spp. identified from North Dakota and Minnesota during the 2018 and 2019 field seasons. Pathogenicity was determined from previous literature.							
Duthium crocioc	Pathogenic on	#Iso	ates				
Pythium species	common bean?	2018	2019				
P. aphanidermatum	Yes ^b	3	1				
P. conidiophorum	Yes ^{ab}	2	0				
P. deliense	No ^b	1	0				
P. heterothallicum	No ^b	3	3				
P. intermedium	Yes ^a	4	0				
P. megacarpum	Not determined	0	1				
P. oligandrum	No ^c	1	0				
P. perplexum	No ^a	5	0				
P. rostratifingens	No ^b	9	1				
P. sylvaticum	Yes ^b	1	3				
P. torulosum	No ^b	0	1				
P. ultimum	Yes ^{ab}	1	2				
P. violae	Not determined	2	0				

fields were sampled in 2018 and 2019, respectively. Isolates of Pythium spp. collected from North Dakota and Minnesota identified using were morphological features and by sequencing of the ITS and Cox1 regions (Plaats-Niterick 1981; Broders et al. 2007; Robideau et al. 2011). Pathogenicity and aggressiveness tests were conducted using an infested rice inoculum as previously described (Rossman et al., 2017) on Montcalm, VAX 3, Dynasty, Cabernet and Talon. Plants were under controlled grown conditions (20°C and 14hr light)

for 14 days and root rot severity

METHODS: Thirty-three and 49

^aLi et al. 2014 ^bRossman et al. 2017 ^cDeacon, JW.1976

was assessed on a 0-9 scale (Van Schoonhoven and Pastor-Corrales, 1987).

RESULTS AND DISCUSSION: To date, 13 *Pythium* spp. have been identified from common beans collected in North Dakota and Minnesota (Table 1). *P. rostratifingens, P. heterothallicum*, and *P. sylvaticum* were recovered most frequently from plants with low root rot severity (rating of 1-3). *P. rostratifingens* and *P. heterothallicum* have been previously demonstrated to be non-pathogenic on common bean (Li et al., 2014; Rossman et al., 2017). *P. sylvaticum* has been demonstrated to be a bean pathogen (Rossman et al., 2017). *P. intermedium, P. conidiophorum*, and *P. violae* were the most frequently isolated species from plants with high root rot severity (rating of 5-9). *P. intermedium* is a known bean pathogen, isolates of *P. conidiophorum* did not cause disease in one study (Rossman et al., 2017) but it has been previously reported as a pathogen (Li et al., 2014). *P. violae* has not been evaluated for pathogenicity on common bean.

Isolate	Root rot	Standard	Germination (%)	Standard
	severity	deviation		deviation
-Control	0.0	0.0	100.0	0.0
P. aphanidermatum-1	0.6	1.2	91.4	12.9
P. aphanidermatum-2	6.7	1.8	91.4	19.2
P. aphanidermatum-3	8.8	0.8	6.3	7.4
P. aphanidermatum-4	3.7	3.1	100.0	0.0
P. heterothallicum-1	6.0	3.1	68.6	27.4
P. heterothallicum-2	5.5	2.9	82.9	15.6
P. heterothallicum-3	4.9	3.3	74.3	18.6
P. rostratifingens	4.3	2.8	88.6	12.0
P. rostratifingens-2	6.0	3.1	94.2	13.0
P. ultimum	5.6	2.9	94.2	13.0
+Control (P. ultimum)	8.0	1.6	59.8	32.6

Table 2. Root rot severity (0-9 scale) caused by *Pythium* spp. collected from North Dakota and Minnesota averaged across five genotypes (Montcalm, Dynasty, Talon, Cabernet and VAX 3) inoculated under greenhouse conditions.

Preliminary results indicate differences in aggressiveness among *Pythium* spp. Inoculation with *P. ultimum*, *P. heterothallicum*, and *P. aphanidermatum* resulted in decreased germination and higher disease severity (Table 2). An interaction between lines and *Pythium* spp. was observed for germination, resulting in high standard deviations. For example, the positive control *P. ultimum* significantly decreased germination in all lines when compared to 100% germination in VAX 3.

Many *Pythium* spp. isolated from common bean in North Dakota and Minnesota are the same species isolated from surveys on other crops in the region as well as from common bean in other parts of the U.S. (Rossman et al., 2017; Broders et al., 2007; Zitnick-Anderson et al., 2011). *P. ultimum* has been the focus of root rot in common bean; however, preliminary results reported here support previous literature that other species may play important roles in causing disease.

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INVESTIGATING MAT HETEROKARYONS IN ISOLATES OF Sclerotinia sclerotiorum IN BRAZIL

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INTRODUCTION: Sclerotinia sclerotiorum is an important pathogen of common bean. It is a homothallic fungus (self-compatible) and the resulting population is expected to be clonal with limited variability (Billiard et al. 2012), but high genetic variability has been reported (Abreu and Souza, 2015). It has been recently found that *S. sclerotiorum* has peculiar MAT alleles for a homothallic species (Chitrampalam et al. 2013). Two MAT alleles are responsible: inversion positive (Inv+) and inversion negative (Inv-). Besides that, *S. sclerotiorum* is a multinucleate fungus, and is known to form heterokaryons under nonrestrictive conditions. Heterokaryons were detected in some isolates by Chitrampalam et al. (2015), providing another possibility of outcrossing. There is no information about this in *S. sclerotiorum* isolates from Brazil. Sample of isolates from Brazil were assessed to verify presence of MAT homokaryon Inv+ or Inv- and MAT heterokaryons.

MATERIALS AND METHODS: A total of 16 isolates were collected in infested common bean fields in Brazil. Each isolate was grown in a liquid medium (M3) and incubated at 23°C in the dark for four days. Mycelia were harvested and ground in liquid nitrogen. DNA extraction was performed using a Wizard Genome DNA Purification kit (Promega), following the manufacturer's recommendations. Aliquots of DNA were quantified with a NanoDrop 1000 spectrophotometer and the concentration was adjusted to 30 ng/ μ L in sterilized water for polymerase chain reaction (PCR). Specific primers were used for identification of Inv+ and Inv- (Chitrampalam et al., 2013). PCR products were resolved on 3% agarose gel. For characterization of S. sclerotiorum isolates, Micelial Growth Rate (mm day⁻¹) were quantified where colonized agar disks, taken from active growth cultures, were placed in 90-mm-diameter dishes containing potato dextrose agar (PDA) medium. The dishes were kept in the dark at a temperature of 22°C. The diameters of the colonies (mm) were measured daily. The MGR was calculated using the formula used by Abreu and Souza (2015). After thirty days of growth in PDA medium, the numbers of sclerotia formed per dish were analyzed for each isolate. The sclerotia of each strain were characterized according to their size as well as the time necessary for the formation of the first sclerotium of each strain was also evaluated. Tests for determination of the mycelial compatibility groups were performed for all possible isolate x isolate combinations. For aggressiveness tests, the method of inoculation utilized was the straw test proposed by Petzoldt and Dickson (1996) and described by Abreu and Souza (2015). For this, G122 (resistant) and Corujinha (susceptible) common bean cultivars were used.

RESULTS AND DISCUSSION

All isolates are from different cities in the state of Minas Gerais (Table 1).

Isolates	Common Bean Lines	Locals of origin (Minas Gerais State)
UFLA3	MAV 5.60	Ijaci
UFLA6	MAV 5.60	Ijaci
UFLA7		Ijaci
UFLA10	Talismã	Ijaci
UFLA11	CNFP15 175	Ijaci
UFLA12		Ijaci
UFLA13		Ijaci
UFLA14	MAV I-21	Ijaci
UFLA15	MAV 3.36	Ijaci
UFLA19	Talismã	Ijaci
UFLA21	Talismã	Ijaci
UFLA23	Talismã	Ijaci
UFLA24	Talismã	Ijaci
UFLA25	Talismã	Ijaci
UFLA27		Lambari
UFLA91	Talismã	Patos de Minas

Table 1 – Local of origin and common bean lines that the isolates were found.

The data presented on Table 2 shows the variation existing among the isolates for all traits evaluated. The evaluation of mycelial compatibility that found only a small percentage of compatible strains (2 to 16%) demonstrates that there was considerable variability among the isolates evaluated. The aggressiveness of isolates was variable in both cultivars.

Table 2 – Variation of traits assessed on 16 isolates

 of Sclerotinia sclerotiorum from this study.

Traits	Means
Micelial Growth Rate (mm day ⁻¹)	15 to 30.2
Days for the first sclerotium formation	4 to 12.3
Number of sclerotium/plate	14 to 43.7
Sclerotium size (mm)	2.03 to 5.3
Micelial compatibility (%)	2 to 16
Aggressivenes (inoculated in G122 line)	1.2 to 5.4
Aggressiveness (inoculated in Corujinha line)	1.3 to 4.9

We found prevalence of Inv- MAT *S. sclerotiorum* isolates (Figure 1). In addition, we observed only one MAT heterokaryon isolate. The presence of the heterokaryon isolate provides the potential to increase genetic variability within the population. Further research on the impact of heterokaryons on the biology of this pathogen would also assist the development of strategies for white mold control.



Figure 1 – MAT locus in *Sclerotinia sclerotiorum* isolates. (A) Amplified bands profile of 673 bp region in the MAT1-1 (Inv-). (B) Amplified bands profile of 1300 bp region in the MAT1-1 (Inv+). 1-UFLA3, 2-UFLA6, 3-UFLA7, 4-UFLA10, 5-UFLA11, 6-UFLA12, 7-UFLA13, 8-UFLA14, 9-UFLA15, 10-UFLA19, 11-UFLA21, 12-UFLA23, 13-UFLA24, 14-UFLA25, 15-UFLA27, 16-UFLA91

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COMPUTATIONAL IDENTIFICATION, PHYLOGENETIC AND SYNTENY ANALYSIS OF RECEPTOR-LIKE KINASES "RLK" AND RECEPTOR-LIKE PROTEINS "RLP" IN LEGUMES

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INTRODUCTION

The plant cell wall is an active structure that mechanically connects cell tissues and controls the shape of the plant cell by sensing external stimuli and transmitting signals to the cytoplasm (Zhou J.M. *et al.*, 2017). The plasma membrane is enclosed by the cell wall and contains RLK and RLP proteins, which play a fundamental roll in cell to cell interaction and are crucial in plant growth, development, and immunity (Monaghan J. *et al.*, 2012, Tor M. *et al.*, 2019). This study attempted to do so using a comparative genomic approach to identify and computationally evaluate RLK and RLP among legumes/non-legumes. The results provide a source of new information for breeders and geneticist considering that extensive genetic and phenotypic studies have reported diverse functional roles of RLK and RLP (plasma membrane receptors) extending from control of cell development to stress responses.

MATERIALS AND METHODS

The RLK/RLP legume analysis used in this study followed a comparative genomic approach based on the three objectives and evaluated the legumes: soybean (Glycine max (L.) Merrill), common bean (Phaseolus vulgaris L.), barrel medic (Medicago truncatula L.), mungbean (Vigna radiata (L.) R. Wilczek), cowpea (Vigna unguiculata L. Walp), adzuki bean (Vigna angularis), and pigeonpea (Cajanus cajan L.), and the non-legumes: Arabidopsis thaliana (L.) Hevnh, tomato (Solanum lycopersicum (L.) H. Karst), and common grape (Vitis vinifera L.). The first objective was the computational identification of RLK/RLP in legumes. A computational strategy integrating multiple tools and including a set of logical conditions was proposed to classify RLK/RLP in legumes/non-legumes. The strategy was statistically evaluated using an independent dataset of resistance RLK/RLP experimentally validated by other authors to evaluate predictive performance. The computational strategy allows practitioners to identify the quantity of RLK/RLP genes present in genomes and to report a catalog of plasma membrane receptors and functional domains per species. The quality of the strategy performance was measured by calculating the specificity, sensitivity, and Matthews coefficient correlation. The predicted RLK/RLP were classified in three categories: ortholog clusters, singletons, and single-copy gene clusters. This analysis followed a pairwise comparison spanning a number of highly similar genes shared within the same or similar genomic regions. The analysis focused only on the regions of the species with RLK/RLP genes for the purpose of identifying patterns of evolutionary conservation and divergence across genomes among synteny blocks that have plasma membrane receptors.

RESULTS AND DISCUSSION

The analysis of both legumes/non-legumes species suggests that about 2% of the proteins of each genome belong to the RLK family and less than 1% belong to RLP family. Almost all of the extracellular domains evaluated were present in most of the species evaluated. More domain diversity combinations existed in the RLK compared with the RLP proteins and LRR domains, and the dual domain combination LRR/Malectin was the most frequent domain for both groups of

plasma membrane receptors among legumes/non-legumes. Legumes exclusively show Pkinase, extracellular domains, and atypical domain combinations in RLK and RLP compared with the non-legumes evaluated. The computational logic approach used for the classification of RLK and RLP allowed the integration of all possible RLK and RLP structural conformations. This shows that the computational logic approach is statistically well supported based on the performance evaluation using an independent set of RLK/RLP experimentally validated, and can be used in other plants.

The analysis of the RLK and RLP suggest a dynamic evolution in the legume family, with between 66% to 85% of RLK and 83% to 88% of RLP belonging to orthologous clusters among the species evaluated; the remaining proteins were classified as singletons. In fact, for the 10-species, a lower number of singleton proteins were reported among RLP compared to RLK, suggesting that RLP are more conserved compared to RLK.

Species	Total proteins	*RLK	RLK-nonRD	*RLP
	reported			
A. thaliana	35,386	555	48	172
C. cajan	48,331	450	61	142
G. max	88,647	1,864	223	468
M. truncatula	62,319	1,060	194	363
P. vulgaris	36,995	842	124	217
S. lycopersicum	34,725	476	83	161
V. angularis	37,769	835	122	215
V. radiata	35,143	770	113	241
V. unguiculata	42,287	992	158	294
V. vinifera	26,346	443	59	172

Table 1. Summary of the RLKs/RLPs identified using probabilistic methods

*non-redundant. RLK-nonRD: absence of an arginine positioned before a catalytic aspartate residue.

The identification of RLK and RLP genes among the synteny blocks in legumes showed that multiple genome segments were highly conserved along different chromosomes. That condition is interesting because plasma membrane receptors do not represent more than 3% of the total number of genes per species. The ratio of the pairwise synteny blocks of RLK/RLP among legumes showed a 1:1 relationship; the exception was *G. max*, which had approximately a 2:1 ratio, demonstrating a similar proportion of plasma membrane proteins among the pairwise clusters. These results provide an overview of the structural organization and distribution of the RLK/RLP among legumes and add to the annotation record of the genes present or absent in synteny blocks.

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QTL ANALYSIS OF A YELLOW PHASEOLUS VULGARIS RECOMBINANT INBRED LINE POPULATION FOR A FAST COOKING, FLAVORFUL, AND FLOURISHING FUTURE OF DRY BEANS

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INTRODUCTION

Dry beans are a nutrient-rich food with diverse culinary attributes, but dry bean consumption in the United States remains low (Mitchell et al., 2009) due in part to their often long cooking times and the preconceived notions held by consumers regarding their flavor (Karlsen et al., 2016). Cooking times and sensory quality are two important traits considered when consumers choose whether to purchase dry beans and which dry bean products to purchase. However, the process of evaluating germplasm for these consumer-valued traits is costly in time and resources, which limits the ability of breeders to incorporate these traits in their programs. In this study, a yellow dry bean (*Phaseolus vulgaris* L.) Recombinant Inbred Line (RIL) population was evaluated for cooking time, flavor, and texture. QTL mapping of cooking time, flavor traits, and texture identified genomic regions influencing these traits. **Objective:** Use QTL analysis to contribute to the growing understanding of the genetic control of cooking time and to lay the foundation for sensory quality improvement in dry bean breeding programs.

MATERIALS AND METHODS

Ervilha and PI527538 are yellow dry bean lines that were selected to develop a RIL population to evaluate the genetic control of cooking time. The parents and population (N = 242) were grown at the Montcalm Research Farm (Lakeview, MI) in 2016 and 2017 in a randomized complete block design. Cooking time evaluation was performed using modified Mattson cookers (Wang and Daun, 1995) with 25 2mm diameter pins weighing 65g each. Following a 12 hour soak in distilled water, 2 reps per genotype were boiled in distilled water. The time required to cook each sample until 80% of the beans were pierced completely was regarded as the cooking time. Sensory evaluation was performed using a modified quantitative descriptive analysis approach with trained evaluators using 5-point attribute intensity scales. The scales indicate no perception to strong presence of a trait. The seed coat perception scale indicates thin to tough seed coat perception. The cotyledon texture scale indicates mushy to firm/gritty cotyledon. Samples were prepared by boiling for the determined cooking times. Each genotype was assessed in 2 sessions with 4 evaluators per session. In each session, 12 genotypes masked with a letter code were evaluated including Ervilha and PI527538 as controls. ANOVAs were performed for each trait using the MIXED procedure in SAS v. 9.4 with genotype, year, and genotype by year as fixed effects and rep as a random effect. For the sensory traits, session(year), reviewer(year), and session(year) by reviewer(year) were included in the random statement. Ervilha, PI527538, and the RILs were genotyped using a GBS approach. Markers exhibiting extreme segregation distortion were excluded from analysis. Linkage mapping was performed and genotyping errors were corrected in MapDisto (Heffelfinger et al., 2017). QTL mapping was performed using the composite interval mapping method in QTL Cartographer (Basten et al., 2002) with data averaged across both years. LOD thresholds were established via permutation testing (N = 1000).

RESULTS AND DISCUSSION

A yellow dry bean RIL population (N = 242) was evaluated for cooking time and sensory characteristics. The cooking times for Ervilha and PI527538 were 20.97 and 29.70 min respectively and ranged from 19.11-33.92 for the RIL population. Ervilha and PI527438 were significantly different for total (3.1 & 3.3), beany (1.3 & 1.8), earthy (2.1 & 2.3), starchy (3.7 & 3.1), sweet (2.2 & 1.7), and bitter (1.3 & 1.8) flavor intensities and seed coat perception (2.9 & 3.5). For the RILs, bitter flavor intensity exhibited the narrowest range (1.0 - 2.3), and beany flavor intensity exhibited the largest range (1.6 - 3.8). The linkage map developed contained 856 markers with a total length of 1896.40 cM. A total of 22 QTL were identified across 10 traits and 7 chromosomes (Table 1). While QTL were identified for vegetative flavor intensity and cotyledon texture, the ANOVAs for these traits were insignificant. There was extensive colocalization across traits, particularly on chromosomes 3, 7, and 10. For Pv03, beany, starchy, and bitter flavor intensity and seed coat perception colocalized. For Pv07, total, vegetative, and sweet flavor intensity colocalized. For Pv10, cooking time and total, beany, and starchy flavor intensity colocalized as did bitter flavor intensity and cotyledon texture. Chromosome 10 appears to be critical for cooking time and many sensory characteristics. With further validation, this information can be used to target faster cooking times and specific sensory profiles, without sacrificing desirable agronomic traits.

Trait	# QTL	Chr	Peak LOD	r ² range	<i>a</i> range
Cooking Time	3	2,8,10	12.24	0.07 to 0.18	-0.82 to 1.31
Total Flavor Intensity	3	7,8,10	10.68	0.04 to 0.14	-0.11 to 0.12
Beany Flavor Intensity	3	3,10	19.95	0.05 to 0.26	0.09 to 0.21
Vegetative Flavor Intensity	2	4	4.01	0.05 to 0.07	-0.08 to 0.07
Earthy Flavor Intensity	2	3,6	4.17	0.07 to 0.09	0.09 to 0.10
Starchy Flavor Intensity	2	3,10	5.76	0.08 to 0.09	-0.09
Sweet Flavor Intensity	2	7,8	4.51	0.06 to 0.07	-0.08 to (-0.07)
Bitter Flavor Intensity	2	3,10	7.38	0.06 to 0.12	0.07 to 0.09
Seed Coat Perception	2	3,10	9.06	0.07 to 0.17	0.08 to 0.13
Cotyledon Texture	1	10	5.33	0.08	0.15

Table 1: Number of QTL, chromosomes containing QTL, peak LOD scores, r^2 value range, and additive effect range for alleles donated by Ervilha for each trait.

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ASSESSING GENOMIC SELECTION PREDICTION ACCURACY FOR YIELD AND END-USE QUALITY TRAITS IN A BLACK DRY BEAN BREEDING POPULATION

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INTRODUCTION

Seed yield and canning quality are crucial traits to be improved in dry bean breeding. Canning evaluation is time, labor and resource demanding. Genomic Selection (GS) could reduce the cycle length, while enhancing the expected genetic gain and the selection response per unit time; it also uses less resources compared with extensive phenotyping¹. The objective of this study was to assess the predictive ability of genomic selection for yield, seed weight, appearance, and canning quality in a black bean breeding population.

MATERIALS AND METHODS

Plant Material: A total of 275 Black Breeding Lines (BBL) were used in this study. The study was composed of lines from Michigan State University and USDA-ARS East Lansing breeding programs.

Phenotyping: Yield, seed weight, color retention, and appearance were measured on seeds growth at the Saginaw Valley Research and Extension Center in Richville, MI. Yield is seed weight reported in kilograms per hectare (kg/ha). Seed weight is determined by weight in grams of 100 seeds. Upon opening, canned beans were evaluated for overall appearance and color retention subjectively by 10–15 bean panelists on a 5-point hedonic scale.

GBS and SNP calling: GBS libraries were based on ApeKI complexity reduction². The NGSEP pipeline was used to identify the SNPs and filter the data for MAF <5%, missing data >50%, and repetitive regions.

Genomic prediction: Prediction accuracies were evaluated using 100 subset validation cycles (50% training:50% testing) in the population with ridge-regression best linear unbiased predictions (rrBLUP). The model was implemented for analysis in R using the package rrBLUP³.

RESULTS AND DISCUSSION

In general, prediction accuracy increased as more data was available to train the model. Prediction accuracies were evaluated using cross-validation. The broad-sense heritability for appearance, color retention, 100 seed weight, and yield in the BBL on 2018 in Michigan were 0.79, 0.84, 0.72 and 0.29 respectively.

The overall prediction accuracies were 0.65 (appearance), 0.67 (color retention), 0.68 (seed weight) and 0.33 (yield) when 50% of the population was used to create the model. Although the prediction accuracies are a standard method to determine the success of GS models, it is more important to assess the power of the models to predict extreme values, and it has been reported that models with overall accuracies of 0.4 to 0.5 are able to select between 60 to 70 % of the top individuals. The above indicates that genomic selection could be integrated into dry bean breeding

to make selections in a wide range of complex traits leading to more efficient and integral breeding programs.



Figure 2. PCA of 275 genotypes determined using 7,132 SNP markers. The PCA separated the two breeding populations. The varieties Eclipse, Zenith, and Zorro are the most common parents in both breeding programs.

Figure 3. Prediction accuracy of 100 cycles of genomic prediction in the four traits assessed in this study. The average prediction accuracy was 0.65, 0.67, 0.68 and 0.33 for appearance, color retention, seed weight and yield respectively.

CONCLUSIONS

The prediction accuracy estimated by the correlation between observed and predicted values for yield and end-use quality traits were moderate for yield to relatively high for appearance, color retention and seed weight. Our results suggest that genomic selection can be used to increase selection intensity in complex traits as end-use quality. Further studies are needed to evaluate the implementation of genomic selection in dry bean breeding.

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WYOMING-GROWN PERUVIAN POPPING BEANS: SENSORY ANALYSIS AND CONSUMER ACCEPTANCE

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INTRODUCTION

American consumers fall short of dietary guidelines for a variety of nutrients including dietary fiber. Beans are a rich source of protein, fiber, starch and vitamins/minerals; however, intake of dry edible beans and legumes is currently contributing only 8% to daily fiber intake in American eating patterns.¹ Nuña beans (*Phaseolus vulgaris* L.) are a class of common beans originated in South America and cultivated in the highland areas of Peru, Ecuador and Bolivia.² They are unique due to their characteristic of "popping" after exposure to heat, producing a toasted, soft-textured edible snack.² Nuña bean preparation is energy-efficient with reduced requirements for fuel and cook time compared to other dry beans. Plant scientists have attempted to eliminate the photoperiod-sensitive gene present in traditional nuña beans, while retaining the popping gene, to cultivate in the U.S.²⁻³ Sensory analysis and evaluation of consumer acceptance can provide insight into factors that influence consumption of dry edible beans and bean extrudates such as taste (including bitter, beany, and/or aftertaste), texture, and mouthfeel.⁴⁻⁵ Consumer testing on food products also includes willingness to pay and purchasing habits. Factors that have been shown to influence willingness to pay include price, quality, taste, and nutritional value.⁶⁻⁷ This study aimed to evaluate consumer perception of nuña beans grown in Wyoming (WY).

MATERIALS AND METHODS

Temperate-adapted nuña bean advanced breeding lines from Colorado (CO) were field-grown using conventional practices in Lingle, WY and hand-shelled for consumer testing. Based on pilot popping method evaluation, CO 49957 beans were prepared by 1) preheating 1 teaspoon (5 mL) canola oil in a cast-iron pan for 5 minutes over medium heat, 2) rinsing and drying the beans, 3) heating ten beans in the pan for 1-2 minutes, 4) removing from pan to cool on a paper towel. Five popped beans were served individually on plates in a brightly lit room with white fluorescent lighting. Samples were evaluated for appearance, flavor, texture, aroma, and acceptability using a 9-point hedonic scale. The scale ranged from "like extremely" (9) to "dislike extremely" (1). Consumers were also asked questions regarding previous knowledge and intent of adding popping beans to their diet using a 7-point Likert scale ranging from "strongly disagree" (1) to "strongly agree" (7). Finally, participants responded to questions about willingness to pay. Participants were seated at separate stations and briefed not to interact with each other during the evaluation. Sensory attribute, consumer acceptability, and willingness to pay mean response main effects were calculated using t-test and analysis of variance (SPSS 26). Differences at *p* < 0.05 were considered significant.

RESULTS AND DISCUSSION

One hundred-and-thirty student, faculty, and staff taste panelists evaluated the samples. The diverse group of panelists, including both men and women of various ages from 18 to 65, showed no clear preference for the popped beans. Overall perception indicated that participants "dislike slightly" the popped beans. Sensory characteristics are presented in Table 1.

Table 1: Sensory evaluation	of popped bea	ns with mean	attributes	and standard	deviations
reported on a 9-point scale (1=dislike extr	emely, 9=like	e extremely	<i>י</i>).	

Sensory criteria	Popped beans
Appearance	4.38 ± 1.86
Aroma	3.85 ± 2.11
Flavor	4.00 ± 2.29
Mouthfeel	4.37 ± 2.28
Texture	4.32 ± 3.0
Overall acceptability	3.98 ± 2.18

There was a significant mean difference between age groups regarding the "overall acceptability" of the popped beans with a lower score among those aged 18-25 compared to those aged 35-44 (p=0.023). There was also a significant mean difference between different race/ethnicity groups with those of Hispanic/Latino, Native American, and Asian/Pacific Islander providing a higher score (p=0.008). Panelist responses to sensory analysis and consumer perception were also compared between two groups, those that enjoyed consuming beans as part of their

regular diet and those that did not. Mean comparison showed that those who enjoyed consuming beans had a higher mean response to "I am aware of the different types of beans and legumes and how to prepare them" and "I am interested in learning more about the different types of beans and legumes". Also, those that enjoyed consuming beans were more likely to pay the same amount for popping beans as they would for kidney beans and peanuts.

Overall, consumer acceptance and willingness to pay did not indicate a positive perception of the popped beans. This may be due to a lower mean age among participants. However, it may also reflect the national trend of higher bean intake among ethnic minority populations and middle-age adults. Additional analysis of sensory attributes among a more diverse panel of consumers may provide a better understanding of the perception of popping beans among U.S. consumers.

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SPEED BREEDING IN DRY BEANS

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INTRODUCTION: Speed breeding is the rapid generation advance of plant populations using long daylengths and increased plant growth temperatures. This technique was primarily pioneered in cereals (Watson et al. 2018), but has been adapted to rapidly advance plant populations in a number of plant species. Dry bean (*Phaseolus vulgaris* L.) is a good candidate for speed breeding approaches due to it being self-pollinated and since Ontario-adapted breeding material is typically photoperiod insensitive. In Ontario, dry bean is cultivated under summer conditions making it tolerant to warm conditions during growth and flowering that is required to reduce generation times through speed breeding. The objective of this paper is to outline steps that the dry bean breeding program at Agriculture and Agri-Food Canada in Harrow, ON has taken to develop a speed breeding platform.

MATERIALS AND METHODS: Daylengths of 22 hrs of light and 2 hrs of dark were used. Initially, high pressure sodium (HPS) lights were used to provide supplemental light and extend daylengths. These lights have been replaced with Illumitex (Austin, TX) LED fixtures which improved light quality and quantity. Targeted photon flux density was 400 µmol m⁻² s⁻¹ over the growing area which appeared to be sufficient. Spectrophotometric analysis showed that HPS lights provided higher photon flux in the far-red (700-800 nm) region which encourages excessive internode elongation. Overall, the shift to LED fixtures increased the red:far-red ratio and produced plants that were more compact and easier to manage in high density planting situations. Temperatures are maintained at 27 °C/22 °C (day/night) to encourage rapid growth. No detrimental effects of long daylengths or high temperatures were observed on plant growth or seed production.

To maximize growing space usage, we use high density containers (Part#:720705C; T.O. Plastics, Clearwater, MN) to grow plants in potting mix (BM6; Berger, St. Modeste, QC) at a density of 200 plants/m². A closed loop ebb and flow irrigation system that uses rolling greenhouse tables was designed to ensure even watering, reduce labour and maximize utilization of greenhouse space. Standard hydroponic nutrient solution used for cucumber production (pH 5.8) was used for irrigation (OMAFRA 2010). Increased plant spacing may be required to maximize seed production. Boron toxicity issues were observed when pH of the hydroponic solution is <6. This was solved by increasing the pH of the solution or reducing the nutrient solution concentration to half. *Pythium* root rot was also an issue for plants where water pooled on benches. A shift to industrial flood bench liners (Zwart Systems, Beamsville, ON) from containers sitting directly on the bench is expected to limit these issues. Nutrient solution was also recycled through holding tanks and a standard household UV filter system was used to reduce disease transmission.

We experimented with harvesting pods from plants at one week intervals after flowering. In our experience, green pods can be harvested 3 weeks after flowering and allowed to dry completely in a 28°C incubator for 5-7 days. Seed viability was approximately 90%.

High density planting and LEDs enabled high throughput screening of breeding populations in a mist chamber at the unifoliate stage. This was effective for selecting Anthracnose (race 73) resistant lines using standard inoculation protocols (Dongfang et al. 2008). Resistant lines were moved to the greenhouse and advanced under speed breeding conditions. The potential exists to include screening at each generation for any trait that can be evaluated at early growth stages, including bacterial blights, or to screen populations using marker assisted selection.

DISCUSSION: In this system, populations can be advanced indoors using bulk or single seed descent with the F_4 generation plants being individually threshed and the resulting F_5 seed planted as single rows in the field (Fig 1a). Traditional generation advance in the field or at off-season nurseries is not required, which saves time and financial resources. For the program at AAFC-Harrow, this system meant restructuring of the breeding program and will reduce time to release of varieties by three years (Fig 1b). Furthermore, it enables the program to react to industry needs and have advanced generation breeding material in multi-location yield trials within three years.



Figure 1: a) Example of breeding program flow for 1 year using the speed breeding system and b) Breeding program flow over a five year period.

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EXPLORING GENETIC IMPROVEMENTS AND INNOVATIVE PROCESSING METHODS IN ORGANIC DRY BEANS

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INTRODUCTION

The consumer demand for organic dry beans has been rising in the United States and globally. However, the major challenges in the production of organic beans include unsatisfactory seed quality (especially seed coat cracking), and unappetizing appearance, texture, and taste of their products. Meanwhile, the canning industry is moving away from cans toward flexible pouches, as the latter is a more efficient use of packaging material and requires less storage space and transportation costs. We propose to establish an organic bean breeding program that develops germplasm with improved seed quality. The improved germplasm will match with improved processing methods such as canning and retortable flexible pouches, which will further enhance the consumer preferred quality traits.

MATERIALS AND METHODS

A field trial with black bean and kidney bean varieties was conducted in 2018 on an organic certified plot provided by collaborating farmer Jim Sattelburg (Everbest Organics). The field is located in Akron, MI. The black bean variety trial consisted of 12 lines (including popular varieties and breeding lines) and the kidney bean variety trial consisted of 12 lines (including white, light red, and dark red kidney varieties and breeding lines). A field trial was conducted in 2019 on another organic plot provided by Sattelburg in Unionville, MI. It consisted of 12 lines of black bean varieties (including eight lines that were the same from previous year and four new breeding lines), 12 kidney bean lines (including nine lines that were the same from previous year and three new breeding lines), and yellow bean varieties of 10 advanced breeding lines. The trials were planted at the same row spacing and managed the same as the rest of the commercial field by Sattelburg. All trials were planted as a randomized complete block design with three replications and each replication consisted of two rows. The plants were harvested by hand and threshed at MSU with an Almaco (Nevada, Iowa) belt thresher model BT14.

The black and kidney beans harvested in 2018 were canned at the Food Processing and Innovation Center (FPIC) in Okemos, MI, using a small-scale canning protocol. The canning quality was evaluated by a group of trained panelists. Kidney beans were scored for appearance with a scale of 1-5 (worst to best appearance) and black beans were scored for appearance with the same 1-5 scale and color with a scale of 1-5 (light brown to dark black). The entries from 2019 will be canned with the same method. All of the samples will also be processed through flexible pouches at FPIC. The pouch process will follow the modified small-scale canning protocol with cans being replaced by pouches. The yield data of 2019 trial and results of the quality evaluation of canning and pouch processing products will be reported in the future.

RESULTS AND DISCUSSION

The kidney bean yields ranged from 14.1 to 29.5 CWT/acre and the top yielding line was the MSU white kidney variety Snowdon according to 2018 yield data. Black bean yields ranged from 19. 5 to 41.5 CWT/acre and the top yielding lines were advanced breeding lines from MSU (B17220) and USDA-ARS (BL1402-15). The NDSU light red kidney Talon, was also one of the top yielding lines (Figure1). Variations were also observed in canning quality among the tested bean lines (Table 1). The MSU dark red kidney variety Red Hawk had the best canning quality among kidney beans. The MSU black bean variety Zenith ranked on top with highest appearance score and color score. The genetic variations observed in both kidney and black beans will provide us a good resource to better study and improve the yield and seed quality of these beans under organic production conditions.



Figure 1. Yield of kidney and black bean lines planted in organic field in 2018.

Construis	Canning	Construns	Canning Quality		
Genotype	Quality	Genotype	Appearance	Color	
Red Hawk	4.25	Zenith	4	4	
K16136	3.75	B17536	3.75	3.67	
Snowdon	3.25	Zorro	3.75	3.33	
Talon	3.25	B17691	3.5	3.67	
Beluga	3	B17922	3.5	3.33	
K16131	2.75	B16501	3.25	4.00	
Red Cedar	2.75	BL1402-15	3	4.33	
K15601	2.25	B16504	3	3.33	
K16640	2.25	B17220	2.75	4.33	
K16957	2	Black Bear	2.75	2.33	
Montcalm	NA	B18504	2.5	3.67	
K15901	NA	Eclipse	2.5	1.67	

Table 1. Canning quality scores of organic kidney and black beans from 2018.

USING THE PULSE CROP DATABASE FOR PULSE CROP RESEARCH AND IMPROVEMENT

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Formerly known as the Cool Season Food Legume database, the Pulse Crop Database (PCD, <u>www.pulsedb.org</u>, Figure 1.) is an online resource for genetics, genomes, and breeding research for common bean, cowpea, pigeon pea, chickpea, pea, lentil, faba bean, lupine, vetch, and bambara bean. It provides access to curated and integrated data and tools to enable pulse crop research, translation and improvement.



Figure 1. PCD homepage features quick access to crops and tools from the menu in the header and other links.

PCD contains publicly available genomics, genetics and breeding data such as genomes, genes, transcripts, genetic maps, markers, QTL, germplasm, phenotype and publications, with integrated tools to easily access, view, filter and download the data. Data can be searched using data specific searches, or a new MegaSearch that allows for more detailed search customization and download. Genetic maps are viewed and compared with the MapViewer tool that allows tailoring of the view of the map and export of publication quality images.

Crop	Markers	Maps	QTLs	Phenotype Measurements
Chickpea	52,735	93	4,648	110,067
Common Bean	8,638	17	509	243,442
Cowpea	38,546	14	367	81,959
Faba Bean	1,836	28	278	22,660
Lentil	20,673	36	316	43,968
Реа	147,144	78	1,291	154,589
Pigeon Pea	7,858	10	30	39,548
Lupin	15,900	7	64	5,729
Bambara Bean	218	1	67	43

Table 1. Number of available data points of each type for each crop in PCD.

Currently, there are eight genomes for which genes and transcripts are searchable within PCD (Table 2). The genome sequences can be searched using BLAST; genome annotations viewed with JBrowse; the structure of the genomes compared with Synteny Viewer with links to paralogs and ortholog; and metabolic pathways explored using PathwayCyc. As new data is published, it is added to the database. Pre-publication datasets, of significant importance for the pulse crop research community, are also made available at the request of the authors. PCD can also provide an accession number and location for data files associated with publications if files do not have a primary data repository or cannot be added to the journal website as supplemental information for the publication.

Table 2. Genomes available in PCD.

Genome	Version
Cicer arietinum cv. CDC Frontier (Kabuli)	1.0
Cicer arietinum cv. ICC 4958 (Desi)	2.0
Cicer reticulatum L. cv. PI489777	1.0
Phaseolus vulgaris cv. G19833	2.1
Vigna unguiculata L. Walp cv. IT97K-499-35	1.1
Pisum sativum L. cv. Cameor	1a
<i>Cajunus cajan</i> acc. Asha	1.0
Lupinus angustifolius L. cv. Tanjil	1.0

The Breeding Information Management System (BIMS) in PCD allows for secure management of breeding program via private user accounts while also enabling access to publicly available pulse phenotype data downloaded from the GRIN database. BIMS also works with the Android app Field Book for streamlined phenotype data collection and upload, or with spreadsheet templates provided for data upload including genotype data. Once the breeding program data is in BIMS, the secure data can be viewed, filtered, analyzed, and archived and used to create templates for data entry. BIMS is being furthered developed to more advanced analysis capability using high performance computing.

BEAN CULTIVARS AND GERMPLASM RELEASED IN CENTRAL AMERICA AND THE CARIBBEAN

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In response to a request at the most recent meeting of the Bean Improvement Cooperative, technical information and descriptions of recent releases of bean cultivars and germplasm in Central America and the Caribbean have been posted on the http://arsftfbean.uprm.edu/bean/web site, and included in Table 1. The table reflects the productivity of bean breeding programs in the Central America and the Caribbean in recent years and represents a valuable group of bean genotypes having unique combinations of traits of economic importance. More information concerning bean releases will be added to the web site as it becomes available.

Table 1. List of bean cultiva	rs and germplasm r	eleased in Central	America and the	Caribbean
since 2010.				

Name(s)	Seed type	Resistances	Countries released	Participating institutions
Aifi Wuriti	Black	BCMV, BGYMV	Honduras, Haiti	Zamorano, UPR, NSS, INTA
(INTA Negro)			Nicaragua	
Amilcar 58	Small red	BCMV, BGYMV	Honduras	Zamorano, FIPAH, ASOCIAL
				Yorito
AO-1012-29-3-3A	Red	BCMV, BCMNV,	Puerto Rico,	UPR, USDA-ARS, Sokoine
	kidney	leafhopper	Tanzania	Univ., Oregon State Univ.
Arbolito Negro	Black	BCMV, BGYMV,	Honduras	Zamorano, PRR, CIAL San
Mejorado		rust		José de la Cuesta
Atillos	Medium	BCMV, drought	Nicaragua	FECODESA, Zamorano,
	red			USDA-ARS-TARS/
				Washington
Badillo	Light red	BCMV, CBB	Puerto Rico	UPR, USDA-ARS-TARS
	kidney			
Bella	White	BCMV, BCMNV,	Puerto Rico	Univ. Puerto Rico, USDA-
		BGYMV, CBB, LF		ARS-TARS
Beníquez	White	BGYMV, BCMNV	Puerto Rico	UPR, USDA-ARS-TARS
Briyo AM	Small red	BCMV, BGYMV	Honduras	Zamorano, PRR,
				ASOCIALAYO
Cardenal	Small red	BCMV, BGYMV,	Honduras	Zamorano, UPR, DICTA,
(Chaparrastique)		drought, heat	El Salvador	CENTA
Campechano JR	Small red	BCMV, BGYMV,	Honduras	CIAT, INTA, Zamorano,
(INTA Fuerte		drought	Nicaragua	PRR, ASOCIALAYO
Sequía)				

Chepe	Small red	BCMV, ALS, ANT	Honduras	CIAT, INTA, Zamorano,
Don Cristábol	Small rad		Handuras	
Don Cristobai	Small red	rust	Honduras	ASOCIALAYO
Don Kike	Small red	BCMV. BGYMV.	Honduras	CIAT, INTA, Zamorano,
		drought		PRR, ASOCIALAYO
ECO Negro	Black	BCMV, BGYMV,	Nicaragua	FECODESA, Zamorano,
		drought, ASB, WB	_	UPR, USDA-ARS-TARS, UPR
Erecto 2222	Black	BCMV, BGYMV	Cuba	INCA, Zamorano
Esperanceño	Black	BCMV, BGYMV	Honduras	CIAT, UPR, Zamorano,
				FIPAH, ASOCIAL Yorito
Flor de Maria	Small red	BCMV, BGYMV,	Nicaragua	FECODESA, Zamorano
		drought		
Guazapa 1	Small red	BCMV, BGYMV	El Salvador	CENTA, Zamorano
Honduras Nutritivo	Small red	BCMV, BGYMV,	Honduras	CIAT, INTA, Zamorano,
		high iron		DICTA
ICTAZAM	Black	BCMV, BGYMV, WB	Guatemala	ICTA, Zamorano, UPR,
ICTA Sayaxché	Black	BCMV, BGYMV,	Guatemala	ICTA, Zamorano, UPR, I
		rust		
ICTA Peten	Black	BCMV, BGYMV	Guatemala	ICTA, CIAT
ICTA Chortí	Black	BCMV, high iron	Guatemala	ICTA, CIAT
ICTA Labor Ovalle	Black	BCMV	Guatemala	ICTA, North Dakota St.
				Univ.
ICTA Superchiva	Black	BCMV, high iron,	Guatemala	ICTA, CIAT
		highlands		
ICTA Utatlán	Black	BCMV, rust,	Guatemala	ICTA, North Dakota St.
		highlands		Univ.
IDIAF Chalona	Black	BCMV, BGYMV,	Dominican Rep.	IDIAF
Negra		rust		
IDIAF DPC-40	Black	BCMV, BCNMV,	Dominican Rep.	IDIAF, UPR, UNL, USDA-
		BGYMV		ARS-TARS, Zamorano
IDIAF Maravilla	Red	BCMV	Dominican Rep.	IDIAF
	mottled			
IDIAF Yaconin	Cranberry	BCMV	Dominican Rep.	IDIAF
INIAP 485-Urcuqui	Black	BCMV, BGYMV,	Ecuador	INIAP, Zamorano, UPR
		ANT, rust, canning		
INTA Zamorano	Small red	BCMV, BGYMV	Nicaragua	INTA, Zamorano, UPR
INTA Centro Sur	Small red	BCMV, BGYMV, LF,	Nicaragua	INTA, Zamorano, UPR, I
		BNF		
INTA Ferroso	Small red	BCMV, BGYMV,	Nicaragua	INTA, CIAT, Zamorano
		high iron		
INTA Tomabú	Small red	BCMV, BGYMV,	Nicaragua	INTA, Zamorano, UPR
		drought		
INTA Rojo Jinotega	Small red	BCMV, BGYMV,	Nicaragua	INTA, Zamorano, UPR
		drought		
INTA Seda 1	Small red	BCMV, BGYMV,	Nicaragua	INTA, Zamorano
		drought		

INTA Seda 2	Small red	BCMV, BGYMV, drought	Nicaragua	INTA, Zamorano
INTA Extrema	Small red	BCMV, BGYMV,	Nicaragua	INTA, CIAT
INTA Nutritivo Rendidor	Small red	BCMV, BGYMV,	Nicaragua	INTA, CIAT
La Presa	Small red	BCMV, BGYMV	El Salvador	CENTA, Zamorano
Lenca Precoz	Black	BCMV, BCNMV, BGYMV	Honduras	Zamorano, UPR, DICTA
La Majada AF	Small red	BCMV, BGYMV	Honduras	Zamorano, PRR, ASOCIALAYO
Maravillosa	Small red	BCMV, BGYMV	Nicaragua	FECODESA, Zamorano
Marcelino	Small red	BCMV, BGYMV	Honduras	Zamorano, ASOCIAL Yorito
Milagrito	Small red	Highland	Honduras	Zamorano, PRR/ASOCIALAYO
Nambí	Black	BCMV, BGYMV, drought	Costa Rica	PITTA-Frijol, CIAT, Zamorano
Negro Tacuba	Black	BCMV, BGYMV, drought, heat	El Salvador	CENTA, Zamorano
Odile (SCR 15)	Small red	BCMV, BGYMV, CBB, rust	Cuba	INCA, CIAT
Paisano PF	Small red	BCMV, BGYMV, heat	Honduras	Zamorano, PRR/ASOCIALAYO
Paraisito Mejorado 2-Don Rey	Small red	BCMV, BGYMV, LF, BNF	Honduras	Zamorano, UPR, DICTA, FIPAH, ASOCIAL Vallecillo
PR0401-259, PR0650-31	Pink, black	BGYMV, BCMV, CBB, WB	Puerto Rico	UPR, USDA-ARS, Zamorano
PR0633-10 and PR0737-1	Red mottled	BGYMV, BCMNV, CBB	Puerto Rico, Haiti, Dom. Rep.	UPR, USDA-ARS-TARS, IDIAF, NSS
PR0806-80 and PR0806-81	White	BCMV, BCMNV, rust	Puerto Rico	UPR, USDA-ARS-TARS, USDA-ARS-BARC
PR1146-138	Yellow	BCMV, BGYMV, leafhopper	Haiti, Puerto Rico	NSS, UPR, USDA-ARS-TARS
PR1572-19 and PR1572-26	Pinto	BCMV, BCMNV, BGYMV, rust	Puerto Rico	UPR, USDA-ARS-TARS, USDA-ARS-BARC
Pueblo viejo 16	Small red	BCMV, BGYMV	Honduras	CIAT, Zamorano, FIPAH, ASOCIAL Yorito
Quebradeño	Small red	BCMV, BGYMV	Honduras	Zamorano, FIPAH, ASOCIAL Yorito
Redoblante	Small red	BCMV, BGYMV	Nicaragua	FECODESA, Zamorano
Rodeo AG	Small red	BCMV, BGYMV	Nicaragua	FECODESA, Zamorano
Rojo Chortí (CENTA EAC)	Small red	BCMV, BGYMV, heat	El Salvador	CENTA, Zamorano, UPR, DICTA
Rojo Delicia	Small red	BCMV, BGYMV	Honduras	CIAT, INTA, Zamorano, PRR, ASOCIALAYO
Rojo Fortificado	Small red	BCMV, BGYMV, high iron	Honduras	CIAT, Zamorano, PRR, ASOCIALAYO

San Antonio FP1	Small red	BCMV, BGYMV,	El Salvador	CENTA, Zamorano
San José	Small red	BCMV, BGYMV	Honduras	Zamorano, FIPAH, ASOCIAL Yorito
Sankara	Black	BCMV, BGYMV	Haiti,	NSS, UPR, UNL, IDIAF,
Azabache 40			Honduras	Zamorano, DICTA
Sequía-1	Black	BCMV, drought	Dominican Rep	IDIAF
TARS-LFR1	Small red	BCMV, CBB, LF, RR	Puerto Rico	USDA-ARS-TARS, UPR
TARS-LH1	Pinto	BCMV, leafhopper	Puerto Rico	USDA-ARS-TARS, Mich. St.
				Univ, UPR, IDIAF
TARS-MST1,	Black	BCMV, CBB,	Puerto Rico,	USDA-ARS-TARS, UPR,
SB-DT1		drought, heat, root	Nebraska	Univ. Nebraska,
		rot		
TARS-HT1, TARS-	Dark red	BCMV (TARS-HT-1),	Puerto Rico	USDA-ARS-TARS, UPR
HT2	kidney,	heat		
	Light red			
	kidney			
TARS-Tep 22,	Off-white,	Tepary: CBB,	Puerto Rico	USDA-ARS-TARS, UPR,
TARS-Tep 32	yellow	drought, heat, rust		Colorado St. Univ.
		(Tep-22)		
Tayní	Small red	BCMV, BGYMV	Costa Rica	PITTA Frijol, Zamorano
Tolupan Rojo	Small red	BCMV, BGYMV, ALS	Honduras	Zamorano, UPR, DICTA
Victoria	Small red	BCMV, BGYMV	Honduras	Zamorano, PRR,
				ASOCIALAYO

Abbreviations used in Table 1:

ALS – Angular leaf spot, caused by *Pseudocercospora griseola*

ANT – Anthracnose, caused by Colletotrichum lindemuthianum

ASB – Ashy stem blight, caused by Macrophomina phaseolina

BCMV, BCMNV – Bean common mosaic virus, Bean common mosaic necrosis virus

BGYMV – Bean golden yellow mosaic virus

BNF – Biological nitrogen fixation

CBB – Common bacterial blight, caused by *Xanthomonas axonopodis*

CENTA - Centro Nacional de Tecnología Agropecuaria y Forestal

CIAT – International Center for Tropical Agriculture, Colombia

DICTA - Dirección de Ciencia y Tecnología Agropecuaria, Honduras

ICTA - Instituto de Ciencia y Tecnología Agrícolas, Guatemala

IDIAF - Instituto Dominicano de Investigaciones Agropecuarias y Forestales, Dom. Rep.

INCA - Instituto Nacional de Ciencias Agrícolas, Cuba

INIAP - Instituto Nacional de Investigaciones Agropecuarias, Ecuador

INTA - Instituto Nicaragüense de Tecnología Agropecuaria, Nicaragua

LF – Low soil fertility

NSS - National Seed Service, Min. of Agric., Min. Nat. Res. and Rural Development, Haiti

PITTA – Programa de Investigación y Transferencia de Tecnología Agropecuaria, Costa Rica

UPR – Dept. of Agroenvironmental Sci., Univ. of Puerto Rico, Puerto Rico

USDA-ARS-BARC – USDA-ARS Beltsville Agricultural Research Center, Maryland

USDA-ARS-TARS - USDA-ARS Tropical Agriculture Research Station, Puerto Rico

RR – Root rot complex

WB – Web blight, caused by Rhizoctonia solani

Zamorano – Zamorano Univ., Honduras

SENSITIVITY IN VITRO OF Pseudocercospora griseola STRAINS TO FUNGICIDES

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INTRODUCTION

The common bean is one of the most important crops for Brazil. Among the factors that reduce yield and cause production instability are pathogens, especially fungi. Angular spot is an important fungal disease in the crop and is caused by the fungus *Pseudocercospora griseola* (Nay et al., 2019). This disease occurs in all regions where this legume is cultivated (Gonçalves-Vidigal et al., 2016). In addition to genetic resistance, another form of angular leaf spot control is the use of fungicides. In Brazil there are 106 registered fungicides (commercial products) that can be used to control the disease, especially the groups of benzimidazois, dithiocarbamates, inorganics (copper and tin), strobilurins, triazoles and some mixtures of these groups (Agrofit, 2019). However, resistant strains of *P.griseola* can arise in the population due to great variability of this pathogen, as well as due to the sporulation, spread and successive applications of the same active ingredient or the same fungicide chemical group (Garcia, 1999). These resistant strains can predominate in the population and the product loses its efficiency. Therefore, it is necessary to monitor the sensitivity of *P.griseola* strains to the main recommended fungicides.

MATERIALS AND METHODS

Leaves naturally infected with symptoms of angular spot were collected in common bean fields in Lavras and Lambari, Minas Gerais-Brazil, in the 2017 dry season. From the lesions, *P. griseola* was isolated to obtain monospore cultures. Strains isolated were preserved using the paper strip method (Botelho et al., 2013).

The experiment was conducted in a randomized block design (RBD) in a 6x15 factorial scheme, with 1 check, 5 fungicides from different chemical groups and 15 strains of *P. griseola*, 7 from Lavras (LV) and 8 from Lambari (LB). Each plot consisted of a Petri dish, totaling 90 plots per replication. The fungicides were incorporated in a culture medium of leaf-dextrose-agar and kept in Petri dishes. After solidification of the medium, a 12-day-old culture mycelium suspension aliquot was added to the Petri dish until it was completely covered. The plates were incubated (B.O.D) for six days at 24°C. The treatments were: 1. Check without fungicide (Check), 2. Pyraclostrobin (P), 3. Methyl thiophanate (MT), 4. Chlorothalonil (C), 5. Piraclostrobin + Epoxiconazole (P + E) and 6. Mancozeb (M), with treatments 2 to 6 being used in commercial concentrations. These fungicides are recommended for control of angular leaf spot of common bean. Sporulation in each plate was evaluated by counting the spores and the concentration obtained in the Neubauer Chamber.

All data were subjected to analysis of variance and averages clustered by Scott-Knott test at 5% probability.

RESULTS AND DISCUSSION

The strains showed differences in the sporulation rate (P < 0.05). In addition, the interaction strains x fungicides was significant, which means that the sporulation of strains varied according to the fungicide used. The strains LV22, LV32, LB23, LB28 and LB30 showed the same behavior for

the evaluated fungicides (Table 2). The strains LV29, LV36 and LB31 showed a reduced sporulation rate for all fungicides (Table 1). The strain LV25 showed the highest sporulation percentage to all fungicides evaluated in relation to the check, except for pyraclostrobin (Table 1). For the evaluated strains, methyl thiophanate showed the least inhibition of sporulation (Table 2).

Table 1: Percentage of sporulation of the strains to different fungicides in relation to the check.

	Fungicides ¹							
Strains	Р	MT	С	P+E	М			
LV22	21.5	50.8	54.2	67.8	33.9			
LV25	19.0	342.9	238.1	257.1	171.4			
LV29	6.3	13.2	10.9	9.5	11.8			
LV31	9.5	41.3	9.5	19.0	38.1			
LV32	48.7	87.2	97.4	59.0	53.8			
LV33	21.3	96.0	53.3	32.0	69.3			
LV36	7.0	23.1	10.5	13.3	16.8			
LB21	18.6	51.6	14.9	17.0	24.5			
LB23	71.4	80.4	75.0	64.3	92.9			
LB28	30.3	30.3	28.9	21.1	21.1			
LB30	60.6	187.9	58.6	42.4	131.3			
LB31	1.9	4.7	2.8	12.7	4.2			
LB32	11.9	44.6	11.3	10.7	10.7			
LB37	27.6	72.7	15.3	15.3	18.4			
LB40	26.5	53.8	12.1	34.8	45.5			

¹Piraclostrobin (P); Methyl thiophanate (MT); Chlorothalonil (C); Piraclostrobin + Epoxiconazole (P+E) e Mancozeb (M)

Table 2:	Ave	rage	straiı	1 sporula	tion	(10^4)
conidia/n	nl) in	diffe	erent	treatmen	ts (c	heck
and fungi	icides)					

	0											
Check and Fungicides ¹												
Strains	Che	eck	I)	M	ΙT	(C	P-	ŀΕ	N	1
LV22	7.4	Aa	1.6	Aa	3.7	Aa	4	Aa	5	Aa	2.5	Aa
LV25	0.9	Ab	0.2	Bb	3	Ab	2.1	Aa	2.3	Aa	1.5	Aa
LV29	29	Aa	1.8	Ba	3.8	Bb	3.2	Ba	2.8	Ba	3.4	Ba
LV31	2.7	Ab	0.3	Bb	1.1	Ab	0.3	Bb	0.5	Ba	1	Aa
LV32	3.3	Ab	1.6	Aa	2.8	Ab	3.2	Aa	1.9	Aa	1.8	Aa
LV33	3.2	Ab	0.7	Bb	3	Ab	1.7	Aa	1	Ba	2.2	Aa
LV36	11.9	Aa	0.9	Ba	2.8	Bb	1.3	Ba	1.6	Ba	2	Ba
LB21	15.7	Aa	2.9	Ba	8.1	Aa	2.3	Ba	2.7	Ba	3.8	Ba
LB23	2.3	Ab	1.7	Aa	1.9	Ab	1.8	Aa	1.5	Aa	2.2	Aa
LB28	6.3	Ab	1.9	Aa	1.9	Ab	1.8	Aa	1.3	Aa	1.3	Aa
LB30	4.1	Ab	2.5	Aa	7.8	Aa	2.4	Aa	1.8	Aa	5.5	Aa
LB31	17.7	Aa	0.4	Bb	0.8	Bb	0.5	Bb	2.6	Ba	0.8	Ba
LB32	14	Aa	1.7	Ba	6.2	Aa	1.6	Ba	1.5	Ba	1.5	Ba
LB37	13.6	Aa	3.8	Ba	9.9	Aa	2.1	Ba	2.1	Ba	2.5	Ba
LB40	11	Aa	2.9	Ba	5.9	Bb	1.3	Ba	3.8	Ba	5	Aa

¹Piraclostrobin (P); Methyl thiophanate (MT); Chlorothalonil (C); Piraclostrobin + Epoxiconazole (P+E) e Mancozeb (M)

*Averages followed by the same capital letters in horizontal belong the same group by Scott-Knott test ($P \le 0.05$).

**Averages followed by the same lower case letters to in vertical belong the same group by Scott-Knott test ($P \le 0.05$).

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STATUS OF ANTHRACNOSE IN MICHIGAN IN 2020

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The race structure of bean anthracnose (caused by *Colletotrichum lindemuthianum*) has been remarkably stable over the last three decades in Michigan. In the early 1990's, races 7 and 73 were first identified (Kelly et al., 1994) and race 73 has persisted over time. Race 7 appears sporadically on kidney beans but is not a major threat. Race 73 is unique in its widespread geographic distribution and persistence unlike other races that are geographically isolated and sporadic in appearance (Padder et al., 2017). Race 73 has been largely contained through certified seed programs and resistance in navy, black and kidney bean varieties, which is conditioned by alleles at the *Co-1* locus on chromosome Pv01. Other races have appeared in Michigan in this era but have not persisted. Race 65 was reported in 1996 (Balardin and Kelly, 1996), but it did not persist and probably was introduced in commercial seed lots. Race 65 differs from race 73, as it lacks virulence to the *Co-2* gene (Cornell 49-242, binary #8, Table 1) and is also controlled by the same alleles at the *Co-1* locus. Isolates of race 65 are widespread in Brazil.

The anthracnose picture changed with the isolation of race 109 on black beans in Northern Michigan in 2017 (Awale et al., 2018). Race 109 first identified in Manitoba in 2007, is very similar to race 105 previously identified in Manitoba in 2005 and later in Ontario in 2015 (Dongfang et al., 2008; Conner et al., 2019). Race 109 differs from 105 based on its virulence to the Co-1³ gene in Perry Marrow (binary #4). Race 109 changed the entire breeding strategy at MSU as it defeated certain genes widely deployed in navy and black bean varieties. With the appearance of race 109, it was determined that the resistant allele widely used in navy and black bean breeding was the Co-1² allele present in the differential cultivar Kaboon (binary #32, Table 1) as race 109 over comes this allele. As a result most current black and navy bean varieties are susceptible to race 109. Race 109 does not defeat the original Co-1 allele assumed to be widely distributed in kidney beans. Certain older kidney beans proved to be resistant to race 109 but many were not, suggesting that the allele in many contemporary kidney bean varieties, such as Red Hawk was actually the $Co-l^2$ allele. The $Co-l^2$ allele conditions resistance to race 7, which favored its deployment in kidney beans in recent years. Breeding for resistance to anthracnose has taken a renewed focus at MSU. Rather than maintaining resistance to race 73 through routine screening, the program is now introgressing other resistance loci into breeding materials.

The current focus has been on the *Co-4* locus on Pv08, which has been widely studied (Melotto et al., 2001; Burt et al., 2015; Oblessuc et al. 2015) and the *Co-4*² allele is recognized as one of the most valuable resistance genes against a wide range of anthracnose races. Despite the depth of knowledge on this allele, it has not been widely utilized in resistance breeding to date. In the MSU program breeders have hesitated to use this valuable genetic resource until such times as the need arises. We have however been moving it through generations of intercrossing into MSU breeding materials and current black and navy bean breeding lines with the *Co-4*² allele now represent five generations of intercrossing from the initial resistance source identified by Young et al. (1998) in SEL1308 and the original landrace Colorado de Teopisca (G2333). Interestingly, preliminary studies of near-isogenic lines w/wo the gene suggest a linkage drag on yield or a fitness cost of resistance despite five generations of intercrossing with the *Co-4*² gene. Further studies are needed to confirm this observation, but caution is merited in its utilization.

In 2019, race 2 appeared in yellow bean nurseries and spread into kidney bean trials. Race 2 defeats the *Co-1* allele but can be controlled by the *Co-1*² allele. Caution in deploying the second allele revolves around its susceptibility to race 109. In Andean beans, Zuiderveen et al. (2016) identified a new locus on Pv02 that conditioned resistance to the virulent Andean races 39 and 55. This locus appears to be the same as the *Co-u* first identified by Geffroy et al. (2008). Resistance to races 39 and 55 in the ADP appears to be tightly linked to the *I* gene. This locus is being studied in detail as it could provide a new source of resistance to anthracnose in kidney beans.

Race	Α	В	С	D	E	F	G	н	I	J	К	L
Binary Code	1	2	4	8	16	32	64	128	256	512	1024	2048
2	R	S	R	R	R	R	R	R	R	R	R	R
7	S	S	S	R	R	R	R	R	R	R	R	R
65	S	R	R	R	R	R	S	R	R	R	R	R
73	S	R	R	S	R	R	S	R	R	R	R	R
109	S	R	S	S	R	S	S	R	R	R	R	R

Table 1. Race designation of *Colletotrichum lindemuthianum* strains from Michigan on twelve differential cultivars of common bean

Differential cultivars: A: Michelite, B: Michigan Dark Red Kidney, C: Perry Marrow, D: Cornell 49242, E: Widusa, F: Kaboon, G: Mexico 222, H: PI 207262, I: TO, J: TU, K: AB 136, L: G2333.

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SIMULTANEOUS INOCULATION OF COMMON BEAN CULTIVARS WITH MULTIPLE RACES OF Collectrichum lindemuthianum

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INTRODUCTION

There is a need for an effective methodology for studies involving the inoculation of common bean plants with multiple isolates or specific races of *Colletotrichum lindemuthianum*, the bean anthracnose pathogen. This methodology could be used for the rapid identification of common bean cultivars or plants from segregating populations with resistance to multiple races of *C. lindemuthianum*. Such a methodology has been used to develop common bean cultivars combining two to four rust resistance genes (gene pyramiding) conferring broad rust resistance to *Uromyces appendiculatus*, the rust pathogen (Stavely, 1983; 2000). The methodology has also been used to elucidate the nature of epistatic interactions between rust resistance genes (Valentini et al., 2015), inheritance of resistance, the validation of molecular markers (Hurtado et al., 2017), and other studies. Thus, we report here the simultaneous inoculation of common bean plants with multiple races of *Colletotrichum lindemuthianum*.

MATERIALS AND METHODS

This study, conducted at the Soybean Genomics and Improvement Laboratory, USDA-ARS, Beltsville, MD, included 10 cultivars: Beija Flor, Corinthiano, Crioulo 159, Cornell 49242, G 2333, Jalo Listras Pretas, Michigan Dark Red Kidney, Ouro Negro, Pitanga, and TO. Ten days after sowing, we prepared five groups of plants. The first group included ten plants per cultivar, and they were inoculated with four races simultaneously. Each of the other four groups, comprised of six plants per cultivar, were inoculated with single races. The races used in this study were from the collection maintained at Beltsville (their origin in parenthesis): race 39 (NL.1.0), 321 (Mx.7.1), 1545 (Gu.8.0) and 3481 (C.R.2.0). The spore solution in all inoculations had a concentration of 1.2×10^6 spores per mL. We used a cotton swab to apply the spore solution of each race to the abaxial side of the leaves. For the simultaneous inoculation (group 1) we cut the tip of one primary leaf and we kept the other leaf uncut. The cut leaves were inoculated with races 39 and 321 and the uncut leaves with races 1545 and 3481. To avoid contamination, we first inoculated with race 39, followed by races 1545, 321 and 3481. The other four groups of plants were inoculated with a single race individually on both primary leaves: group 2 with race 39, group 3 with race 321, group 4 with race 1545, and group 5 with race 3481. After inoculation, the plants were transferred to a mist chamber ($20 \pm 1^{\circ}$ C and 95% humidity, under darkness) for 48 hours. Then, the plants were transferred to a greenhouse bench. Seven days later, these plants were evaluated for their anthracnose reaction using a 1 - 9 scale, where grades 1-3 were considered resistant, and grades 4 - 9 were considered susceptible.

RESULTS AND DISCUSSION

The resistant and susceptible reactions of the common bean cultivars to races 39, 321, 1545 and 3481 of *C. lindemuthianum* were identical in the simultaneous inoculations with multiple races and in the individual (single race) inoculations (Table 1). These results also show that no interaction occurred between the races in the simultaneous inoculations (Figure 1). This methodology could be used to identify common bean cultivars with resistance to several races of

C. lindemuthianum, to study inheritance of resistance, allelism tests, and to elucidate epistasis interaction among different anthracnose resistance genes. This methodology could also be used to develop cultivars with multiple anthracnose resistance genes (pyramiding), and for fine mapping to identify genomic regions with anthracnose resistance to multiples races of *C. lindemuthianum*. Moreover, this methodology will save time, labor, space, costs, and will require less seeds. This is the first report of a simultaneous inoculation of common bean plants with multiple races of *C. lindemuthianum*. This is an effective methodology for genetic studies with *C. lindemuthianum*.

Cultivar ¹		Inoculated	d with multiple	e races ²	Inoculated with single races ³					
	39	321	1545	3481	39	321	1545	3481		
Corinthiano	S	S	S	S	S	S	S	S		
Pitanga	S	S	R	S	S	S	R	S		
JLP	S	S	R	R	S	S	R	R		
Beija Flor	S	R	R	S	S	R	R	S		
MDRK	S	R	R	R	S	R	R	R		
Crioulo 159	R	S	S	R	R	S	S	R		
ТО	R	S	R	S	R	S	R	S		
Cornell 49242	R	R	S	S	R	R	S	S		
G 2333	R	R	R	S	R	R	R	S		
Ouro Negro	R	R	R	R	R	R	R	R		

Table 1. Comparison of inoculation methods on common bean cultivars inoculated simultaneously with multiple races and single races of *Colletotrichum lindemuthianum*

¹The first five cultivars are Andean and the other five are Middle American. MDRK=Michigan Dark Red Kidney; JLP = Jalo Listras Pretas. ²This group of plants (group 1) was inoculated with four races simultaneously. ³Each of the next four groups of plants were inoculated with a single race individually; group 2 with race 39, group 3 with race 321, group 4 with race 1545, and group 5 with race 3481.



Figure 1. Reaction of selected common bean cultivars to races 39, 321, 1545 and 3481 inoculated simultaneously; A = Corinthiano; B = Pitanga; C = Beija Flor, and D = MDRK.

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PHENOTYPE AND SNPs REVEALED AN ANTHRACNOSE RESISTANCE LOCUS IN ANDEAN COMMON BEAN LANDRACE BEIJA FLOR

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INTRODUCTION

Brazil is the world's largest producer and consumer of common beans (*Phaseolus vulgaris*). In this country, the anthracnose disease, caused by *Colletotrichum lindemuthianum*, reduces yield and quality of seeds and pods. Host resistance is the most cost-effective method to manage anthracnose. The Andean bean landrace Beija Flor has been reported as resistant to multiple Mesoamerican races of *C. lindemuthianum* (Vidigal Filho et al., 2008; Marcon, 2017; Souza et al., 2018). It has been suggested that anthracnose resistance genes of Andean origin confer resistance to Mesoamerican races of *C. lindemuthianum* (Gonçalves-Vidigal et al. 2009). In this study, we report an anthracnose resistance locus in Beija Flor and used bulk segregant analysis and single nucleotide polymorphism (SNPs) markers to position the resistance locus of Beija Flor in the common bean genome.

MATERIALS AND METHODS

This study was conducted at the Núcleo de Pesquisa Aplicada a Agricultura, Maringá, PR, Brazil and at the Soybean Genomics and Improvement Laboratory, ARS-USDA, Beltsville, MD, USA. The reaction of Beija Flor to eight races (7, 39, 89, 321, 449, 453, 1545 and 3481) of C. lindemuthianum was evaluated, in the USA. Two populations were used for the inheritance resistance study; one with 164 F_{2:3} families from the Beija Flor×Cornell 49242 cross, and the other with 188 F₂ plants from the Beija Flor×Crioulo 159 cross. Both populations were inoculated with races of C. lindemuthianum using a 1.2×10^6 spores per mL⁻¹ concentration. The trifoliate leaves of each 10 plants per F_{2:3} family from the Beija Flor×Cornell 49242 cross were inoculated with race 1545 in Brazil. After inoculation, the plants were transferred to a mist chamber for 72 hours and then to a bench. In the study conducted in the USA, the primary leaves of F₂ plants of the Beija Flor×Crioulo 159 cross were simultaneously inoculated with races 321 and 1545, one race on each leaf. The leaves were inoculated on the abaxial side using a cotton swab. After inoculation, the plants were transferred to a mist chamber for 48 hours and then transferred to a greenhouse bench. The plants were evaluated for their anthracnose phenotype about 10 days after inoculation using a 1-9 scale, where grades 1-3 were resistant and grades 4-9 were susceptible. The newly emerged trifoliate leaves from each of the F₂ plants of both crosses were collected for DNA extraction. Based on the anthracnose reaction of the $F_{2:3}$ families from the Beija Flor×Cornell 49242 cross, we prepared three resistant and three susceptible homozygous bulks. Each bulk, containing DNA from six different plants, were used for bulk segregant analysis (BSA). The DNA of each bulk and two samples from each parent were analyzed with 11,292 single nucleotide polymorphism (SNPs) markers on the Illumina BeadChip BARCBean12K.

RESULTS AND DISCUSSION

In the study conducted in the USA, Beija Flor was resistant to Mesoamerican races 321, 449, 453 and 1545, and susceptible to Andean races 7 and 39 and Mesoamerican races 89 and 3481 (Table 1). Beija Flor has also been reported in Brazil as resistant to Mesoamerican races 65, 73 and 2047
(Vidigal Filho et al., 2008; Marcon, 2017; Souza et al., 2018). The seven races to which Beija Flor is resistant, overcome most known Mesoamerican anthracnose resistance genes. Race 2047 alone overcomes the resistance present in 11 of the 12 anthracnose differential cultivars. The inheritance of resistance conducted in two populations indicate that a single dominant locus is present in Beija Flor (Table 2). Marcon (2017) also reported a single locus in the F₂ population from the Beija Flor×TU cross. We observed cosegregation in the Beija Flor×Crioulo 159 cross; plants that were resistant or susceptible to race 321, were also resistant or susceptible to race 1545, respectively. These results indicate that a single locus in Beija Flor confers resistance to both races. Based on the BSA, 21 SNPs were associated with the resistance locus. These SNPs were polymorphic between Beija Flor and Cornell 49242; the resistant bulks clustered tightly with the resistant Beija Flor and the susceptible bulks clustered with the susceptible Cornell 49242. The 21 SNPs revealed that the physical location of the resistance locus of Beija Flor is on chromosome Pv04, between 3,592 bp and 1,996,445 bp, spanning a 1.99 Mb region. There is a resistance cluster on Pv04 that includes anthracnose, rust, angular leaf spot, and other disease resistance loci. Future studies, will use fine mapping to determine the physical location of the resistance locus on Beija Flor and to develop molecular markers tagging this resistant locus for marker assisted selection by breeding programs.

 Table 1. Reaction of Andean landrace Beija Flor and other common bean cultivars to Andean and

 Mesoamerican races of *Collectorichum lindemuthianum* evaluated in the USA

Cultivore*	Resistance	Races of C. lindemuthianum*								
Cuttivars	Gene	7	39	89	321	449	453	1545	3481	
Beija Flor	?	S	S	S	R	R	R	R	S	
Crioulo 159	<i>Co-16</i>	S	R	R	S	S	S	S	R	
Michigan Dark Red Kidney	Co-1	S	S	R	R	R	R	R	R	
TU	<i>Co-5</i>	R	R	R	R	R	R	S	R	

*Cultivars and races of *C. lindemuthianum* highlighted in blue are Andean; the other are Mesoamerican.

Table 2. Inheritance of resistance in a F_2 population from the cross Beija Flor×Crioulo 159 inoculated with races 321 and 1545 and in a $F_{2:3}$ population from the cross Beija Flor×Cornell 49242 inoculated with race 1545 of *Colletotrichum lindemuthianum*

Population	Observed	Expected	Chi square	P value
F _{2:3} Beija Flor×Cornell 49242	44R: 82H: 38S	41R: 82H: 41S 1:2:1	0.439	0.8029
F2 Beija Flor×Crioulo 159	132R: 56S	141R: 47S 3:1	2.298	0.1296

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GENE Co-12 OF JALO VERMELHO CULTIVAR CONFERRING RESISTANCE TO RACES 55 AND 1545 OF Colletotrichum lindemuthianum

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INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) yield can be affected by several disease-promoting microorganisms. The fungus *Colletotrichum lindemuthianum*, which causes anthracnose in common beans, is one of the most relevant bean pathogens (Singh and Schwartz, 2010). Breeding programs develop strategies to identify genetic sources of disease resistance considering the genetic variability of the fungus (Ferreira et al., 2013). The Andean cultivar Jalo Vermelho possess the anthracnose resistance gene *Co-12* and stands out as being resistant to races 9, 23, 31, 55, 64, 65, 81, 83, 89, 95, 449, 453 and 1545 (Vidigal Filho et al., 2007; Gonçalves-Vidigal et al., 2008; Castro et al., 2017). The objective of the present study was to perform co-segregation test for resistance to *C. lindemuthianum* races 55 and 1545 in the Jalo Vermelho cultivar by phenotyping $F_{2:3}$ families with each race independently.

MATERIALS AND METHODS

Experiments were conducted under greenhouse conditions and at the Laboratório de Melhoramento do Feijão Comum e de Biologia Molecular of the Núcleo de Pesquisa Aplicada à Agricultura (Nupagri) at the Universidade Estadual de Maringá, Paraná, Brazil. The Andean cultivar Jalo Vermelho was crossed with the Mesoamerican cultivar Crioulo 159 to obtain F_1 seeds and by self-pollination the generations F2 and F2:3 were obtained. Co-segregation analysis was performed in 84 $F_{2:3}$ families divided into two identical sets independently tested with C. *lindemuthianum* races 55 and 1545. The seeds were sown in $48 \times 30 \times 11$ cm plastic trays containing peat substrate and kept in greenhouse until inoculation, when seedlings reached the first trifoliolate leaf stage. Inoculum preparation was performed according to the methodology proposed by Cárdenas et al. (1964). The races were grown in pod tubes and incubated in the growth chamber (BOD) at 20 \pm 2 ° C for 14 days for pathogen sporulation. Ten days after inoculation, visual evaluations of seedling symptoms were performed using the severity scale from 1 to 9. Scores from 1 to 3 indicate a resistant plant, whereas scores from 4 to 9 indicate a susceptible plant (Pastor-Corrales, 1995). Chi-square test (χ^2) were applied at 5% level of probability to define goodness of fit for the observed to expected phenotypic ratio for segregating in F_{2:3} families. Statistical analyses were performed with software Genes (Cruz, 2013).

RESULTS AND DISCUSSION

Evaluation data of Jalo Vermelho, Crioulo 159 and $F_{2:3}$ families are shown in Table 1. The Andean Jalo Vermelho cultivar is resistant to *C. lindemuthianum* races 55 and 1545, as reported in the literature, and Crioulo 159 was susceptible to both races. A total 84 $F_{2:3}$ families derived from the Jalo Vermelho × Crioulo 159 cross were separated into two groups that were inoculated separately, one with races 55 and the other with race 1545. These two groups exhibited identical co-segregation of resistance/susceptibility to both races. All plants that were homozygous resistant to race 55 were also homozygous resistant to race 1545, whereas all plants that were susceptible to race 55 were also susceptible to race 1545. No recombinants were observed in these $F_{2:3}$ families. The observed co-segregation ratio of 23RR:40Rr:21rr individuals ($\chi^2 = 0.285$, P value = 0.867),

fitted an expected 1RR:2Rr:1rr ratio (Table 1). This result shows that the 84 $F_{2:3}$ families cosegregated for both races, indicating that the *Co-12* gene of Jalo Vermelho cultivar confers resistance to both races 55 and 1545 of *C. lindemuthianum*.

Parent and cross	Generation	Observed ratio (RR:Rr:rr)	Expected ratio (RR:Rr:rr)	χ^2	<i>P-value</i>					
C. lindemuthianum race 55										
Jalo Vermelho	RP ^a	10:0								
Crioulo 159	SP ^b	0:10								
Jalo Vermelho × Crioulo 159	F _{2:3}	23:40:21	21:42:21	0.285	0.867					
		C. lindemuthianu	<i>m</i> race 1545							
Jalo Vermelho	RP	10:0								
Crioulo 159	SP	0:10								
Jalo Vermelho × Crioulo 159	F _{2:3}	23:40:21	21:42:21	0.285	0.867					

Table 1. Reaction of $F_{2:3}$ plants from the cross Jalo Vermelho × Crioulo 159 inoculated with *C. lindemuthianum* races 55 and 1545

^a Resistant Parent; ^b Susceptible Parent.

The co-segregation test allows the study of the cultivar resistance to different pathogenic races. The first co-segregation analysis was conducted by Gonçalves-Vidigal et al. (2001), wherein it was possible to identify the dominant monogenic inheritance of cultivar AB 136 for *C. lindemuthianum* races 31 and 69. Similarly, the present study deepens the understanding of the monogenic inheritance of gene *Co-12* in the common bean Andean cultivar Jalo Vermelho, inoculated with races 55 and 1545 of *C. lindemuthianum*, and shows its importance as a promising source of anthracnose resistance.

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REACTION TO FUNGAL DISEASES AND FIELD PERFORMANCE OF BLACK COMMON BEAN GENOTYPES ASSESSED IN THE HIGH MOUNTAINS OF VERACRUZ, MEXICO

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INTRODUCTION: In 2019, artificial inoculation with *Colletotrichum lindemuthianum* and *Uromyces appendiculatus* var. *appendiculatus* collected in commercial fields of Veracruz and Chiapas, Mexico, was carried out in a group of tropical black common bean breeding lines to identify genotypes that display resistance or tolerance to anthracnose and rust in controlled environmental conditions. Based on the results obtained, 11 genotypes were selected for their resistance or high tolerance reaction to both plant diseases. This group of breeding lines was arranged as a uniform yield trial to assess their field performance across natural environments known to favor the presence of rust and anthracnose diseases during yrs. 2019 and 2020 in Veracruz and Chiapas, Mexico. Preliminary results obtained are presented from a site located in the high mountains of the state of Veracruz, Mexico.

MATERIALS AND METHODS: A yield trial was sown on September 2, 2019, in Rincón Grande, municipality of Orizaba, located at an altitude of 1,248 m in the high mountains of Veracruz, Mexico. The group of 11 breeding lines was evaluated, along with three susceptible commercial cultivars used as checks: Negro Medellin, Negro Jamapa and Verdín. The field trial was arranged in a RCBD with three replications, with experimental plots of three rows 5 m long using a 250,000 ha⁻¹ plant density. Days to flowering and physiological maturity were recorded as well as the reaction of genotypes to the incidence of rust. The rust disease was present from R5 stage (pre-flowering) to R8 (filling pod stage), mainly due to conditions of high relative humidity (> 80%) and temperatures that varied between 17 and 27 °C. Anthracnose disease was not present; in contrast, genotype reaction to web blight (*Thanatephorus cucumeris*) was taken, since this fungus was present at the R8 stage of development. This was mainly due to the conditions of high relative stage of the crop and moderately high temperatures (20 and 30°C). Reaction to both diseases was visually scored using a scale of 1 to 9 (CIAT, 1987). Seed size and grain yield were also recorded. ANOVA's were performed and LSD (0.05) was used for the separation of means.

RESULTS AND DISCUSSION: Highly significant ($p \ge 0.01$) differences were detected between common bean genotypes for all measured variables. Nine breeding genotypes, including the check cultivars, Negro Medellin and Negro Jamapa, flowered later than Verdin cultivar, which was the earliest to flower and mature (Tosquy *et al.*, 2014; 2018). Regarding field reaction to rust, check cultivars Verdín and Negro Jamapa, had an intermediate reaction to this disease, with 5.3 and 4.3 ratings, respectively, while the rest of the genotypes, including Negro Medellin, a check cultivar, showed high field resistance (Table 1). However, rust did not significantly affect bean yield (r = 0.20 ns). In turn, the Negro Jamapa cultivar was highly affected by web blight, whose damage was statistically similar to those displayed by the three breeding lines derived from the Negro Citlali/ RAV-187-3 population, and higher than the rest of the genotypes. It is important to highlight that the three breeding lines derived from the Jamapa Plus/XRAV-187-3 cross, together with Papaloapan/SEN 46-3-2, Papaloapan/SEN 46-2-6 breeding lines and Verdín cultivar showed resistance to this disease; moreover, web blight significantly affected 100 seed weight (r = -0.65 *) and consequently the grain yield (r = -0.81 **). Yield performance of genotypes indicated that Jamapa Plus/XRAV-187-3-4-4 was the most productive breeding line and statistically similar to the other three breeding lines (Jamapa Plus/XRAV-187-3-4-1, Jamapa Plus/XRAV-187-3-1-2 and Papaloapan/SEN-46-7-12) and to the Verdín cultivar, but higher than the rest genotypes (Table 1). It is important to point out that all these three lines also showed resistance to both diseases. On the other hand, the Negro Citlali/XRAV-187-3-1-6, Negro Citlali/XRAV-187-3-1-5 and the check cultivar, Negro Jamapa, produced the lowest seed yields, due in large part to web blight.

varua	ted in Kineon Grande, Orizaba, ve		o. Summer	1 ull 2017	growing seaso.		
т	Constyne	F	PM	Rust	Web blight	100SW	Yield
I	Genotype	(d)	(d)	(1-9)	(1-9)	(g)	(kg ha⁻¹)
1	Papaloapan/SEN 46-2-6	43.3 *	82.7 *	3.33	3.33	17.93	1167
2	Papaloapan/SEN 46-3-2	42.3 *	84.0 *	3.33	2.00	18.93	1233
3	Papaloapan/SEN 46-7-7	43.3 *	81.0	2.33	4.33	16.10	1222
4	Papaloapan/SEN 46-7-1	42.3 *	80.7	2.33	4.33	17.80	1117
5	Papaloapan/SEN 46-7-12	42.0	80.7	3.00	4.33	16.73	1327 *
6	Negro Citlali/XRAV-187-3-1-5	43.7 *	80.0	2.00	5.67 *	12.20	789
7	Negro Citlali/XRAV-187-3-1-6	42.7 *	81.0	1.00	5.33 *	12.33	715
8	Negro Citlali/XRAV-187-3-1-8	41.3	81.0	2.33	4.67 *	13.60	1131
9	Jamapa Plus/XRAV-187-3-1-2	42.0	81.3	2.67	3.33	15.33	1389 *
10	Jamapa Plus/XRAV-187-3-4-1	42.3 *	84.0 *	2.00	2.00	18.53	1462 *
11	Jamapa Plus/XRAV-187-3-4-4	41.0	81.0	3.00	3.00	16.73	1498 *
12	Negro Medellin (check)	42.3 *	80.0	3.33	4.33	17.47	1069
13	Negro Jamapa (check)	42.3 *	83.0 *	4.33 *	6.00 *	16.80	849
14	Verdín (check)	36.3	74.0	5.33 *	3.00	22.50 *	1244 *
	Average	41.9	81.0	2.88	3.98	16.64	1158
	ANOVA	**	**	**	**	**	**
	CV (%)	2.31	1.62	23.11	22.11	5.54	13.46
	LSD (0.05)	1.627	2.200	1.118	1.476	1.549	261.65

 Table 1. Phenological traits, disease reaction, seed size and seed yield of black common bean genotypes field

 evaluated in Rincón Grande, Orizaba, Veracruz, Mexico. Summer-Fall 2019 growing season.

T = Treatment. F = Days to flowering. PM = Physiological maturity. 100SW=100 seed weight. * Significance within column. ** Highly significance (0.01). Correlation coefficients: Rust *vs* Yield (r = 0.20 ns), Web blight *vs* Yield (r = -0.81**) and Web blight *vs* 100SW (r = -0.65*).

CONCLUSIONS: Based on the results obtained in this evaluation site, all three lines derived from the Jamapa Plus/XRAV-187-3 cross were the most productive, had an intermediate growing cycle and showed resistance to both rust and web blight, diseases that commonly affect tropical and subtropical bean production areas of the state of Veracruz, Mexico. Web blight significantly affected seed yield and to a different extent seed size of bean genotypes.

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REACTION OF TROPICAL BLACK BEAN BREEDING LINES TO THE ARTIFICIAL INOCULATION OF RUST AND ANTHRACNOSE IN SOUTHEAST MEXICO

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INTRODUCTION: In the humid tropics of southeastern Mexico, Chiapas and Veracruz are the main bean-producing states, but their productivity is low (700 kg ha⁻¹). Various factors cause low yields, among them are the incidence of fungal diseases such as rust and anthracnose, which can cause losses of 20 to 100% of the grain yield, (López et al., 2006) and the sowing of landraces and cultivars of unknown origin which are of low yield and susceptible to diseases (Tosquy et al., 2014). The objective of this research was to evaluate the reaction of 53 recombinant lines plus three tropical black bean cultivars to artificial inoculation with *Colletotrichum lindemuthianum* and *Uromyces appendiculatus* var. *appendiculatus*, which cause anthracnose and rust, respectively.

MATERIALS AND METHODS: The 56 bean genotypes were inoculated separately with each of the pathogens, rust and anthracnose. On May 28, 2019, 20 seeds of each genotype were sown individually in single-celled vessels with peat moss: agrolite (1: 1) and once the seedlings emerged, they were kept in the greenhouse until inoculation, which was carried out June 7. Ten seedlings of each genotype were inoculated with C. lindemuthianum and the other 10 with U. appendiculatus var. appendiculatus. In the case of anthracnose, the inoculation was performed on primary leaves, using a conidial suspension at a concentration of 1.2×10^6 conidia / mL, made from monosporic cultures of C. lindemuthianum. For rust, the inoculation was performed with a mixture of urediospores made up of individual pustules, which were selected from leaf samples collected in 2018 in Veracruz and Chiapas, Mexico, from which a suspension was prepared at a concentration of 2×10^4 urediospores / mL and sprayed on the primary leaves. In both cases, the inoculated plants were incubated in a humid chamber and subsequently transferred to the greenhouse. At 14 days after inoculation, the reaction of the genotypes to anthracnose was evaluated on a scale of 0 to 4 proposed by Garrido and Romero (1989) and to rust on a scale of 1 to 6 described by Stavely et al. (1983). The experiment used a completely randomized design with 10 repetitions per treatment, and LSD (0.05) was used for separation of means.

RESULTS AND DISCUSSION: Highly significant differences between treatments were detected for both variables, indicating that the genotypes showed different reactions to inoculation with *C. lindemuthianum*, and with *U. appendiculatus* (Table 1). In total 45 genotypes showed resistance to anthracnose, of which 18 recombinant lines (nine from the Papaloapan / SEN 46 population, five from the Negro Citlali / XRAV-187-3 population, and three from the Jamapa Plus / XRAV-187-3 population, and the ELS-15-55 elite line) stood out for presenting minimal symptoms in leaf veins, with a reaction value of 1.0. That group was statistically similar to the Negro Jamapa cultivar and three other lines, and superior to the rest of the genotypes. The Negro Medellín cultivar and the NGO-17-99 line had a susceptible reaction to anthracnose with values greater than 3.0, similar to the other nine lines. Regarding rust, 41 genotypes presented only small necrotic spots without sporulation (ratings <2.4), that is, they showed a hypersensitivity reaction

out of which 25 lines stood out for presenting the least damage with a reaction value of 2.0. This group included 13 lines derived from the Papaloapan / SEN 46 cross, seven from Negro Citlali / XRAV-187-3, three from Jamapa Plus / XRAV-187-3 and the ELS-15-55 elite line, that were statistically superior to the rest of the genotypes, including Negro Medellin, Negro Jamapa and Verdín cultivars. The latter two were moderately susceptible to rust, since they obtained reaction values less than 5.0 (Table 2).

Table 1. Square means and significance among treatments detected in both variables, reaction of black common bean genotypes to anthracnose and rust.

Variation factor	DF	Reaction to anthracnose	Reaction to rust
Treatments	55	4.450617 **	2.767139 **
Error	504	0.134325	0.130556
Total	559		
_CV (%)		22.14	15.35
** TT' 11 ' 'C' (0.01)			

** Highly significant (0.01)

Table 2. Reaction of black common bean genotypes to artificial inoculation with *Colletotrichum lindemuthianum* and *Uromyces appendiculatus* var. *appendiculatus*, air borne fungal diseases that cause anthracnose and rust, respectively.

No.	Breeding populations	Anthracnose	Reaction	Rust	Reaction
1	Nagra Danalaanan / SEN 46 (24)†	1.0 - 1.3	R	2.0 - 2.4	HR^{\ddagger}
1	Negro Fapaloapali / SEN 40 (54)	2.6 - 2.8	S	2.5 - 3.0	R
2	Nagra Citlali / VDAV 197 2 (12)	1.0 - 1.0	R	2.0 - 2.3	HR
2 Negro Citian / 2	Negro Ciuali / XRAV-18/-3 (12)	2.7 - 2.7	S	2.5 - 3.1	R
3 Jamapa Plus / XI	Lamona Dlug / $\mathbf{VD}\mathbf{AV}$ 197.2 (2)	1.0 - 1.0	R	2.0 - 2.0	HR
	Jamapa Plus / XRAV-18/-3 (3)	2.0 - 2.0	R	-	-
	Check genotypes				
1	NGO 17-99	3.2	S	2.7	R
2	SCN-2	2.0	R	3.8	MR
3	ELS-15-55	1.0	R	2.0	HR
4	Negro Medellín	3.4	S	2.5	R
5	Negro Jamapa	1.2	R	4.2	MS
6	Verdín	1.6	R	4.5	MS
	LSD (0.05)	0 3213		0 3167	

[†]Numbers in parenthesis indicate the amount of breeding lines evaluated. [‡]HR= Hypersensitivity reaction, MR= moderately resistant, MS= moderately susceptible

CONCLUSIONS: Black common bean breeding lines resistant to rust and anthracnose were identified in the three populations evaluated, 45 lines were resistant to anthracnose and 41 to rust, of which 18 stood out in the first group and 25 in the second, for presenting the least damage to these diseases.

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EPISTASIS BETWEEN RUST RESISTANCE GENES IN TWO COMMON BEANS OF ANDEAN ORIGIN

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INTRODUCTION

Epistasis, from the Mendelian point of view, defines a gene interaction in which the effects of one allele at one gene (epistatic) masks the effect of alleles at another gene (hypostatic). This type of epistasis is common among rust resistance genes of common bean. Recent reports indicated that the Ur-5 rust resistance gene is epistatic to the Ur-4 gene (Valentini et al., 2015) and the Ur-3 gene is epistatic to Ur-5 (Hurtado et al., 2019). In addition, the Ur-3 and Ur-6 genes have both been reported to be epistatic to the Ur-11 gene (Stavely, 2000). When epistatic and hypostatic rust genes are combined into a single cultivar, it is difficult to confirm the presence of the masked hypostatic gene using single races of the rust pathogen as phenotypic markers. This is also why it is difficult to pyramid rust resistance genes that interact in epistasis using phenotypic markers. This study reports a new epistatic interaction between the Ur-4 rust resistance gene present in Andean Early Gallatin and an unnamed rust resistance gene present in the landrace PI 260418.

MATERIALS AND METHODS

A total of 226 F_2 plants from the Early Gallatin x PI 260418 cross and 30 plants of each of these parents were inoculated on the primary leaves. The tip of the primary leave was cut and the leaf was inoculated with race 22-6 (also named race 49) of *Uromyces appendiculatus*. The other uncut primary leaf was inoculated with race 22-52 (also known as race 108). After inoculation, the plants were transferred to a mist chamber ($20 \pm 1^{\circ}$ C and 95% humidity, under darkness) for about 18 hours. Then, the plants were transferred to a greenhouse bench. Seven days later, these plants were evaluated for their reaction to both races. Early Gallatin and PI 260418 were resistant to both races. The resistant reaction of PI 260418 was expressed as tiny (>3.0 mm in diameter) sporulating rust pustules (TP) accompanied with faint chlorotic spots (f2). The resistant reaction of Early Gallatin was necrotic spots without sporulation, known as the hypersensitive reaction or HR. The HR reactions were observed in about two to three days and the TP reactions in about 10 days, after inoculation, respectively. The large susceptible pustules of the control cultivars produced large sporulating pustules after 10 days.

RESULTS AND DISCUSSION

The reactions of the Early Gallatin and PI 260418 parents, of the F_2 population from the Early Gallatin x PI 260418 cross, and of the control cultivars are shown in Table 1. The inoculation of the 226 F_2 plants from the Early Gallatin x PI 260418 cross with races 22-6 (49) and 22-52 (108) resulted in three groups with distinct types of reactions:1) 171 resistant plants exhibiting the TP and f2 types of reactions, 2) 42 plants were also resistant but with the HR type of reactions, and 3) 13 susceptible plants with large (4, 5, 6) types of sporulating pustules (Table 2). The production of three phenotypes, rather than the four phenotypes (9:3:3:1) that characterize the dihybrid crosses of two independent genes, suggest an epistatic interaction is occurring when the unnamed gene in PI 260418 and the *Ur-4* gene in the Andean snap bean Early Gallatin are combined. These results (171 F_2 plants with TP resistant reactions: 42 F_2 plants with HR resistant reactions:13 F_2 plants with S susceptible reactions) correspond to a 12:3:1 phenotypic ratio that supports complete

dominance in both gene pairs and a type of epistatic interaction between the rust resistance genes in PI 260418 and the *Ur-4* gene in Early Gallatin. The "12" part of the ratio includes two groups of F₂ plants with TP: (9) plants with the TP phenotype and the A_B_ genotype and (3) plants with the TP phenotype and A_bb genotype. The "3" part of the ratio includes plants with the HR phenotype and the aaB_ genotype. The "1" part of the ratio includes plants with the susceptible (S) phenotype and the aabb genotype. The complete dominance at both genes reveals that the rust resistance gene in PI 260418 (A_), with the TP phenotype, is epistatic to the hypostatic *Ur-4* (B_) rust resitance gene in Early Gallatin. The dominant TP phenotype hides the effect of HR and S phenotypes.

Table 1. Reactions of the common beans Early Gallatin and PI 20418 to races 22-6 (49) and 22-52 (108) of *Uromyces appendiculatus* crossed to develop a F_2 population to study the epistasis between the rust resistance genes present in these cultivars. Three common bean cultivars with known reaction to races of the rust pathogen were also included in this study.

Cultivars	Resistance	Gene	*Reaction to appendiculatus	o races U .
	Gene	POOL	49	108
Parents				
Early Gallatin	Ur-4	А	2,2+; HR	2,2+; HR
PI 260418	Unknown	Α	3,f2; TP	3,f2; TP
Controls				
Pinto 114	Unknown	MA	4,5; S	4,5; S
Aurora	Ur-3	MA	4,5,6; S	2,2+; HR
Golden Gate Wax	Ur-6	А	5,6; S	5,6; S

*The reactions of common bean cultivars were evaluated using a Standard bean rust grading scale. Resistant reactions: 2, 2^+ = Necrotic spots without sporulation (HR) and 3, f2 = tiny sporulating pustules (TP) less than 0.3 mm in diameter. Susceptible: 4, 5, 6, = large pustules.

Table 2. Observed and expected ratios of the F_2 population from the Early Gallatin x PI 260418 cross inoculated with races 22-6 (49) and 22-52 (108) of *Uromyces appendiculatus* to study epistasis.

		F ₂ Observed		F ₂ Expected			<u>_</u> ,		
Parents and F ₂ Population	N° of Plants	Resist. TP	Resist. HR	Susc	Resist. TP	Resist. HR	Susc	χ^2	P-value
Early Gallatin	30	0	30	0	-	-	-		
PI 260418	30	30	0	0	-	-	-		
$F_2 \: E.G \times PI260418$	226	171	42	13	169.5	42.375	14.125	0.106	0.9483

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AGRONOMIC PERFORMANCE AND REACTION TO ASHY STEM BLIGHT OF COMMON AND TEPARY BEANS UNDER HEAT-STRESS ENVIRONMENTS

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INTRODUCTION: Heat sensitivity and ashy stem blight (ASB) disease [causal agent: *Macrophomina phaseolina* (Tassi) Goidanich (Mp)] cause yield reductions in *Phaseolus* species (Kaur et al., 2012; Liu et al., 2019). Partial resistance to ASB and tolerance to heat stress have been reported in common bean (*P. vulgaris* L.) and tepary bean (*P. acutifolius* A. Gray) (Rainey and Griffiths, 2005; Viteri and Linares, 2017). Our objective was to evaluate the agronomic performance and response to ASB of 23 bean genotypes to the aforementioned stresses.

MATERIALS AND METHODS: Twenty common beans and three tepary beans were planted in a randomized complete block design, with three replicates at the Isabela and Lajas Research Experimental Stations of the University of Puerto Rico in May, 2019. Data collected for agronomic traits included: days to flowering, days to maturity, and seed-yield. All traits were collected in both environments during the summer of 2019. Additionally, ASB incidence and severity were scored for each genotype at the mid-seed filling stage. A scale from 1 to 9 was used to score the disease severity, where 1= no sign of Mp infection, and 9= the fungus infection passed the third node above or below the stem and may cause plant death (Viteri and Linares, 2017).

RESULTS AND DISCUSSION: The mean for days to flowering varied in both locations (Table 1). All genotypes were harvested at 105 days after planting in Lajas and at harvest maturity in Isabela (Table 1). In general, lower mean seed-yield (134 kg/ha) was observed in Lajas as compared to Isabela (585 kg/ha). Higher mean temperatures of 27 °C at night during the reproductive stages could be one of the causes of the lower agronomic performance in Lajas compared with Isabela, where mean temperatures of 25 °C at night were recorded. Previous studies also mentioned that higher night temperatures reduced pollen viability, ovule fertilization, and pod and seed set (Gross and Kigel, 1994; Konsens et al., 1991). All these variables are associated with a decrease in common bean production. Furthermore, the presence of heat-stress environmental conditions could promote the development of ASB disease (Pastor-Corrales et al., 1988). In fact, higher ASB incidence and severity were observed at Lajas in this study (Table 1). 92BG-7, 'Bella', 'Jaguar', 'Matterhorn', SB-DT1, SEN 3, SEA and SER breeding lines, 'Tio Canela-75', 'UI 320', USPT-WM-1, VA 19, 'Verano', and XAN 176 had lower seed-yield (< 150 kg/ha) in Lajas. In contrast, all these genotypes produced over 400 kg/ha in Isabela. Tepary beans 88, PI 464025, and Sowi; and common bean TARS-MST 1 had the highest seed-yield (345-512 kg/ha) in Lajas where higher ASB pressure was observed. However, only TARS-MST 1 had the lowest ASB incidence (< 10%).

	Isabela					Lajas			
Genotype	DTF ^a	DTM ^b	Yield kg/ha	Ashy ste	m blight	DTF ^a	Yield kg/ha	Ashy ster	m blight
				Incidence	Severity ^c			Incidence	Severity
88	34	69	601.1	0.0	1.1	35	359.5	34.9	4.0
92BG-7	35	68	688.9	7.3	1.6	41	76.3	36.2	4.4
BAT 477	34	66	471.0	30.0	2.4	36	0.0^{d}	29.5	4.3
'Bella'	34	66	862.2	10.0	1.7	35	41.7	35.1	4.3
'Jaguar'	36	74	546.0	45.0	4.3	41	30.0	66.0	5.8
'Matterhorn'	32	62	451.2	89.7	7.7	35	0.0^{d}	80.1	7.7
PI 462025	34	74	150.0	14.2	1.9	35	510.8	44.3	6.6
PRA 154	29	66	233.9	9.2	3.4	31	234.8	37.3	6.5
'Roza'	32	66	93.7	92.5	8.3	35	39.8	77.4	7.1
SB-DT1	33	68	447.1	63.3	5.0	35	125.2	38.6	4.7
SEA 5	31	72	192.7	49.2	4.3	35	53.5	31.8	4.2
SEA 16	31	66	719.9	55.6	5.1	35	146.1	24.0	3.9
SEN 3	34	72	562.3	30.0	3.0	35	57.4	47.3	5.6
SER 16	32	71	638.8	38.3	3.6	35	90.7	57.7	5.9
SER 22	31	72	591.4	44.2	4.1	35	75.0	53.8	5.6
Sowi	33	69	207.5	4.2	1.3	35	512.1	48.3	5.5
TARS-MST 1	34	68	1247.1	33.3	3.5	35	345.8	8.4	3.0
'Tio Canela-75'	33	66	940.4	39.2	3.7	37	86.1	33.3	4.1
'UI 320'	29	63	226.8	100.0	9.0	33	64.6	67.8	6.5
USPT-WM-1	33	71	855.5	17.5	2.3	30	64.6	57.6	5.5
VA 19	30	71	618.6	18.3	2.0	35	65.9	47.1	5.3
'Verano'	34	66	1023.1	15.0	1.7	37	11.1	49.3	5.6
XAN 176	34	66	1084.2	30.8	3.1	35	98.3	27.6	4.4
Mean	33	68	584.9	36.4	3.7	35	134.3	44.9	5.2
LSD ($P \le 0.05$)	2.5	8.1	574.9	17.5	1.3	5.9	261.4	22.6	1.5

Table 1. Days to flowering, days to maturity, seed-yield, and response to ashy stem blight caused by *Macrophomina phaseolina* (Tassi) Goidanich of 23 common and tepary beans planted at the Isabela and Lajas Research Experimental Stations in May 2019.

^aDTF, days to flowering.

^bDTM, days to maturity.

^cAshy stem blight severity was measured in a scale of 1 to 9 where 1 = no sign of Mp infection, 5 = Mp passed the first node below the point of infection and the fungus reached 50% of the second internode, and 9 = the fungus infection passed the third node above or below the stem and may cause plant death.

^d Plants did not produce viable-seed.

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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

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INTRODUCTION: Our research goal is to identify putative sources of resistance to white mold in adapted backgrounds at multiple sites located in major bean-production areas of the northern United States. In 2019, greenhouse and field evaluations were used to screen eight lines for putative sources of WM resistance in adapted backgrounds. These lines included black, great northern, pinto, red, and small red bean types, with two of these lines having been evaluated previously in these trials, and six being new this year. Evaluations were performed in six states: MI, NE, ND, OR, WA, and WI, which collectively represent the major bean-production area of the U.S. Multi-site testing is essential for robust evaluation under different environmental conditions and with different pathogen populations that our previous research has shown are significantly different in both genetic variation and aggressiveness (Kamvar *et al.* 2017).

MATERIALS AND METHODS: A straw test that consistently identifies sources of resistance in adapted and unadapted bean germplasm (Teran *et al.*, 2006) was used for greenhouse tests, which requires only a small number of seeds to confirm resistance. Eleven bean lines, including controls G122 (partially resistant), Bunsi (mostly field avoidance) and Beryl (susceptible), were evaluated in the greenhouse at all locations except ND. Ten bean lines, including controls, were evaluated in the field at all six locations. Data was collected from only five locations due to severe damage from multiple hailstorms in August in Nebraska, demonstrating the necessity of multiple sites for generating data despite weather complications in field trials.

RESULTS AND CONCLUSIONS:

Although no lines scored as more resistant than the resistant control G122 in either the greenhouse or field trials this year, two lines (SR9-5 and USPT-WM-12) performed equal to G122 in the greenhouse (Table 1), and two lines (NDF120287, and SR9-5) performed equal to G122 in field screenings (Table 2). Across the past four years, several lines have been found to be equal to G122 in the field, with at least one line repeated across years (Table 3). In the field trials, three lines outperformed the moderate control, indicating either resistance or escape mechanisms in the field. One line (NDF120287) scored as susceptible in the greenhouse performed equally to G122 in the field, suggesting some type of escape

Table 1.	Greenhouse test results*	for 2019.	Mean	disease rating**
(controls	highlighted in blue).			

Line	WI	NE	MI	OR	WA	Mean	t Grouping
G122	4.8	5.0	5.3	3.4	5.4	4.80	a
USPT-WM-12	4.4	5.7	6.7	3.9	4.7	5.06	а
SR9-5	6.2	6.1	4.7	4.4	5.5	5.38	a b
Bunsi	6.8	7.7	6.0	4.7	7.3	6.48	b c
R17604	7.8	5.7	9.0	5.1	7.4	7.00	c d
Beryl	5.3	7.6	9.0	7.1	6.6	7.11	c d e
P16901	7.8	6.3	9.0	7.4	6.0	7.30	c d e
NDF120287	8.9	7.3	7.3	7.3	8.2	7.82	d e
ND112929	8.0	8.6	9.0	7.3	8.2	8.22	d e
ND121315	8.3	8.3	8.7	8.0	8.3	8.31	e
G16351	8.8	8.5	9.0	8.0	7.4	8.33	e

P = <0.001; LSD = 1.289

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

mechanism. Additionally, the loss of field data from Nebraska due to August hailstorms demonstrates the need for multiple site evaluations each year.

Table 3. Lines equal to G122(moderately resistant line) in field test inone or more years

Line	'16	'17	'18	'19
SR9-5				\checkmark
NDF120287				\checkmark
N14229			\checkmark	
VCP-13*		\checkmark	\checkmark	
NE5-16-101		\checkmark		
N14229		\checkmark		
B15430		\checkmark		
NDZ14083		\checkmark		
ASS 1865	\checkmark			
R12844	\checkmark			
R13752	\checkmark			
PS08-039-A5	\checkmark			
USPT-WM-12	\checkmark			
* Previously nan	ned NE	25-16-9	98	

 Table 2. Field test results* for 2019. Mean disease rating** (controls highlighted in blue).

Line	WI	WA	MI	OR	ND	Mean	t Grouping
G122	3.7	2.6	2.0	2.3	5.5	3.22	a
SR9-5	7.7	4.0	3.3	3.3	6.6	4.99	a b
NDF120287	9.0	3.8	3.0	4.3	7.5	5.53	a b
USPT-WM-12	9.0	4.8	4.0	3.3	7.3	5.69	b
Bunsi	9.0	4.5	6.3	5.0	7.3	6.43	b c
R17604	8.0	6.2	5.0	5.7	7.4	6.45	b c
ND112929	9.0	5.5	4.7	5.5	8.1	6.55	b c
P16901	7.3	6.7	5.0	6.7	7.9	6.72	b c
G16351	9.0	7.2	7.0	4.0	7.3	6.90	b c
Beryl	9.0	8.0	8.7	5.8	8.8	8.06	с

* P = 0.0187; LSD = 2.319

**CIAT scale is 1-9, wherein 1= no disease, 9 = dead plant

In the past four years, data generated by this research have been used for publications and release of six lines (**Table 4**), which includes two pintos (Singh et al., 2016), one small red (Kelly et al., 2018), one great northern (Baumann, 2020), and one white kidney (Baumann, 2020). One other was published prior to that (USPT-WM-12) and one is forthcoming (NDF120287).

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Line	Year(s) evaluated	Seed class	Released as:	Citation:
NDF120287	2019	-	ND Twilight (forthcoming)	-
VCP-13*	2017, 2018	Pinto	Reg. No. GP-301, PI 676974	Singh et al., 2016
PRP-153	2018	Pinto	Reg. No. GP-300, PI 676973	Singh et al., 2016
Cayenne	2017	Small red	Reg. No. CV-322, PI 685022	Kelly et al., 2018
ND121630	2017	Great Northern	ND Pegasus	Baumann, 2020
ND122386	2017	White kidney	ND Whitetail	Baumann, 2020
USPT-WM-12	2016, 2019	Pinto	Reg. No. GP-294, PI 668537	Miklas et al., 2014

*Note: This line named NE5-16-98 in 2017

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CHEMICAL TREATMENT OF WHITE MOLD (Sclerotinia sclerotiorum) IN COMMON BEAN

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INTRODUCTION

Several fungicides are active against white mold caused by *Sclerotinia sclerotiorum*, that is one of most important diseases affecting beans. In general, it is recommended that fungicides be applied during early bloom and if conditions continue to be favorable for the disease, an additional application may be necessary. Timing of fungicidal application is critical to protect blossoms from infection (Lehner, 2017). The objective of this work was to verify the chemical control of *white mold* in common bean.

MATERIALS AND METHODS

The field experiment was conducted in the agricultural year 2017/2018 at Fazenda Ubiratã, in the city of Londrina, Brazil. The region is located between the geographical coordinates $23 \circ 20'23$ "S, $51 \circ 12'32$ " O, 532 m. The work was carried out on an eutropheric Red Latosoil, with smooth undulating relief and a humid subtropical mesothermal Cfb climate (Iapar, 1994; Olmos-Iturri et al., 1984). During the 2017 agricultural year, cultivation was carried out with forage turnip. The previous infestation of the area with sclerotina from production fields was also carried out, to ensure a high initial inoculum. Direct sowing on straw was carried out on 11/21/17, using the bean cultivar IAPAR 81, at a density of 12 seeds per linear meter in a spacing of 0.40 m between the sowing lines, in experimental plots of 5 X 5.6 m, with a total area of 28 m². The application of the fungicide Fluazinam was evaluated, applied sequentially, with the first application at the phenological stage R4 and the second at the phenological stage R5. Throughout the cycle, the crop was irrigated to increase the predisposition for the development of white mold. The statistical design was completely randomized with four replications, with or without the use of the fungicide (fluazinam).

The observation of the incidence and severity of the disease was carried out simultaneously during the phenological stage R8 (beginning of physiological maturation), with all plants having three rows in each plot. The scale of 0-3 was adopted, adapted from Dann et al. (1999), where: Score 0 = plant apparently healthy; 1 = Plants with up to two side branches affected; 2 = Plants with two or more affected side branches or central branch without the death of the plant; 3 = Central branch affected and dead plant.

Based on the scores assigned and using the formula below, the Severity Index * was calculated using the weighted average. * IS = $[(N \circ 1 \times 1) + (N \circ 2 \times 2) + (N \circ 3 \times 3)]$ TOTAL N \circ OF PLANTS. Where; N \circ 1 = Number of plants that received score 1. N \circ 2 = Number of plants that got score 2. N \circ 3 = Number of plants that got score 3. The data obtained were analyzed by the Tukey test at 5% for the comparison of averages.

RESULTS AND DISCUSSION

The fungicide Fluazinam applied reduced the progress of white mold. An incidence of 0.68% was observed in the applied plots and 8.97% in the control. Differences were also observed in the

severity of the disease, where the use of fluazinam provided a severity of 1.73 against 23.83 in the untreated area (figure 1).

Figure 1. Effect of the fluazinam fungicide (Frowncide) on the severity and incidence of white mold in sprayed plots.



* Different letters differ by the Tukey test at 5%.

The time of application of the fungicide in the stages of R4 and R5 was favorable because they represent stages considered critical for infection by the fungus *S. sclerotiorum* in common bean. This is mainly due to the high numbers of flowers on the plants and petals on the soil, which favors the germination of the ascospores, through the supply of nutrients to them (Sutton and Deveral, 1983). Due to the fungicide applied, apothecium emission and the germination of the spores may be reduced. The sequential application of the fungicide fluazinam proved to be an important practice since it can act during several cycles of the disease that occurs in the field at different times thus ensuring more efficient control for a longer period.

CONCLUSIONS

The fungicide fluazinam applied sequentially from the R4 stage controls white mold and reduces the residual inoculum in the post-harvest.

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SEED COAT FRACTION AND YIELD POTENTIAL FOR POLYPHENOL EXTRACTION IN COMMON BEAN GERMPLASM GROWN IN MÉXICO

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INTRODUCTION: Common bean seeds represent a sustainable source for extracting polyphenols and other multiple bioactive compounds (Heredia *et al.*, 2017), commonly used in food and pharmaceutical industries. Variations have been detected for the content of bioactive compounds in common beans, according to the cultivar and its interaction with environmental conditions registered in production areas (Herrera *et al.*, 2019). Differences have also been observed based on seed color, due to higher levels for polyphenols detected in the seed coat, mainly in common bean cultivars with black seeds (Chávez and Sánchez, 2017). Identification of better options for efficient polyphenol extraction is necessary in order to ensure the industry supply, to increase the economic benefits in common bean production and to manipulate traits related to accumulation of polyphenols and other bioactive compounds. The main objective was to estimate fractions and the seed coat yield in common bean breeding lines and cultivars grown in Durango, México.

MATERIALS AND METHODS: Twelve common bean improved lines and four commercial cultivars were planted under irrigation in the state of Durango, México. Lines and cultivars were sown in July 10th using a completely randomized design with five replications. Experimental plot consisted of eight rows with 10 m in length and 0.81 m apart. Fertilizer was applied during the sowing, at the rate of 35-50-00 (for N-P₂O₅-K₂O). Irrigation was applied three times in order to avoid severe water stress in plants and to optimize seed yield in common bean breeding lines and cultivars. Insecticide (Dimethoate or Spinetoram) was applied in five opportunities for bean beetle (Epilachna varivestis) and bean pod weevil (Apion spp.) control. At maturity, five plant samples were taken per cultivar and breeding line for seed yield determinations. Plant samples consisted of two rows, 5 m in length by 0.81 m in width (8.1 m²). Seeds obtained in each field sample were cleaned and weighted for yield estimation, and then a sub-sample was taken for seed size determination (100 seed weight). Two 25 seeds sub-samples were also obtained in order to separate seed coat and to determine seed coat and cotyledon fractions. The analysis of variance was obtained under a completely randomized design with five replications. When statistically significant differences were observed, means comparison were performed using Tukey's honest significant difference test ($p \le 0.05$). For the analysis of variance and means comparison the SAS ver. 9.4[®] computer program was used.

RESULTS AND DISCUSSION: Significant differences ($p \le 0.05$) were observed for all the variables included in the study (Table 1). Higher seed yield was observed in the breeding line NGO14013 (4,165 kg ha⁻¹), which also showed intermediate values for seed size (29.6 g/100 seeds), lower coat fraction (9.2 %) and higher seed coat yield (383.2 kg ha⁻¹). Other cultivars and breeding lines showing outstanding seed yield and seed coat yield were Negro San Luis (4,142 kg ha⁻¹; 389.3 kg ha⁻¹), NGO14014 (3,714 kg ha⁻¹; 341.7 kg ha⁻¹), and PT14036 (3,661 kg ha⁻¹; 336.8 kg ha⁻¹). Breeding lines showing the largest seed size were PT14059 (40.4 g/100 seeds) and

PT14036 (40.1 g/100 seeds), but no significant relation (r= -0.03) was observed with seed coat yield. Significant variation was observed for seed coat fraction among common bean breeding lines and cultivars. Cultivar and breeding line showing higher seed coat fraction were Flor de Mayo Media Oreja (11.1 %) and NGO14063 (10.4 %), both including genes from the Jalisco Race (Río Grande was included as a parent of NGO14063). Results suggest that genetic advances are possible in breeding programs for increased yield and enhanced seed coat fraction. Optimization of bioactive compounds for extraction and increased economic benefits could also be achieved. Simple selection criteria are required for genetic improvement of common beans with increased values for seed coat fraction and the content of nutraceutical compounds. Successful strategies are also needed to encourage common bean consumption, through the development of food and dietary supplements, in order to enhance human health naturally (Chávez y Sánchez, 2017).

CONCLUSIONS: Significant variation was observed for seed coat fraction and seed coat yield in common bean. Several cultivars showed potential for extraction of bioactive compounds, based on seed coat yield. Selected germplasm included black (NGO14013, NGO14014, NOD 1, NGO14063 and Negro San Luis) and pinto (PT14036, PT14053 and PT14029) breeding lines and cultivars.

Lino/cultivar	Seed Yield (kg	100 Seeds	Seed Coat Fraction	Seed Coat Yield
Lille/Cultival	ha⁻¹)	Weight (g)	(%)	(kg ha⁻¹)
¹ NGO14013	4,165ª	29.6 ^{hi}	9.2 ^g	383.2 ^{ab}
Negro San Luis	4,142ª	33.4 ^g	9.4 ^f	389.3ª
PT14036	3,661 ^{ab}	40.1 ^{ab}	9.2 ^g	336.8 ^{abc}
NGO14014	3,714 ^{ab}	31.2 ^h	9.2 ^g	341.7 ^{abc}
PT14053	3,117 ^{bc}	37.3 ^{de}	9.7 ^d	302.3 ^{abcde}
PT14029	3,436 ^{ab}	39.8 ^{abc}	9.1 ^h	312.7 ^{abc}
NOD 1	3,219 ^{abc}	28.2 ⁱ	9.5 ^e	305.8 ^{abcd}
PT14055	3,081 ^{bc}	37.8 ^{cd}	9.5 ^e	292.7 ^{bcde}
PT Saltillo	2,944 ^{bc}	35.4 ^{efg}	8.6 ^k	253.2 ^{cdef}
NGO14063	2,898 ^{bc}	23.2 ^j	10.4 ^b	301.5 ^{abcde}
FM14011	2,914 ^{bc}	35.1 ^{fg}	9.9 ^c	288.5 ^{cde}
Negro 80 25	2,969 ^{bc}	20.4 ^k	8.8 ^j	261.3 ^{cde}
PT14061	2,426 ^{dc}	38.1 ^{bcd}	8.9 ⁱ	215.9 ^{def}
PT14059	2,338 ^{dc}	40.4 ^a	9.1 ^h	212.8 ^{ef}
PID 1	1,878 ^d	36.8 ^{def}	8.9 ⁱ	167.1 ^{fg}
FM Media Oreja	741 ^e	29.0 ^{hi}	11.1 ^a	82.3 ^g
Average	2,978	33.5	9.4	277.9

Table 1. Traits related to seed coat yield observed in several breeding lines and cultivars grown under irrigation in Durango, México.

 1 NGO = opaque black, PT = pinto and FM = flor de mayo (pink).

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DIFFERENCES IN THE SEED COAT DARKENING PROCESS AMONG LIGHT COLORED DRY BEANS

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INTRODUCTION

Dark seed coat in light colored dry beans is associated with loss of culinary quality and this leads to a decrease in commercial value, therefore slow darkening dry beans are desirable. Different propensity to seed coat darkening between dry bean varieties has been observed. Accelerated aging tests (Jacinto-Hernandez *et al.* 2006, 2007) and darkening stimulation using UV light have been performed in order to select genotypes with greater color stability during storage. In 2017, we reported that the color changes in seed coat that occur naturally can be detected using reflectance spectrophotometry during the first days post-harvest. The objective of this work was to anticipate propensity for seed coat darkening by comparing the color change that occurred in dry bean varieties of the Bayo, Canario and Azufrado classes during 77 days post-harvest.

MATERIALS AND METHODS

During 2018, six dried bean genotypes, including four of the Bayo type (Bayo-Mecentral, Bayomex, Bayo-Azteca and Altiplanomex), which are beige; one Canario type (Canario-107) and one azufrado (Azufradoro -which was bred for slow darkening), were sown at Texcoco, state of Mexico under rainfed conditions. The experimental plot was a 4 m long row. Upon reaching maturity, the plants of each plot were threshed by hand. The grains of two plants were mixed to form a replica. Three replicas were left exposed to natural light at room temperature in Petri dishes in the lab for 77 days. The controls were kept refrigerated. Color measurements were made weekly. The first measurement was made when removing the grains from the pods and the subsequent ones were carried out weekly, using a CM-5 spectrophotometer (Konica Minolta, Inc., Osaka, Japan). The color reflectance was recorded in the CIE Lab color coordinate system, with D65 Illuminant and 10th observer. The activity of polyphenol oxidase (PPO) was determined in control samples as well as in experimental samples 77 days after harvest.

RESULTS AND DISCUSSION

The value of L* (lightness) in the control samples ranged from 58.7 (Bayomex) to 66.3 (Altiplanomex). The value a* ranged between -1.6 (green tones in Azufradoro) to 5.3 (red tones in Altiplanomex), The value b* (yellow tones) ranged from 23.3 (Bayo_Mecentral) to 48.3 (Azufradoro) (fig. 1).

Change in the color of the genotypes was detected from the first seven days after removing the grains from the pods (in the first week the varieties reached their characteristic grain color). The results show differences in varieties starting from the first 14 days after harvest. Darkening of the seed coat was detected, which was associated with a decrease in the L* value, an increase in red tones (a*) and a decrease in yellow tones (b*). Bayo-Azteca and Canario-107 were the varieties with the greatest decrease in L* (Δ L* = 3.9 and 1.4 units respectively) at 14 days and after 77 days these same varieties showed the greatest decrease (Δ L* = 7.4 and 6.5 units respectively) compared

to their control samples. The variety with the least color change was Azufradoro, which after 77 days decreased its L* value by one unit. In the red tones (a*), Bayomex, Canario-107 and Bayo-Mecentral, increased their red tones the most ($\Delta a^* = 0.9, 0.5$, and 0.9 respectively after 14 days; and 3.1, 3.1, and 1.8 after 77 days). In the variable b*, Bayomex was the one that lost most of its yellow tones ($\Delta b^* = 4.8$ units at 14 days and 5.2 units after 77 days). The activity of the PPO was consistently higher in the samples 77 days after harvest, compared to its controls. The varieties with highest PPO activity were Bayomex, Bayo Mecentral and Bayo Azteca.



Figure 1. Color changes (L*, a*, b*) of the seed coat of 6 improved varieties of *Phaseolus vulgaris* L. determined 77 days after harvest

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COMMON BEAN LINE SELECTION FOR DROUGHT RESISTANCE

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INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is a legume grown worldwide for its economic, social importance and high nutritional value. However, abiotic factors, such as drought, can compromise the development of the crop and reduce its production. The effects of water deficit have been studied, supporting selection of lines more adapted to this adverse condition. Thus, the determination of drought resistance parameters is essential to ensure efficiency in the process of selecting potential genotypes for drought resistance. This work aimed to evaluate 121 common bean lines collected in Brazil in field conditions, and to select bean lines with drought resistance.

MATERIALS AND METHODS

Two treatments, drought stress and non-stress, were evaluated in a 11x11 triple lattice design (k = 121), during the dry season of 2019 in Cáceres city, Mato Grosso state, Brazil. The plots were planted in rows with fifteen plants per meter and 0.5 m between rows. In the flowering stage, the irrigation of the drought stress field was suspended and the water tension in the soil maintained at -50 KPa at 0.20 m depth, measured by tensiometers installed in the field. After ten days of water deficit, the irrigation was reestablished in the drought stress field. The yield (kg ha⁻¹) was measured under drought and non-stress. The Drought Susceptibility Index (DSI) according to Fischer & Maurer (1978), was estimated by DSI = (1 - Yd / Yp) / D, where Yd is the yield of the genotype in the experiment with drought, Yp is the yield of the genotype in the experiment without drought and D = 1 - (average yield of the genotypes under drought / average yield no-drought). Drought Tolerance Efficiency (DTE) was estimated by the equation of Fischer and Wood (1981), DTE (%) = (genotype yield in experiment with drought / yield of the genotype in the experiment no-drought) * 100. The average yield was analyzed using REML / BLUP. This approach was adopted to predict genotypic values using the estimates of variance components as random effects, obtained by REML, using model 17 (Resende, 2016)

RESULTS AND DISCUSSIONS

The deviance analysis (ANADEV) revealed significance differences (LRT \geq 6.63) for grain yield in both experiments, allowing select ten bean lines greater in drought resistance parameters (e.g., DTE and DSI) when compared with the others 107 bean lines tested in this field experiment. However, we observed the ordering of the lines (by DTE and DSI) does not follow the order of greater yield bean lines in both experiments (Table 1), this happened because DTE and DSI is related with the among of yield losses. To select the bean lines by their drought resistance we considered the highest DTE values (97.4 to 61.02) and the lowest DSI values (0.04 to 0.58) among 121 bean lines. Bean lines BGF107 and F71 stands out by their drought resistance, which presented DTE of 97.4, 88.96 and DSI of 0.04 and 0.16, respectively, and it can be promising to use in plant breeding for drought resistance.

Table 1. Rank of drought resistance parameters (DTE - drought tolerance efficiency, DSI - drought susceptibility index) and respective genotypes codes (Gen.), genotipic value (u+g), adjusted average of yield (Kg há-1) under drought (Yd) and no drought (Yp) experiments.

			DSI	Yield (kg ha-1)							
Dank	Gen.	DTE (%)		Yp**			Yd**				
Nalik				Rank	u+g	Adjusted	Rank	u+g	Adjusted		
1	BCF107	97.40	0.04	21	3109.6	4060 7	1	2801.9	2801.9		
2	F71	88.96	0.04	21 17	203/3	3222 5	1	1707.0	2001.5		
2	Г/1 DCE22	88.90 84.56	0.10	40	1072.0	2172.0	4	1550 1	1072.2		
3	DGF32	84.30	0.23	49	19/2.0	51/2.0	4	1338.1	18/3.3		
4	BGF93	84.03	0.24	87	465.2	2256.4	50	396.8	878.3		
5	BGF5	66.41	0.50	40	2499.6	3406.4	3	1575.7	1952.0		
6	BGF113	64.70	0.52	55	1850.3	3033.2	9	1120.7	1467.4		
7	BGF74	63.77	0.54	78	1104.7	2516.5	33	672.9	1046.2		
8	BGF91	63.30	0.54	85	489.5	2294.4	58	325.5	822.5		
9	BGF197	61.54	0.57	72	1372.7	2676.7	21	809.5	1196.3		
10	BGF36	61.02	0.58	54	1868.3	3055.1	11	1089.0	1405.5		

Drought Intensity Index for the experiment, DII (Ramírez-Vallejo and Kelly, 1998) = 0.76; ** Significant Deviance at 1% (LRT \geq 6.63).

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GAS EXCHANGE AND CHLOROPHYLL CONTENT IN COMMON BEAN cv. "BRSMG MADREPÉROLA" UNDER RARE EARTH ELEMENT FOLIAR APPLICATION

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INTRODUCTION: Rare Earth Elements (REE) have been applied in China to several crops to increase agricultural productivity. In this country, for example, crops treated with commercial leaf fertilizers including ETRs showed an increase of about 10 to 20% in productivity. However, the effects of these elements on plant physiology have not been elucidated. Therefore, it is necessary to study the effect of REE on plant physiology in order to verify how these elements result in increased productivity, through acting as bio-stimulants of growth and development. In view of this, the present study aimed to evaluate REE effects on the physiology of common bean (*Phaseolus vulgaris* L.) cv. BRSMG Madrepérola, in order to elucidate how these elements act as bio-stimulants of plant growth and development. For this reason, two products containing Ce and La separately, and one product containing a mixture of REE (REE mix), were tested by measuring the effects on gas exchange and chlorophyll relative index in common bean.

MATERIALS AND METHODS: An experiment was carried out to study the effects of foliar application with REE on gas exchange and chlorophyll relative index (SPAD) of common bean cv. BRSMG Madrepérola. Plants were exposed to full sunlight for 68 days. At 56 days, 600 g ha-¹ (foliar application) of two products containing 41.38% Ce and 23.95% La, separately, as well as a mixture of REE (REE mix) containing 41.38% Ce, 23.95% La, 13.58% Pr, and 4.32% Nd was applied. On the last day of the experiment the relative chrolophyll index (SPAD Index), using a portable chlorophyll meter, was measured. A fully expanded leaf on the newest trifoliolate was standardized for SPAD evaluations and the analyses were conducted in three regions of each leaf (basal, medium and apical). On the same day that SPAD was measured, the analysis of gas exchanges was performed, 12 days after the REE application. An infrared gas exchange analyzer (IRGA) was used to make measurements on the same leaf on which SPAD was evaluated between 08:00 to 10:00 am. The density of the photosynthetically active photon flux irradiation inside the camera was fixed at 1000 µmol m⁻² s⁻¹. The following variables were assessed: photosynthetic rate (A - μ mol CO₂ m⁻² s⁻¹), transpiration (E - mmol H₂O m⁻² s⁻¹), and stomatal conductance (g_s - mol $H_2O m^{-2} s^{-1}$). Five replicates were arranged in a completely randomized design. Data were subjected to analysis of variance (ANOVA) and the means compared by the Tukey's Test at a 0.05 significance level of probability using software Sisvar 5.3 statistics.

RESULTS AND DISCUSSION: The common bean photosynthetic rate with application of REE was increased (Figure 1A) when compared to the plants in the absence of REE. This increase in the photosynthetic rate contributed directly to increases in productivity such as those found in China. The transpiration rate and stomatal conductance were not influenced by the presence of REE (Figures 1B and 1C). The transpiration rate and the stomatal conductance, although they also contribute to the photosynthetic rate, in this present study were not affected by the presence of REE. The relative chlorophyll index (SPAD Index) was increased with application of isolated REE (Ce and La) to leaves, compared to the control treatment (without REE) and was even greater in the treatment with the REE Mix application (Figure 1D). This indicates that when combined, rare

earth elements further enhance chlorophyll biosynthesis in common bean. This increase in the relative chlorophyll index promoted an increase of the photosynthetic rate observed with application of REE, because the higher the chlorophyll content, the higher the possibility to perform photosynthesis. However, because of the several factors that make up the photosynthetic rate, it did not increase further with application of REE Mix compared to the other REE treatments (Ce and La), as observed for the relative chlorophyll index. In order to increase the photosynthetic rate, besides the chlorophyll content, it depends on stomatal conductance, which was not altered by REE, as well as on the decarboxylation rate of ribulose-1,5-bisphosphate, among others. Thus, REE was found to increase the relative chlorophyll index in bean plants, enhancing photosynthetic rate and potentially enabling an increase in productivity.



Figure 1. Physiological characteristics of the common bean under foliar application of REE. A: Photosynthetic Rate; B: Transpiration Rate; C: Stomatal Conductance; and, D: Relative Chlorophyll Index (SPAD Index). Graphical Bars followed by the same letter did not differ significantly by Tukey's Test, p <0.05. Mean Standard Error Bars were utilized (n = 5).

CONCLUSIONS: The application of REE in common bean does not influence stomatal conductance and the transpiration rate, but increases the photosynthetic rate due to other factors, such as the increase in the relative chlorophyll index. Leaf application of REE, as isolated Ce and La, increases the relative chlorophyll index but the application of REE Mix increases the relative chlorophyll index to a greater extent. Thus, REE can function as bio-stimulants of common bean photosynthesis.

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IMMEDIATE AND RESIDUAL EFFECTS OF AN ECOLOGICAL FERTILIZATION MIXTURE ON THE CONCENTRATION OF NUTRIENTS IN BEAN LEAVES

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INTRODUCTION

Beans are the main source of vegetable protein for the Brazilian population. They are also an important source of iron and zinc, in addition to calcium, magnesium and phosphorus. Common bean production requires balanced nutrition to ensure its production potential and to generate plants with adequate levels of mineral elements. In low-fertility soils, crop nutrition must be supplemented with mineral fertilization (Araujo et al., 1996). Rock powders are characterized by multi-element composition and slow solubilization capacity, which are suitable for use in alternative production systems and in conditions favorable to the leaching of nutrients and in degraded soils (Van Straten, 2006). As the chemical and texture composition of the rocks is quite varied, each one releases its elements at different speeds. Granodiorite is an igneous rock with a chemical composition similar to granite, but containing more plagioclase than alkaline feldspar or orthoclase (Fernandes et al., 2010), and is a good source of potassium. Results obtained by Grecco et al. (2013) with treatments based on granodiorite, presented satisfactory results when compared with the control that used soluble NPK fertilization. The objective was to evaluate the immediate and residual effect of the use of an ecologically based fertilizer mixture on the accumulation of nutrients in bean culture.

MATERIALS AND METHODS

The work was carried out at the Embrapa Clima Temperado, at Terras Baixas Station, located in the city of Capão do Leão, in October 2013. Cv. Iraí, with a determinate habit and early maturity, was used. The soil, a planossol with drainage deficiency, was sieved and placed in pots with a total volume of ten liters of soil. Doses of 0, 500, 1,000, 2,000, 4,000, 8,000 and 16,000 kg.ha⁻¹ of the fertilizing compound were used and a control with soluble fertilizer (NPK) was added at a dose of 200 kg.ha⁻¹ of fertilizer in the formulation 5-30-15. The rock compound was a mixture of rock powders, with granodiorite and natural phosphate plus tung cake in the same quantities. The rock powder represented a comprehensive composition, regarding K, Ca, P, Mg, N and micronutrients. The trials were evaluated in two seasons, evaluating the immediate effect (sowing in October) and the residual effect (sowing in February). The design used was a factorial between dose of the mixture and time of evaluation, and was completely randomized with three repetitions. In the pre-flowering period, the plants were ground, acid digestion was conducted to evaluate the mineral composition of the plants including the following elements: phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) through the method of flame spectrophotometry (Silva, 1999).

RESULTS AND DISCUSSION

Table 1 shows the content of the nutrients evaluated, the interaction between the times of evaluation (immediate and residual) and dose of the mixture. As for Ca, in the evaluation of the immediate effect, the mixture showed a consistent increase in the calcium content of the leaves, that decreased when it was evaluated in the second trial (residual effect). In the second trial, the

mixture overcame the control with soluble NPK. This is due to the slower release of these nutrients in relation to this fertilizer. In the case of Mg, the difference is not so noticeable when comparing between the cultivation right after the application of the agrominerals and the residual effect. For the K content, in the evaluation of the immediate effect, the NPK treatment exceeded the rock fertilizer mixture, however in the second year the rock mixture exceeded the soluble fertilizer. As for the P content, there was an increase in the element content in leaves in the second trial analyzed, however in the first year, there was no increase in P content. This fact was expected due to the effect of solubilization that occurs in the application of reactive natural phosphates whose effect becomes more pronounced in the second year of cultivation.

Table 1. Calcium (Ca), magnesium (Mg), potassium (K) and phosphorus (P) present in pre-
flowering bean leaves grown in soil fertilized with a rock powder mixture containing natural
phosphate, granodiorite and tung cake evaluating the immediate (first trial) and residual (second
trial) to the dose applied to the soil.

Dosis t.ha ⁻¹	Ca g.kg ⁻¹		Mg g.kg ⁻¹		K g.kg ⁻¹		P g.kg ⁻¹	
	immediate	residual	immediate	residual	immediate	residual	immediate	residual
0	9,4 b B	20,8ab A	3,79 cd B	4,50 b A	27,89 c A	23,75 a A	1,70 b B	2,97 ab A
0,5	11,7 b B	20,3ab A	3,49 d B	5,16 ab A	26,60 c A	23,26 a A	1,77 b B	2,93 ab A
1	11,4 b B	20,3ab A	4,22 cd B	5,25 ab A	30,12 c A	24,27 a B	1,77 b B	3,17 ab B
2	10,5 b B	22,5a A	4,00 cd B	5,53 a A	29,52 c A	23,32 a B	2,00 b A	2,79 ab A
4	11,8 b B	23,0a A	4,47 c B	5,67 a A	30,20 c A	24,41 a B	2,13 b B	4,02 a A
8	13,3ab B	23,8a A	4,67 bc B	5,36 ab A	31,66 bc A	22,57 a B	1,66 b B	4,08 a A
16	18,6a A	22,3ab A	5,55 ab A	5,26 ab B	36,04 b A	24,65 a B	1,48 b B	3,37 ab A
NPK	15,2ab A	15,8b A	6,14 a A	4,73 ab B	41,87 a A	23,24 a B	3,91 a A	2,49 b B
Media	12,74 B	21,10 A	4,56 B	5,18 A	31,74 A	23,68 B	2,05 B	3,23 A
C.V. (%)	14.4		7.5		7.7		18.4	

*Values follow for the same letter in lines and columns not differ significantly in the Tuckey test at 5%.

CONCLUSIONS: The use of rock powder mixture based on natural phosphate and granodiorite, and tung cake is an alternative to the chemical fertilization of bean. The dose of 4 t. ha⁻¹ shows a good fertilizing effect during two seasons with adequate levels of Ca, Mg, P and K present in the plants.

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GENETIC DIVERGENCE OF ANDEAN BEAN LINES AND CULTIVARS REVEALED BY MORPHOAGRONOMIC TRAITS

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INTRODUCTION: Common beans (*Phaseolus vulgaris* L.) of Andean origin, also known as special grains, have larger grains, and a variety of seed colors, such as cream, white, yellow and red (Blair et al., 2010). They are appreciated in international markets, have greater added value and can be an alternative source of income for Brazilian farmers who are interested in exporting (Thung et al., 2009). For the development of new, more productive and adapted cultivars, characterization is necessary, which allows the differentiation of genotypes both for diversity studies and for the protection of new cultivars (Collares et al., 2002). Thus, this study aimed to evaluate the contribution of the descriptors proposed by the National Cultivar Protection System – SNPC to the genetic divergence of Andean common bean cultivars and breeding lines.

MATERIALS AND METHODS: Sixteen genotypes were evaluated, of which 13 were breeding lines developed by the IDR–IAPAR–EMATER breeding program and three cultivars - IPR Garça, BRS Embaixador and BRS Radiante. The experiment was established in the 2017/18 season, at the IDR–IAPAR–EMATER experimental station in Londrina, Paraná, Brazil. The design used was a randomized block with four replications, plots of four rows, four meters long, with 0.5 m between lines and a population of 12 plants per linear meter, with the two central rows being considered as a useful plot. The morpho-agronomic characterization was performed using the 56 minimum descriptors proposed by the Ministry of Agriculture, Livestock and Supply – MAPA through the SNPC and 9 descriptors related to yield components. To assess genetic diversity between accessions, from the data obtained, principal component analysis (PCA) and hierarchical grouping using the Gower dissimilarity matrix and the UPGMA grouping method were performed. The analyzes were performed using the R program (R Core Team, 2019).

RESULTS AND DISCUSSION: The first two components of the PCA together explained 52% of the variability present between the breeding lines and cultivars evaluated in this study (Figure 1). The variables that contributed most to the formation of the first component (Figure 1A) were the characteristics related to the color of the pods and seeds, namely: presence and density of the secondary color in the pods and the number and distribution of colors in the seeds. In the formation of the second component, the characteristics that contributed most were related to the dimensions of the pods and seeds: width of the pod, relation between width and thickness of the seeds and width of the cross section of the seeds.

In both analyzes (PCA and dendrogram) the genotypes were grouped into three main groups (Figure 1B and 2). The first group was the most numerous, formed by 11 accessions of which two were cultivars that presented greater weight of 1000 seeds, especially the LPES-10 breeding line with 399 g, and greater seed brightness. On the other hand, LPES-10 had the lowest yield among the three groups, with an estimated average of 896.24 kg ha⁻¹. The second group consisted of only one genotype, as this was the only one that showed an indeterminate type of growth habit, in addition to having a longer guide length and number of nodes, elliptical seeds in the longitudinal section, and opaque color. Finally, the third group was composed of four

genotypes, one cultivar and three breeding lines, which presented intermediate values for most variables, however it showed the highest yield among the three groups, with an average of 1206.68 kg ha⁻¹. In particular, the LPES-16 genotype presented the highest yield, 1451.04 kg ha⁻¹.



Figure 1. Principal component analysis (PCA) of breeding lines and cultivars of Andean common beans. Contribution of variables (A) and distribution of accesses in relation to the first two components (B).

Q1- leaf size; Q2- cycle until flowering; Q3- plant height; Q4- pod length; Q5- pod width; Q6- pod thickness; Q7- thickness / width ratio (pod); Q8- length of the apical tooth (pod); Q9- weight of 1000 seeds; Q10- shape of the seed in cross section; Q11- width in cross section of the seed; Q12- seed length; Q13- length/width ratio (COEF J) (seed); Q14- thickness/width ratio (COEF H) (seed); Q15- total cycle; Q16- insertion height of the 1st pod; Q17- length of the guide (cm); Q18- number of nodes; Q19- number of locus/pod; Q20- number of seeds/pod; Q21- total number of pod/plant; Q22- total number of seeds/plant; Q23- weight of seeds/plant; Q24- yield; M1- plant size; M2- intensity of green color on the leaf; M3- location of inflorescence (determined habit); M4- size of flower bracts; M5- flower banner color; M6- flower wing color; M7- intensity of the primary color in the pod; M8- secondary color of the pod at physiological maturation; M10- degree of curvature of the pod; M11- shape of the pod; M12- curvature of the apical tooth; M-13 secondary color of the pod at the point of harvest; M14- density of secondary spots at the point of collection; M15- constriction of the pod; M16- shape of the seed in longitudinal section; M17- degree of seed curvature; M18- number of colors in the seed; M19- main color of the seed; M20- secondary color of the pod; M21- secondary color distribution in the seed; M22- seed venation; M23- seed shine; B1- growth habit; B2- type of plant; B3- presence of secondary color in the pod; B4- position of the apical tooth in the pod.



Figure 2. Dendrogram generated from Gower's dissimilarity matrix and Ward's grouping method of 16 Andean common bean genotypes through qualitative and quantitative variables.

CONCLUSIONS

evaluated The traits allowed for the discrimination and the diversity study between the cultivars and breeding lines. It was possible to observe the superiority of some breeding lines for desirable characteristics (e.g. vield) in relation to the cultivars present in this study. The morphoagronomic characteristics evaluated proved to be efficient for characterization and will enable the protection of new cultivars, which are different from cultivars already protected, that are homogeneous in each cycle and stable over the generations.

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QTL MAPPING OF AGRONOMIC TRAITS IN BLACK BEANS

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INTRODUCTION AND METHODS

Two half-sibling black bean recombinant inbred line (RIL) populations segregating for color retention after canning were developed from elite black bean breeding lines adapted to Michigan growing conditions (Bornowski et al. 2017). The commercial variety 'Zenith' (Kelly et al. 2015) was crossed with the B14311 breeding line that exhibited resistance to common bacterial blight (CBB). B12724 was also crossed to B14311 to confirm QTL data from the first population. Agronomic traits were evaluated in replicated plots during the 2017 growing season at the Saginaw Valley Research and Extension Center (SVREC) and included days to flowering, days to maturity, canopy height, lodging, foliar effects of CBB and ozone damage, and a desirability score that represented perceived agronomic potential. After harvest, seed yield was taken for each RIL as the least square mean across field replications and seed weight was recorded as the weight of 100 randomly-selected seeds from each RIL. The BARCBean6k_3 BeadChip microarray (Song et al. 2015) was used to genotype the RIL populations and a QTL (quantitative trait loci) analysis was conducted on all agronomic traits.

RESULTS AND CONCLUSIONS

Distributions of agronomic traits varied since parental lines were not significantly different (p > 0.05) for most agronomic traits, as the focus of the study was primarily concerned with canning quality traits. However, QTL for agronomic traits were detected in both populations but should be interpreted with caution because of their polygenic nature and single year of measurement. Several QTL co-localized to the first 1.5 Mb of Pv08 in the B14311/B12724 (BB) population. In this region, QTL for seed yield, canopy height, and agronomic desirability explained 12%, 22%, and 15% of the phenotypic variation for these traits, respectively. A seed weight QTL from the B14311/Zenith (BZ) population was located over 11.5-11.8 Mb on Pv03 and explained 22% of the phenotypic variation for the trait; there was a corresponding seed weight OTL located over 9-12 Mb in the BB population. Surprisingly, a QTL explaining 20% of the phenotypic variation for days to flowering mapped to 61 Mb on Pv08 in the BZ population, a location previously reported by Kamfwa et al. (2015) who found a significant SNP ss715646088 at a physical position of 61.16 Mb in the v2.1 dry bean genome. That SNP was not included in the current linkage mapping study, but its physical position is centered within the QTL interval (60.08-61.73 Mb) from this study. Foliar bronzing attributed to ozone damage was observed in the 2017 field trials, however bronzing was not evenly distributed across the field, and ratings may have been confounded by CBB lesions, which could explain proximity of these QTL with known QTL associated with CBB. The most significant QTL for the foliar bronzing trait was located between 2.7-4.3 Mb on Pv07 in the BB population. This QTL explained 16% of the phenotypic variation and RILs with the B14311 allele had less severe foliar symptoms. Interestingly, this bronzing QTL on Pv07 is extremely close to a CBB resistance locus near 4 Mb on Pv07 that is currently being fine-mapped (P. Miklas, pers. comm.). In the BZ population, a bronzing QTL was located at the proximal end of Pv08 near 61 Mb along with QTL for days to flowering, seed weight, and seed yield. Although this bronzing QTL is in the same general region as SU91 marker, they are most likely different QTL since the

B14311 allele for BRZ8.1^{BZ} was associated with increased foliar bronzing, whereas the B14311 allele for SU91-CG11 was associated with a reduction in foliar symptoms of CBB. Overall, the QTL identified in the two related populations of black beans offer insight on the genomic regions associated with control of these agronomic traits and will be useful in future black bean breeding efforts.

		Peak		Peak	R ²		Map interval	Physical interval
QTL ID	Chr	сM	Peak SNP	LOD	%	a	(cM)	(Mb)
HT1.1 ^{BB}	1	17.1	ss715646260	4.75	7	-0.34	0.0-37.0	0.16-2.85
SW3.1 ^{BB}	3	0.0	ss715646396	3.71	8	-0.33	0.0-1.5	1.19-1.30
$SW3.2^{BB}$	3	54.43	ss715648109	3.66	8	0.35	53.8-58.4	9.45-12.04
$DM4.1^{BB}$	4	0.8	ss715647817	5.85	13	-0.42	0.0-5.2	2.19-2.56
$SW4.1^{BB}$	4	4.0	ss715646247	3.59	8	-0.35	0.7-4.3	2.2-2.55
HT4.1 ^{BB}	4	10.8	ss715646232	10.58	17	-0.49	5.2-14.6	2.56-3.04
DS4.1 ^{BB}	4	12.0	ss715646229	3.60	7	-0.12	9.4-12.0	2.58-2.89
CBB5.1 ^{BB}	5	131.8	ss715645324	3.26	7	-0.19	128.6-139.0	39.26-39.34
CBB7.1 ^{BB}	7	43.4	ss715646472	6.44	16	-0.30	37.4-43.9	2.71-4.25
$SY8.1^{BB}$	8	0.0	ss715646680	5.35	12	-1.03	0.0-12.9	0.37-1.50
DS8.1 ^{BB}	8	0.0	ss715646680	6.81	15	-0.19	0.0-12.9	0.37-1.50
HT8.1 ^{BB}	8	0.0	ss715646680	12.96	22	-0.59	0.0-12.9	0.37-1.50
CBB9.1 ^{BB}	9	19.4	ss715645628	3.47	7	0.18	12.7-19.6	31.41-33.35
DM11.1 ^{BB}	11	10.8	ss715647770	4.33	9	0.37	0.0-10.9	49.59-51.12
HT2.1 ^{BZ}	2	82.5	ss715648525	5.15	12	-0.50	82.5-84.5	25.4-25.98
DM2.1 ^{BZ}	2	98.7	ss715647744	4.69	11	-0.43	84.5-103.7	25.98-34.73
$SW3.1^{BZ}$	3	117.6	ss715646286	12.10	22	0.78	112.1-121.0	11.47-11.82
HT3.1 ^{bz}	3	131.8	ss715646292	3.77	7	0.37	117.6-142.3	11.48-12.04
SW4.1 ^{BZ}	4	12.3	ss715649427	8.11	13	-0.61	7.0-16.7	0.07-1.84
SY8.1 ^{BZ}	8	136.2	ss715646529	3.79	9	-1.00	136.2-142.8	60.08-60.56
DF8.1 ^{BZ}	8	144.1	ss715646515	9.66	20	-0.47	136.2-147.9	60.08-61.73
BRZ8.1 ^{BZ}	8	149.8	ss715646750	4.02	8	0.28	144.0-154.4	60.98-62.21
SW8.1 ^{BZ}	8	186.7	ss715647407	4.52	7	-0.40	184.7-187.6	62.89-62.9
DS9.1 ^{BZ}	9	14.9	ss715647038	5.52	13	-0.19	6.2-16.8	0.53-7.87
DF11.1 ^{BZ}	11	127.7	ss715649909	3.20	5	0.24	127.7-128.0	48.21-51.96
HT11.1 ^{BZ}	11	128.3	ss715649459	3.33	7	0.38	128.3-133.4	52.16-52.66

QTL names are assigned according to the dry bean QTL nomenclature established by Miklas and Porch (2010). Abbreviations: SY: seed yield, SW: 100-seed weight, DF: days to flowering, DM: days to maturity, HT: canopy height, DS: desirability score, CBB: common bacterial blight resistance, BRZ: ozone bronzing

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THE RELATIONSHIP BETWEEN COLOR AND RELATIVE CHLOROPHYLL CONTENT IN SNAP BEAN LEAVES AND PODS

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INTRODUCTION

The common bean is one of the most important vegetable and grain legume crops consumed globally. Leaf and pod color are important phenotypic traits affecting quality and productivity. Chlorophyll is the main factor affecting plant color, and plays an important role in human health, such as odor control, cancer prevention, blood detoxification, and immune system support. To determine the factors affecting the genetic architecture of leaf and pod color, a genome-wide association study (GWAS) is underway using the snap bean association panel (SnAP) of 382 accessions.

MATERIAL AND METHODS

For the current preliminary study, 10 accessions, five green and five wax bean lines were selected from the snap bean panel. 'BBL 110', 'Bush Romano 71', 'Corbett Refugee', 'Renegade' and 'Roma II' represent green cultivars with different leaf and pod colors from different market classes while 'Brittle Wax', 'Earliwax', 'Gem', 'Refugee Wax', and 'Romano Gold' are wax bean cultivars. Both a colorimeter and the MultispeQ were used to take measurements. While color measurements were taken in the greenhouse by colorimeter, relative chlorophyll content was obtained in the field with the MultispeQ. Colorimeter values were obtained as CIE L*a*b* values and were converted to RGB and referenced to Royal Horticultural Society (RHS) colors using an Excel macro developed by Lattier and Contreras (2020).

Accessions	Туре	RHS Group for Leaves	RHS Value for Leaves	RHS Group for Pods	RHS Value for Pods
BBL 110	Green	Green	N137D	Yellow-Green	146C
Bush Romano 71	Romano	Green	N137B	Yellow-Green	146D
Corbett Refugee	Refugee	Green	N137D	Green	139D
Renegade	Green	Yellow-Green	146A	Yellow-Green	146B
Roma II	Romano	Green	139A	Yellow-Green	146D
Brittle Wax	Wax	Yellow-Green	146A	Greyed-Yellow	160D
Earliwax	Wax	Green	N137D	Greyed-Yellow	160D
Gem	Wax	Green	N137D	Greyed-Yellow	160D
Refugee Wax	Wax Refugee	Green	N137D	Greyed-White	156A
Romano Gold	Wax Romano	Green	N137D	Greyed-Yellow	162D

Table 1: Royal Horticultural Society (RHS) color values for leaves and pods of ten snap bean accessions from the SnAP diversity panel.

RESULTS AND DISCUSSION

Both green and wax bean cultivars had mostly green leaves compared to pod color (Table 1) While pod color had a yellow-green appearance in green pods, wax bean cultivars tended towards greyed-yellow on the RHS scale. For relative chlorophyll content, the leaves of green types had more relative chlorophyll content than the leaves of wax bean types, although the difference was not large (Figure 1). Of the green types, the Romano accessions had the highest relative chlorophyll

while 'Corbett Refugee' was the lowest. Overall, the lowest relative chlorophyll content belonged to 'Refugee Wax' (37.5) suggesting that the combination of being a refugee type with wax pods negatively affects chlorophyll content. In contrast to the leaves, there were large differences between the pods of green and wax bean cultivars (Figure 1). Among the pods of green accessions, 'Renegade' had the highest relative chlorophyll content (34.5), while 'BBL 110', 'Bush Romano 71', and 'Roma II' had almost the same value (~28). Moreover, there was almost no difference in the pods of wax bean accessions (~-4). Overall, it appears that leaves with in the green RHS color groups have more relative chlorophyll content compared to pods, which have lower relative chlorophyll content.

The negative values for wax bean pods would suggest that while green bean pods have the ability to photosynthesize, wax bean pods lack this ability. These results are congruent with our previous work on mapping and identifying a potential candidate gene that is targeted to chloroplast metabolism (Myers et al., 2018). In addition, using transmission electron microscopy we found that chloroplasts in wax bean pods have a rudimentary structure and are probably nonfunctional (Myers et al., 2019).





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DIVERSITY OF SNAP BEAN ACCESSIONS FOR NUTRITIONAL CONTENT

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INTRODUCTION: Within the group of vegetables, the snap bean (*Phaseolus vulgaris* L.) is one of the most economically relevant species, occupying the sixth place in relation to the volume produced in Brazil (Ramalho, 2003). The pods are harvested at an immature stage and can be consumed *in natura* or after processing, being a source of vitamins, fibers, minerals and antioxidant compounds (Filgueira, 2000; Blair et al., 2010). In the snap bean plant breeding programs, nutritional quality is an important attribute to be considered, since the increase in nutritional value will better meet the needs of consumers who choose a healthier lifestyle. Therefore, the objective of the present study was to evaluate the diversity of accessions of snap beans in relation to nutritional content.

MATERIALS AND METHODS: Twenty snap bean accessions of determinate growth habit were evaluated in the Fall/Winter season of 2018 in Londrina, Paraná, Brazil. The evaluated accessions are divided between breeding lines from the International Center for Tropical Agriculture - CIAT: HAB404, HAB407, HAB415, HAB427, HAB428, HAB432, HAB440, HAB441, HAB443 and HAB447; commercial cultivars: Alessa, Clarke, Macarrao Baixo, Napoli, Savannah, Zigane, UEL 2 and Vicenza; and landraces: Idaho Refugee and Saxa. The experimental design was randomized blocks with three replications and plots of four rows of two meters in length, with a spacing of 0.5 meters between lines and ten plants per linear meter. The pods were harvested between tender grades 1 and 2, that is, until the seed occupied ³/₄ of the locule space (PBMH, 2010).

A sample of 20 pods from each experimental plot was washed in water, dried in an oven with air circulation at 65 °C for 72 hours and ground. The levels of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn), boron (B), manganese (Mg), iron (Fe), sulfur (S) and nitrogen (N) were measured with the flour obtained. The mineral contents were determined by nitroperchloric digestion with HNO3: HClO4 solution and by atomic emission spectrophotometry (ICP-AES) (Thermo Jarrell Ash ICAP 61E). The nitrogen content was quantified using the Kjeldahl method and the samples were read on a UV-VIS spectrophotometer. The analysis was developed according to the methodology described by Miyazawa et al. (1999). From the averages, the heatmap analysis was performed, using the R software (R Core Team, 2019) and the Pheatmap package (Kolde, 2019). For the elaboration of the heatmap, the Euclidean distance was used to generate the dissimilarity matrix, and for grouping, the UPGMA method was used.

RESULTS AND DISCUSSION: In the heatmap analysis (Figure 1), the formation of two large groups of genotypes was observed. When compared, the second presented higher nutritional contents for most of the evaluated nutrients. The first was the most numerous, composed of 11 accessions, and grouped most of the CIAT breeding lines, the local varieties and the cultivar Clarke. The second group, on the other hand, was formed mostly by commercial cultivars, with

two subgroups. The first group was formed by the cultivars Alessa, Vicenza, and Macarrão Baixo and by the breeding line HAB 440, which presented in general the best nutritional contents. The second group was formed by the cultivars Napoli, Savannah, Zigane, UEL 2 and the breeding line HAB404, while cultivar Savannah had the highest iron content among all genotypes. The cultivars belonging to group two, mainly to the first subgroup, can be indicated for consumption as food with a high nutritional content, as well as be included as parents in breeding programs with the objective of developing biofortified cultivars.



Figure 1. Heatmap with nutrient content in the pod of 20 snap bean accessions of determinate habit grown in 2018 in Londrina, Paraná, Brazil.

groups were formed. The first was composed of nutrients, zinc, potassium, phosphorus and nitrogen; the second, sulfur, calcium and -1 manganese; the third, boron and magnesium, and the last two formed separately by iron -2 (Fe) and copper (Cu). A relationship was observed between nutrients within the same group, and the fluctuation of the levels implies equal behaviors within the same group. This relationship may be favorable for genetic improvement, in which the efforts to increase the content of a nutrient can result in a concomitant increase in other related nutrients.

When considering the nutrient traits, five

CONCLUSIONS: A great diversity was observed for the nutritional contents between the accessions of snap beans evaluated. Cultivars with better nutritional contents can be consumed in the human diet in order to meet nutritional needs. These cultivars, as well as the breeding lines that stood out, can be used in breeding programs to obtain new cultivars.

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PLANT ARRANGEMENTS AND SEEDING TIMES IN THE PRODUCTION OF SNAP BEAN SEEDS

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INTRODUCTION: The arrangement of plants, that is, the way plants are distributed in the field represents a practice of easy adoption and low cost, as it results from the combination of the spacing between lines with the number of plants per linear meter, which determines the total plants per area. It should also be considered that the sowing time, due to different meteorological characteristics, may or may not enhance the competition between plants depending on the plant arrangement adopted. Little is known about the effect of the plant arrangement and sowing time, as independent or interacting effects, on seed production. Thus, the objective was to evaluate the production of bean pods with determinate growth according to the plant arrangement and sowing time.

MATERIALS AND METHODS: In Londrina, State of Paraná, Brazil, the cultivar Feltrin Macarrão Napoli® was evaluated in autumn-winter (sowing on 03/30/2017) and summer-autumn (sowing on 12/20/2017), in 10 arrangements (combination between the spacing of 0.45 and 0.90 m between lines, with densities of 133,000, 177,000, 221,000, 265,000 and 309,000 plants ha⁻¹ in a 2x5 factorial scheme). The experimental design was randomized blocks, with three replications. In R9, pods were harvested manually in 0.9 m² of the useful area of the plots. The average number of pods per plant (NMVP), the mass of seeds per plant (MSP), the number of seeds per pod (NSV), the crude protein content of the seeds (TPBS) and the seed yield (REND) were evaluated. After verifying the normality and homogeneity of variances, the data were subjected to the individual analysis of variance for each season and then the joint analysis of the experiments was carried out. The means of the sowing time and line spacing factors were compared using the Tukey test (p <0.05), whereas, for the plant density factor, the means were submitted to regression analysis (p <0.05).

RESULTS AND DISCUSSION: Except for REND, the other characteristics were affected by the sources of variation. In the 0.45 m spacing between rows, the NSV was higher in autumn-winter. In the summer-autumn, the superiority of the NSV was found when the spacing was doubled to 0.90 m (Table 1). In autumn-winter, 279.4 mm of rainfall was recorded during the reproductive phase of the crop, which, for the same period in summer-autumn, was 389.3 mm. In addition to the differences in quantity, the distribution of rainfall was different between sowing times. Between phases R8 and R9, that is, in the pods' filling and maturation phases, rainfall of 8.2 mm was recorded in autumn-winter, and 222.1 mm in summer-autumn. In this scenario, we suggest that the largest number of pods produced in autumn-winter results from the occurrence of pods with higher health quality and thus, with reduced rates of seed abortion. It is also important to highlight that, with the use of 0.45 m between rows, the space between the plants in the row is smaller, and the closing speed of the plants between the rows is greater, resulting in a microclimate unfavorable to plant health. This occurred in this study, favored by the conditions of high humidity, which, in both cases, may have contributed decisively to the lower NSV in the summer-autumn season. The isolated effect of plant density allowed a significant quadratic adjustment of the NSV response, with a maximum production of 4.02 seeds per pod, using the density of 213,750 plants per hectare, with a subsequent decrease due to population increase (Figure 1).

		NSV					
Sowing season (EP)	Spacing between lines (m)						
	0,45	0,90	Average (EP)				
Autumn-Winter	4,28 aA	3,92 aA	4,10 a				
Summer-Autumn	3,24 bB	3,95 aA	3,60 b				

Table 1. Number of seeds per pod (NSV) of the growing bean determined according to the sowing time and the spacing between rows.

* Averages compared by the same lowercase letter, in the column, and uppercase in the row, do not differ by the Tukey test at 5%.



Figure 1. Number of seeds per pod (NSV) as a function of plant density.

In Table 2, it is observed that the cultivation of pod beans in the summer-autumn favored NMVP and TPBS. We suggest the occurrence of the dilution effect to explain the opposite relationship between NSV and NMVP and TBPS. In our analysis, plants that produce a greater number of pods have fewer photoassimilates for seed formation, and thus, greater NMVP and TBPS, and less NSV, as observed in this study.

Table 2. Average number of pods per plant (NMVP) and crude protein content of the seeds (TPBS)
of the growing bean determined according to the sowing time.	

00	0	0
Sowing season (EP)	NMVP	TPBS(%)
Autumn-Winter	6,34 b	16,25 b
Summer-Autumn	7,54 a	29,72 a

* Averages compared by the same lowercase letter, in the column, and uppercase in the row, do not differ by the Tukey test at 5%.

Table 3 shows that the use of the 0.45 m spacing between lines provided greater NMVP and MSP for determinate growth beans. With 0.45 m spacing, it is suggested that photosynthetic activity was favored, which resulted in the greater interception of solar radiation, greater floral retention and, as a consequence, greater NVMP and MSP.

Table 3. Average number of pods per plant (NMVP) and seed mass per plant (MSP) of the growing pods determined as a function of row spacing.

4	1 0	
Spacing between lines (m)	NVMP	MSP (g)
0,45	7,61 a	4,39 a
0,9	6,27 b	3,57 b

* Means compared by the same lower case letter, in the column, do not differ by the Tukey test at 5%.

CONCLUSIONS: Seed yield is not affected by plant arrangement and sowing time. In the 0.45 m spacing, the number of seeds per pod is higher when the bean is grown in autumn-winter. The use of 0.90 m of line spacing provides a greater number of seeds per pod compared to 0.45 m in summer-autumn. Maximum seed production per pod occurs at a density of 213,750 plants ha⁻¹. The average number of pods per plant is favored by the cultivation in autumn-winter and the use of 0.45 m of spacing between lines. The crude protein content of the seeds is favored in autumn-winter. The mass of seeds per plant is favored with the use of 0.45 m of spacing between rows.

A MAJOR REPRODUCTIVE ISOLATION QTL IS ASSOCIATED WITH F₁ STERILITY IN COMMON BEAN × TEPARY BEAN HYBRIDS

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INTRODUCTION: The current projections of climate change predict more frequent incidents of heat and drought stresses. These changes will have major negative impacts on common bean (*Phaseolus vulgaris* L.), which is adapted to temperate regions and is highly susceptible to heat and drought stresses. One strategy to improve common bean tolerance to these abiotic stresses is to introgress useful genetic diversity from a sister species, tepary bean (*P. acutifolius*). Originating in the Sonoran Desert, tepary bean is adapted to high temperatures and drought, and thus is more resilient to extreme environments (Porch et al., 2013). Hybridization between these two species requires the use of a specific common bean bridge line as a parent as well as embryo rescue through dissection of F_1 hybrid seeds. The interspecific F_1 hybrids are sterile and require extensive backcrosses to common bean to achieve fertile introgression lines. Our current study was designed to identify genomic regions that are associated with hybrid sterility in a cross between common and tepary bean. Identification of such loci will provide fundamental knowledge to overcome these barriers and facilitate the introgression of useful genetic diversity from tepary bean into common bean more efficiently.

MATERIALS AND METHODS: *Developing introgression lines*: The common bean genotype Ica Pijao was used as the female parent in the interspecific cross with the tepary bean genotype Frijol Bayo. Both genotypes were acquired from the National Genetic Resources Program of the USDA. Initial crosses were made in December 2018 in the Michigan State University greenhouse facilities. About 23 days after pollination, pods were separated from the plants and surface sterilized using 70% ethanol for 3 min and then 10 min in 0.2% hypochlorite sodium. Immature embryos were dissected out of the seeds in a laminar flow and transferred to Murashige and Skoogmedia basal media containing 1 % Agar. Once the first trifoliate leaf began to develop, the seedlings were transferred to the pots containing soil. The inter-specific hybrids were confirmed using two sets of molecular markers. Sterile hybrids were backcrossed extensively to the common bean parent to develop BCF₁ plants. The successful backcrosses developed normal pods within ~45 days after pollination and embryos did not need to be rescued. No selfed pods were observed in F₁ hybrid plants, which indicates a high level of sterility.

Bulked Seqregant Analysis: Whole genomic DNA was shallow sequenced from BCF₁ plants and the Ica Pijao parent using the Illumina NovaSeq 6000 platform at the Texas A&M Agrilife Research Center. After trimming, the reads from BCF₁ were aligned to the Ica Pijao reference genome using BWA-MEM. SNPs for each line were called using GATK4. For each line, the proportion of reads supporting reference (REF) and alternative allele (ALT) were calculated for all the SNPs. Then the means of alleles for the REFs and ALTs were calculated, separately for
fertile and infertile BCF_1 plants. These data were analyzed with Bulked Segregant Analysis (BSA) using QTLseqr (Mansfeld, et al, 2018). BSA was performed by two analytical methods: Δ SNP index and G².

RESULTS AND DISCUSSION: Overall, 51 interspecific hybrids were developed that reached complete maturity. These lines were sterile (both male and female) and needed to be backcrossed to either parents to produce seeds. Out



Figure 1. Identification of a major QTL associated with interspecific hybrid sterility between common bean and tepary bean. Bulk Segregant Analysis was performed using two statistical methods; **a**) Δ SNP index, red lines indicate 95% confidence interval and **b**) G', red line indicates FDR = 1% threshold

of 950 backcrosses to the common bean parent, 37 BCF₁ plants were successfully generated. Eighteen plants suffered from high levels of sterility (average seed per plant = 2 ± 2.9) and nineteen plants were fertile (average seed per plant = 158.4 ± 117.5).

Each of the BCF₁ plants was sequenced to an average depth of 7X. In total, 1,605,819 SNPs were detected between the BCF₁s and the Ica Pijao genome. BSA revealed a major QTL on Chr09/22,165-31,970,64 (Figure 1). Interestingly, we were not able to detect any recombination within the first 22 Mb of Chr09 among the BCF₁s. In contrast, 18 recombination events were detected in the 22 Mb towards the end of chromosome (38.2 Mb). Lack of recombination at the first half of Chr09 suggests that there is a chromosomal rearrangement for tepary bean relative to common bean which suppresses recombination and contributes to sterility in interspecific hybrids. Although it did not pass the significance threshold, a potential second contributor to hybrid sterility was identified on Chr02 in the BSA. Similarly, no recombination was detected on the first 33 Mb of Chr02, however, 22 recombination events were detected for the rest of Chr02.

CONCLUSIONS: In this study, we detected a major QTL for hybrid sterility on Chr09 and a potential QTL on Chr02 in a cross between common bean and tepary bean. Lack of recombination in these regions suggest that chromosomal rearrangements, might be the major cause of hybrid sterility between these two bean species. These reproductive isolating barriers could be overcome by future breeders through adoption of a chromosomal engineering approach.

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GRAIN YIELD RESPONSE OF *Phaseolus acutifolius* A. Gray TO PLANT DENSITY

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INTRODUCTION: Legumes are an important source of nutrition, and their consumption helps to control and prevent diseases. Tepary bean (*Phaseolus acutifolius* A. Gray) is adapted to arid regions during the summer when the temperature is high and is considered drought resistant (Miklas et al., 1994). Mexico is the primary center of tepary bean diversity (Jiménez and Acosta, 2012) and its grain can be a source of resistance to abiotic elements, as well as food. This study was conducted under glasshouse conditions with the objective of determining the effect of plant density per pot on the phenology, grain yield and yield components of tepary bean.

MATERIALS AND METHODS: This study was conducted in a glasshouse (intercepts 27% incident solar radiation measured at 12:00 hours) in Montecillo, Municipality of Texcoco, State of Mexico, Mexico (19 ° 29'N and 98 ° 53'W and 2250 m of altitude) with a temperate climate (García, 2005). The sowing of a tepary bean brown seeded accession was on June 2, 2017, in 5 kg capacity pots with silty-clay soil, pH 7.6, CE of 1.9 dS m⁻¹ and organic material of 3.5%. The treatments consisted of 1, 2, 3, 4 and 5 plants per pot (PP). Two irrigations were applied per week. The experimental design was a randomized block with 5 repetitions. In accordance with the criteria indicated in Escalante and Kohashi (2015), the grain yield (GY, g), the number of grains (GN), grain size (GS, mg), and number of pods (PN) were recorded per plant. The number of grains per pod (GP) and the pod filling index (IV), which was calculated by IV = [dry weight of grain / (dry weight of grain + dry weight of leaflets) * 100] were also collected. In addition, the maximum and minimum temperatures (° C) were recorded. An analysis of variance (ANDEVA), the Tukey test and a correlation analysis were applied using the SAS 9.0 package (SAS, 2003).

RESULTS AND DISCUSSION: The emergence of the tepary bean was at 8 days after sowing (dds), the beginning of flowering was at 50 and physiological maturity was at 95 dds. The average minimum and maximum temperatures were 15 and 48°C, respectively, with an average relative humidity of 71%. Masaya and White (1991) indicate 20 to 25 ° C as the optimum temperature for bean growth, which indicates that the temperature conditions during crop development were limiting for the growth of the tepary beans. ANDEVA showed significant changes due to the effect of PP for GY, GN and PN (Table 1). The highest GY (22 g plant⁻¹), GN (150) and PN (46) were found with 5 plants per pot, and the lowest with 1 (8 g, 20 and 6, respectively). The GY was related to the GN (r = 0.99 **) and PN (r = 0.99 **). The GS and GP showed no significant changes due to the number of plants (Table 1). The mean GS was 0.154 g and the GP was 3.4. The IV presented significant differences due to the effect of PP. The highest IV was found with 2 to 5 plants per pot (0.80 to 0.85) and the lowest (0.69) with one plant per pot. This indicates that with the sowing of greater PP, greater PN, GN and GY is achieved, because with a greater number of plants there is a greater number of roots that more efficiently take advantage of water and soil nutrients.

Plant number	GY (g)	GN	GS (g)	PN	GP	IV
1	4 e	20 d	0.189	6 d	3	0.69 b
2	10 d	72 c	0.142	20 c	4	0.80 a
3	12 c	76 c	0.153	28 b	3	0.85 a
4	16 b	126 b	0.136	32 b	4	0.81 a
5	22 a	150 a	0.148	46 a	3	0.80 a
Mean	13	89	0.154	26	3.4	0.79
Tukey 0.05	1.7	15	0.07	4.2	1	0.09

Table 1. Grain yield in grams (g) and its components in tepary beans (*Phaseolus acutifolius* A Gray) according to the number of plants per pot at Montecillo, Municipality of Texcoco, State of Mexico, Mexico during the Summer of 2017.

GY = grain yield; GN = Grains number; GS = grain size; PN = Pod number; GP = grains by pod; IV = pod filling index.

CONCLUSIONS: Phenological stages were not affected by changes in the number of plants per pot. As the number of plants increased, the number of pods, grains, grain yield and pod filling index increased. The number of grains per pod and grain size were not affected by changes in number of plants.

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OBSERVATION ON THE POTENTIAL USE OF *MOGETTE DE VENDEE* AS AN INTERSPECIFIC BRIDGE

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In the summer of 2019, I was growing a greenhouse increase of Mogette de Vendee. Mogette de Vendee a type I *Phaseolus vulgaris* white kidney like bean that is used in France and classified as a Lignot type. Mogette de Vendee was of particular interest as Dr. Paul Gepts identified a sample of Mogette de Vendee as an "S" phaseolin type (Gepts, 1984). The "S" phaseolin type contrasted with the "T" phaseolin of many kidney beans lines from the United States and the Lignot types from France characterized in Dr. Gepts thesis.

At the same time I had breeding lines of type IV climbing *Phaseolus coccenius* in the field related to a project focused on developing Haricot Geant types better adapted to growing in Wisconsin. When Mogette de Vendee began to flower in the greenhouse the racemes were extremly long compared to other *P. vulgaris* materials in the greenhouse including: Alaric, Snowden, Aries. The length of the racemes reminded one of *P. coccenius*. Harvesting *P. coccenius* flowers in the field from: Wolven Pole, Judion de la Granja, KG040 (F₃ lines of Corona/8005), KG043 (F₃ lines of Moonlight/Wolven Pole) and 8005, pollinations were made to Mogette de Vendee in the greenhouse. The success rate of pollination was near 90% and all combinations produced seed.

 F_1 seed of Mogette de Vendee x *P. coccenius* was planted in the February 2020 greenhouse cycle. All F_1 seed germinated and produced indeterminate plant types. There were two distinct F_1 plant types. A "normal" type resembling the Type IV *P. coccenius* male parent that was indeterminate and an aggressive climber, 300-400 cm. The second type of F_1 was a sipindly, indeterminate, with highly compressed nodes that might reach 30-50 cm (SCI). F_1 Observations of the crosses are summarized in Table 1. All the F_1 's exhibited an intermedite hypogeal/epigeal emergence where the cytoledeans remained close to the soil surface. The F_1 from "normal" plants started to flower approximately 30-40 DAP and currently are setting selfed seed without any manipulation. As of this writing (50 DAP) none of the SCI plants have flowered.

The success of using Mogette de Vendee with *P. coccenius* has been surprising. Mogette de Vendee has been crossed with a number of *P. vulgaris* lines during the spring and summer of 2019. Crosses included both "S" and "T" phaseolin types. Nothing abnormal has been observed in progeny of *Phaseolus vulgaris* crosses with Mogette de Vendee. Progeny of the P.v. x P.c. crosses will be planted for further observations.

Table 1. Male Pollen Source and F ₁ Observations. Wisconsin, 2020						
P.c. Male Pollen Source	P.v. Female	F ₁ observation				
Wolven Pole	Mogette de Vendee	7 Normal & 2 SCI				
F3 Moon Light / Wolven Pole	Mogette de Vendee	10 Normal, no SCI				
F3 Corona / 8009	Mogette de Vendee					
8009	Mogette de Vendee	2 Normal & 7 SCI				
Judion de la Granja	Mogette de Vendee	8 Normal & 1 SCI				

SCI= spindly compressed internodes

Table 2. <i>P. coccenius</i> Lines and Source				
P. coccenius Lines	Source			
Wolven Pole	Ken K. accession from Minnesota			
Moon Light	Tozer Seeds, UK (derived from interspecific cross)			
Corona	Rancho Gordo - USA			
8009	Tozer Seeds, UK (derived for interspecific cross)			
Judion de la Granja	Spain - Strube Espana			

F1 P.v. x P.c. Normal

F₁ P.v. x P.c. Spindly Compressed Internodes (SCI)



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YIELD COMPONENTS OF RUNNER BEAN (*Phaseolus coccineus* L.) IN TWO TYPES OF TRELLIS SYSTEMS

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INTRODUCTION

The runner bean (*Phaseolus coccineus* L.) is a legume native to Mexico with food potential. Trellising is a method to achieve greater distribution, capture more solar radiation and increase production of dry matter. Climbing beans need trellising systems, however, wooden posts come with a high economic investment, while using another crop, e.g. maize or sunflower, is effective as long as the supporting crop is resistant to the weight of the legume. In intercropping systems, the competition of water, solar energy and nutrients can result in reduced grain yield for both crops (Delgado et al., 2015; Escalante et al., 2015). The aim of the study was to determine the effect of trellis type on grain yield components in runner bean grown under rain fed conditions in a temperate climate.

MATERIALS AND METHODS

This study was established at Montecillo, Texoco, State of Mexico, Mexico ($19 \circ 29'$ N and $98 \circ 54'$ W, 2,250 m of altitude). It is characterized by a temperate climate with a clay loam soil with pH 7. The sowing of the purple grain 'Juchitepec' cultivar was on May 2, 2016. The population density was four plants per m², planted in a conventional trellis or with sunflower. Data recorded included: phenology (Escalante and Kohashi, 2015), grain yield (GY), number of pods (PN) and grains (GN), the grain size (SG) and grains per pod (GP). An analysis of variance (ANDEVA), the Tukey test and a correlation analysis were applied using the SAS 9.0 package (SAS, 2003).

RESULTS AND DISCUSSION

In both types of trellis systems, the phenology of runner bean was similar. Thus, emergence was at 8 d, flowering at 65 d and physiological maturity at 130 d. Seasonal precipitation was 420 mm, of which 40% occurred during the reproductive stage. With the sunflower intercropping system, the GY was reduced by 57%, as a result of reduction in GN, PN, SG and GP (Table 1), but this reduction can be compensated by sunflower yield (Díaz-López *et al.*, 2010). Likewise, GN and PN presented a high correlation with the GY (r = 0.97) and to a lesser extent with SG and GP (r = 0.60). These results indicate that according to their ontogeny, yield components to increase in order of priority would be PN, GN, GP and SG to achieve high GY.

T	able	1. Y	lield	and	con	npor	nents	of ru	nner	bean	(<i>P</i> .	cocci	neus I	Ĺ.)	accord	ling t	to th	le type	of	trellis	
sy	/stem	at	Mon	tecil	lo, 7	Гехс	oco,	State	of M	lexico	, M	exico	during	g th	e Sum	mer o	of 2	016.			

TRELLIS	GY (g m ⁻²)	GN m ⁻²	SG (g)	PN m ⁻²	GP
CONVENTIONAL	136 a	190 a	1.24 a	49a	2.3 a
SUNFLOWER	58 b	52 b	0.99 b	26 b	1.9 b
MEAN	93	88	1.12	37	2.9
F PROB.	*	*	*	*	*
TUKEY 0.05	45	48	0.13	18	0.18

* Prob > 0.05. GY = grain yield, GN = grains number, SG = grain size, PN = pod number, GP = grains per pod.

CONCLUSION: Using the trellis system with sunflower, reductions were observed in GN, PN, SG, GP and consequently in GY. This reduction can be compensated for through the lower cost of the intercropping system and through the grain yield obtained from sunflower.

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ACCESSIBILITY OF IMPROVED BEAN SEEDS TO SMALLHOLDER FARMERS: EXPERIENCES FROM BEAN SEED SYSTEMS PROJECT IN NORTHERN TANZANIA

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INTRODUCTION

Common bean production level in Tanzania has increased over the years since 1995 with decreases in 2008 and 2011 (FAOSTAT, 2013). The area under bean production has been increasing at an average rate of 11% per annum and yield growth rates have shown modest increases from 0.48 in 1970 to 0.77 ton/ha in 2001-2007 (Katungi et al., 2009). This upward trend was attributed to national research efforts in collaboration with CIAT/PABRA which have resulted in development and identification of several improved and farmer Preffered bean varieties tolerant to environmental stresses. The lower yields experienced by smallholder farmers is attributed to an array of factors including lack of access to good quality seed in improved bean varieties, poor crop management practices, susceptibility of commonly grown varieties to major diseases and pests, variability in climate patterns, and lack of access to extension information. Other factors include poor return as a result of lower productivity and producing/ marketing non-market preferred varieties. Consequently, farmers get low farm-gate prices due to long value chains and poor-quality standards of their produce. Also, there was no single company engaged in the bean seed business prior to this initiative. To sustainably harness the potential of beans for food and nutritional security, efforts need to be intensified towards improving accessibility and utilization of good quality seed of improved bean varieties through creation of awareness and training on the importance of using good quality bean seed and recommended production techniques, as well as provisions of start-up seed-coupled with field demonstrations.

MATERIALS AND METHODS

This project was conducted stepwise by i) exposing the available improved bean varieties to bean value chain actors through stakeholder meetings, plot demonstrations and field days so that they can identify varieties of preferences; ii) supporting the capacity of research centers, public seeds agency and privates seed companies for producing breeder seeds, basic seeds and certified seeds, respectively, of the marketable and preferred bean varieties; iii) Linking agro-dealers to seeds companies and farmers so that small seed packs can be delivered to them for easier accessibility and affordability to small farmers; and iv) conducting seed business and farming as business trainings to seed value chain actors for better income generation to all actors.

RESULTS

A total of three stakeholders' meetings were conducted which brought all value chain actors on board from 10 districts of the project area (Figure 1). Three improved bean varieties, namely Lyamungo 90, Njano Uyole and Jesca (Figure 2), were identified as the most demanded varieties due to their higher yield, marketability, early maturity and disease resistance. A total of 522 on-farm demonstrations were conducted and 1,704 farmers and other stakeholders participated in demos and business training while 6,750 farmers participated in field days and agricultural shows. Production of certified seeds was increased by 150% while 34% were marketed in small seed packs of 2kgs (Figure 3). Three seed companies namely, Agricultural Seed Agency (ASA), Meru agro and Beula seeds were fully engaged in the production of certified bean seeds. A total of 36,480

farmers, mostly from remote villages, accessed certified seeds through agro-dealer networks and market days and a total of 13,188 leaflets and posters about bean production, agronomy, integrative pest management, value addition and farming as a business were distributed to create awareness. About 143 private and public extension officers were capacitated on dissemination of agricultural technologies (Figure 4), while 50 agro-dealers were linked to seed companies for sustainable seed systems.



Fig. 1: Project area Northern Tanzania



Fig. 2: Preferred improved bean varieties



Fig. 3: Small seed packs (2kgs)



Fig. 4: Training for technology transfer

CONCLUSIONS

The innovation of small seed packs through this project stimulated the demand for improved bean seeds by farmers. Many agro-dealers showed to be ready to continue with seed distribution along the bean value chain actors if there is a constant supply of seed from companies. More seed companies (East Africa Seeds and Seed Co.) observed the opportunities for business in other regions targeting hard-to-reach farmers.

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SITE-SPECIFIC SELECTION OF COMMON BEAN (*Phaseolus vulgaris* L.) CULTIVARS

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INTRODUCTION

Genotype x environment interaction is one of the main constraints in the plant breeding environment since its existence results in a decrease of genotypic performance when submitted to a given distinct location from where this genotype has been selected. In order to counteract the effects of this interaction, the common bean breeding research team of Embrapa Clima Temperado designed, in the early 90s, the Common Bean Demonstration Units System - SUDF (Villela et al., 2016), which is comprised of common bean cultivars released by Brazilian research institutions dealing with common bean breeding. Common bean production in Brazil is under temporal changes in area and volume as the commercial value of commodities changes and production systems acquire distinct components, also as an adaptation process to the market. In Rio Grande do Sul State, where common bean production is traditional since the early 1900's, the soybean production has had a significant increase in the last ten years, mainly due to the growth of the international market. As a result, in traditional growing areas, common bean has been replaced by soybean production and moved to the region where apple production has attained a significant increase in the last twenty years. This region, known as "Campos de Cima da Serra", is nowadays the main area of common bean production. Aiming to identify common bean cultivars suited to the environmental conditions found in this new region, Demonstration Units of beans that have been sown within this region have been analyzed in order to provide information to farmers on the most adapted cultivars. As checks, traditional cultivars under use by farmers have been used. This paper, which reviews the results from experiments conducted under 'Caxias do Sul' extension service supervision, aims to contribute to the selection by farmers of the best adapted cultivars among much of those presently available.

MATERIAL AND METHODS

The municipality of Caxias do Sul, located in the central hilly region of Rio Grande do Sul, with geographical coordinates 29.1634°S, 51.1797°W, is the headquarters of Emater's administrative region and comprises 49 municipal offices. Methodology follows the description by Villela et al. (2016), where the Demonstration Unities (UD) were composed of seventeen cultivars already recommended by research institutions located in southern Brazil, having as check the cultivars in use by farmers. From the 49 municipalities, 16 carried out 23 UDs. The testing period ranged from 1994/95 to 2006/07. UD's, for the most part, were installed in properties of farmers selected by Emater / RS employees. Statistical analysis involved the analysis of variance for the variable grain

yield and the Dunnett's test mean comparison, having the farmer's cultivar as a term of comparison. Twelve of the seventeen cultivars presented the required amount of data for statistical analysis.

RESULTS AND DISCUSSION

As shown in Table 1 of the twelve cultivars tested, two of them, Macotaço and Minuano, displayed significant yield differences in relationship to the farmers' cultivar, with 44.0 and 38.1 % yield advantage over the check, respectively. Both cultivars are black seeded, which is the color seed type preferred in Rio Grande do Sul State. An important characteristic associated with the cultivar Macotaço is its favorable performance when subjected to water stress. This testing concept has been developed from accumulated experience by farmers. As a solid conclusion, the SUDF has shown, as it was detected for the Soledade region (Villela et al., 2016), to be a valuable methodology for cultivar selection in different production areas, at a low cost.

Table 1. Mean seed yield (kg.ha⁻¹) and releasing year of SUDF cultivars in comparison to farmer's cultivar. Emater /RS' Caxias do Sul region, RS, Brazil.

Cultivar	Seed yield (kg.ha ⁻¹)	Release year
Farmer's cultivar (check)	2,037.7	-
Rio Tibagi	1,899.3	1976
Carioca+	2,528.7	1976
Guateian 6662	2,625.3	1979
Iraí+	1,847.0	1981
Macanudo	2,659.2	1989
FT 120	2,734.4	1989
Minuano	2,815.1*	1991
Iapar 31+	2,695.2	1994
Macotaço	2,938.9**	1994
Iapar 44	2,706.7	1994
Guapo Brilhante	2,438.2	1995
Pérola+	2,734.5	1999

*: Cultivar differs from the check by Dunnett's test at α =0.05; **: Cultivar differs from the check by Dunnett's test at α =0.01; +: Cultivar with no black seed coat.

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AGRONOMIC PERFORMANCE AND YIELD STABILITY OF COMMON BEAN IMPROVED LINES DEVELOPED IN DURANGO, MÉXICO

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INTRODUCTION: Yield variations registered over locations and years make selection difficult among common bean (*Phaseolus vulgaris*) breeding lines evaluated in multi-environment trials (Agyeman *et al.*, 2015). Several methods have been developed in order to improve efficiency in breeding line selection, including evaluation under irrigation and the use of the Additive Main Effects and Multiplicative Interaction Model (AMMI) (López *et al.*, 2011). The AMMI method is a multivariate model that combines variance and principal component analyses into one. This method requires few replications, its effectiveness increases with the size of the trial and a large number of lines can be evaluated without losing accuracy or increasing the cost of the experiments. The objective was to evaluate agronomic performance and yield stability in common bean breeding lines developed in Durango.

METHODS: Twelve common bean improved lines were planted at seven environments under irrigation in the state of Durango. Lines were sown from June 21th to July 20th using completely randomized design with two to five replications. Experimental plots consisted of five to eight rows, 50 m to 100 m in length and 0.81 m apart. Fertilizer was applied at the rate of 35-50-00 (N-P₂O₅-K₂O). In each growing cycle, irrigation was applied one to three times in order to avoid severe water stress in plants. At maturity, two to five plant samples were taken for yield determinations. Plant samples consisted of two rows 5 m in length by 0.81 m in width (8.1 m²). The analysis of variance was obtained using a completely randomized design with two to five replications. Mean comparisons were performed using Tukey's test ($p \le 0.05$). The parameters of stability were also calculated, using the AMMI model (Vargas and Crossa, 2000). Analysis of variance, means comparison and the parameters of stability in SAS® ver. 9.4 were used.

RESULTS: Highly significant differences (p < 0.01) were found for seed yield among environments, lines and their interactions, revealing differential response of breeding lines across environmental conditions. The Environment explained largely the observed variance (42 %), compared to Line x Environment interaction (28 %) and the Line effect (8 %). Breeding line response was strongly affected by planting sites, which showed differences for soil and weather conditions. The full-season improved lines (NGO14013 and NGO14014), which included parents from the Jalisco Race (Negro San Luis), showed high seed yield average (Table 1). High yielding lines were NGO14014 (3,184 kg ha⁻¹), NGO14013 (3,081 kg ha⁻¹), NGO14035 (3,039 kg ha⁻¹) in the opaque black group; while in the pinto class, higher seed yield was observed for PT14053 (3,013 kg ha⁻¹). Genetic advance, mainly related to interracial crosses, were observed for disease tolerance and seed yield in opaque black and pinto common bean lines developed in northern México. According to the AMMI model analysis, the first three principal components (PC) were significant and accumulated 76.9 % of the variance explanation, showing their importance for the representation of the Line x Environment interaction (Table 2). Environmental variability was higher (great dispersal) than genetic differences observed among the common bean lines included in the study (Figure 1). Environmental conditions registered in Durango 2015 helped to identify breeding lines adapted under stress (low rainfall, 227 mm, and several periods with high maximum temperature > 29 °C), while in Durango 2019 and 2018 high expression of the yield potential was detected in full-season lines due to the favorable meteorological conditions. The breeding lines

NGO14035 (cv. NOD 1) and PT14053 showed higher seed yield and displayed specific adaptation under irrigation in Durango. Lines with black opaque seeds, NGO14014 and NGO14013, registered wide adaptation mainly in high yielding environments, while PT14059 showed adaptation under restrictive conditions.

	U		U					
Line	A1	A2	A3	A4	A5	A6	A7	Mean
NGO14014	3,363	1,233	3,508	3,280	4,108	2,993	3,713	3,184ª
NGO14013	3,744	857	3,031	3,078	3,767	2,848	4,165	3,081 ªb
NGO14035	3,482	2,033	2,628	3,695	3,578	2,456	3,219	3,039 ^{ab}
PT14053	5,365	1,544	3,499	2,965	3,386	2,624	3,117	3,013 ^{abc}
PT14055	4,640	1,403	2,987	2,757	3,237	2,515	3,081	2,815 ^{abcd}
Mean	3,848	1,723	2,828	2,703	3,080	2,498	3,070	2,750

Table 1. High yielding common bean breeding lines evaluated in seven environments.

Environment: A1= Durango14, A2 = Durango15, A3 = Durango16, A4 = Durango17, A5 = Durango18, A6 = Nombre de Dios19 y A7 = Durango19.

Table 2. Results of the sum of squares in AMMI¹ terms.

				Variance Explanation (%)			
Source	² DFAMMI	SSAMMI	MSAMMI	Individual	Accumulated		
³ PC1	27	20.5	0.76**	27.7	27.7		
PC2	25	19.4	0.78**	26.3	53.9		
PC3	23	17.0	0.74**	23.0	76.9		
Error	252	49.5	0.19				
CV (%) = 16.1	$R^2 = 0.79$						

 $^{1}\overline{\text{AMMI}}$ = additive main effects and multiplicative interaction model; $^{2}\text{DFAMMI}$ = degrees of freedom; SSAMMI = sum of squares; MSAMMI = mean squares; ^{3}PC = principal component; CV = coefficient of variation. **= highly significant (p< 0.01).



Figure 1. Main effects and interaction of the environments with the average yields of common bean breeding lines. DGO = Durango, ND = Nombre de Dios; PT =pinto, NGO = opaque-black, FM = flor de mayo (pink).

CONCLUSIONS: Environmental conditions such as irrigation, temperature, accumulated rain, fertilization and pests control lead to higher yields in common bean germplasm. Breeding lines NGO14035 and PT14053 registered high agronomic performance, showing yield stability under the typical irrigation producing environments observed in Durango, México.

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2019 FINANCIAL STATEMENT BEAN IMPROVEMENT COOPERATIVE

BALANCE AS OF January 1, 2018

\$ 42,595.00

INCOME

	2019
2019 Dues	\$ 220.00
2020 and 2021 Dues prepaid	\$ 340.00
2019 BIC meeting sponsorship received	\$ 1,000.00
Back Issues	\$ 60.00
Bank Interest	\$ 175.00
TOTAL INCOME	\$ 1,795.00
EXPENSE	
Labor charges	\$ 425.00
Postage, Copy Charges and Office Supplies	\$ 695.00
Pdf & Book editing and publishing fees	\$ 681.00
PayPal Fees	\$ 24.00
2019 BIC Meeting Costs	\$ 12,548.00
Hotel down payment \$2000	
Student travel awards \$6000	
Technical Merit and Oral/Poster awards \$450	
Invited speaker costs \$1686	
Miscellaneous meeting costs paid out \$2412	
TOTAL EXPENSE	\$ 14,373.00

BALANCE AS OF December 31, 2019

\$ 30,017.00