

**Feed the Future Innovation Lab for
Collaborative Research on Grain Legumes
(Legume Innovation Lab)**

**FY 2016 Annual Project Technical Progress Report
(October 1, 2015 – September 30, 2016)**

Project Code and Title: S01.A4 -Development and implementation of robust molecular markers and genetic improvement of common and tepary beans to increase grain legume production in Central America and Haiti

Lead U.S. Principal Investigator and University:

James Beaver - University of Puerto Rico, Mayaguez, PR

Collaborating Host Country and U.S. PIs and Institutions:

Consuelo Estévez de Jensen - University of Puerto Rico, Mayagüez, PR

Timothy Porch - USDA/ARS/TARS, Mayaguez, PR

Phil Miklas - USDA/ARS, Prosser, WA

Juan Osorno and Phil McClean – North Dakota State University (NDSU), Fargo, ND

Juan Carlos Rosas - Escuela Agrícola Panamericana (Zamorano), Honduras

Julio Cesar Villatoro - Instituto de Ciencia y Tecnología Agrícola (ICTA), Guatemala

Emmanuel Prophete - National Seed Service, Ministry of Agriculture, Haiti

I. Abstract of Research and Capacity Strengthening Achievements

Conventional plant breeding techniques and marker-assisted selection were used to develop dry bean cultivars with enhanced levels of disease resistance and greater tolerance to abiotic stresses. During the past few years, the Bean Technology Dissemination project multiplied and distributed seed of improved bean cultivars developed by S01.A4 plant breeders to thousands of farmers in Central America and Haiti. The small red cultivar 'Paraisito Mejorado 2- Don Rey' with excellent seed type, disease resistance and abiotic stress tolerance was released in Honduras. Seed of multiple disease resistant black bean cultivar 'XRAV-40-4' and red mottled bean breeding line PR0737-1 was multiplied in Haiti. The BGYMV and BCMNV resistant black bean line MEN-2201-64ML from Zamorano had superior performance under drought conditions in Haiti, Nicaragua and El Salvador. The yellow bean germplasm line PR1146-138 with BGYMV, BCMV and leafhopper resistance was released. The bruchid, BCMV and BCMNV resistant red kidney germplasm AO-1012-29-3-3A was also formally released. Red mottled, cranberry and pinto bean

lines with BGYMV and BCMNV resistance are ready for testing in field trials. Black bean breeding lines that combine resistance to BGYMV, BCMNV and bruchids are also ready for field testing. Angular leaf spot isolates from Honduras and Puerto Rico were found to have high levels of virulence. Populations are being developed to identify a molecular marker for the *Bgp-1* gene that confers resistance to pod deformation in the presence of BGYMV. Novel sources of resistance to BCMNV in tepary bean were identified in a newly developed Tepary Diversity Panel (TDP). Tepary bean populations are under development to increase seed size, improve agronomic traits, and combine disease resistance (BCMNV, rust, common blight). Tepary adaptation trials have been conducted in Honduras, Nicaragua, El Salvador, Tanzania and Burkina Faso. Molecular markers of the *I* gene that confers resistance to BCMV and the *Ur-3* and *Ur-11* genes for resistance to rust have been developed and are being used for indirect selection for resistance. GWAS enabled fine mapping of the recessive *bc-1²* gene and discovery of the genomic location for the *bc-u* gene for resistance to BCMV and BCMNV. GWAS was also used to identify new genes and QTL for halo blight resistance on chromosomes Pv04, Pv05 and Pv10. When data from the alpha-amylase inhibitor (AAI) amplification was analyzed, it was discovered that the products differed by 45bp, and that the polymorphism was completely diagnostic between bean lines known to be susceptible and resistant to the common bean weevil. A workshop dealing with abiotic stress was offered to bean researchers from Central America and Haiti at the University of Puerto Rico. Héctor Martínez from Guatemala, Carl Didier Joseph from Haiti and Iveth Rodríguez from Honduras are pursuing M.S. degrees at the UPR and Carlos Maldonado from Guatemala and Lucy Lund from the U.S. are receiving M.S. degree training at NDSU. Several B.S. degree students have opportunities to work with the bean research program at Zamorano.

II. Project Problem Statement and Justification

Increased bean production during the past 30 years in Central America and Haiti has been due, in large part, to expansion of production in the lowlands (< 1000 m). Greater heat tolerance combined with resistance to BGYMV increased bean seed yield and production in El Salvador. Bean production in Guatemala and Nicaragua has expanded into more humid lowland regions whereas a significant portion of the beans in Haiti continues to be produced in the lowlands. Bean production in Africa could be expanded if lines with better lowland adaptation were developed. This Legume Innovation Laboratory project will address several of the biotic and abiotic constraints often encountered by bean producers in the tropical lowlands.

BCMNV threatens bean production in warmer bean production regions of Mexico, Central America, the Caribbean and Africa. The recent arrival of BCMNV in the Caribbean made the selection for resistance to this virus a priority breeding objective in Haiti, the Dominican Republic and Puerto Rico. Collaborative research supported by the Legume Innovation Laboratory has resulted in the development and release of black bean cultivars and breeding lines such as DPC-40, XR4V-40-4 and MEN-2201-64ML that combine resistance to BCMNV and BGYMV. Small red bean breeding lines with the same combination of resistances are currently being developed at Zamorano. These BGYMV and BCMNV resistant black and small red bean lines will be available in the event that BCMNV emerges as a threat to bean production in Central America. The availability of small red bean breeding lines with BCMNV resistance will permit the field testing of this seed type in Eastern Africa.

Small red and black beans tend to have greater yield potential and heat tolerance than Andean beans. Middle American beans also tend to have greater resistance to diseases in Africa, since pathogens in this region have co-evolved with Andean beans. Increased resistance to common bacterial blight and web blight is needed for beans produced in warm and humid lowland regions such as the Petén in Guatemala. This combination of resistances may also permit increased production of beans in Central America during the first growing season when rainfall is generally more abundant and reliable. The previous Dry Grain Pulse CRSP project (UPR-1) developed Middle American and Andean bean breeding lines having adaptation to the lowland tropics and different combinations of resistance to diseases (common bacterial blight, rust, angular leaf spot, web blight and root rot) and tolerance to edaphic constraints (low N soils, high temperature). During the past three years, the Legume Innovation Lab project has used these elite breeding lines as the base for the continued improvement of beans for our target countries. Several improved black and small red bean germplasm lines and cultivars are expected to be released in Central America and the Caribbean during the next year. This Legume Innovation Laboratory project will continue, in collaboration with CIAT, to support bean research network activities in Central America and the Caribbean. Collaborative activities such as the regional performance nurseries will help to extend the impact of this project through the release of improved cultivars throughout the region.

The project plans to release in Haiti red mottled, yellow and white bean cultivars with enhanced levels of disease resistance. These seed types are produced in regions in Haiti where the CRSP project has had less impact. This effort is consistent with the FtF 2011-2015 multi-year strategy in Haiti to increase the production of staples such as beans to increase food security. In a previous Pulse CRSP project, Dr. Phil Miklas developed Andean bean breeding lines with resistance to BCMNV and anthracnose that should be useful to Legume Innovation Lab breeding projects in Africa and the Caribbean. Yellow, red mottled and white bean breeding lines having BCMNV resistance will be available for Legume Innovation Lab or Feed the Future projects to test in Eastern Africa.

Andean bean breeding lines developed by Dr. Paul Kusolwa at Sokoine University of Agriculture have a unique combination of traits that confer a high level of resistance to bruchids. These breeding lines include the APA locus derived from *P. acutifolius* and possibly the null phaseolin trait from *P. coccineus*. These bruchid resistant breeding lines have been used as progenitors by the University of Puerto Rico bean breeding program to introgress this resistance into black, small red and white beans that also have resistance to BCMV, BCMNV and BGYMV. Evaluations have been conducted in Central America and the Caribbean to measure the durability of the resistance when exposed to different genera and ecotypes of bruchids.

The project continues to screen germplasm to identify additional sources of resistance to diseases that limit bean production in Central America and the Caribbean. For example, more resistance to ashy stem blight, caused by *Macrophomina phaseolina*, is needed to improve adaptation to hot and dry environments such as the dry corridor in Guatemala and southwestern Haiti. Ninety three genotypes from the BASE 120 trial were screened against a *Macrophomina phaseolina* isolate from Juana Diaz, Puerto Rico (Mp-JD). Six of the 93 genotypes evaluated had low disease

severity. Greater resistance to web blight, caused by *Rhizoctonia solani*, is required to increase yield and seed quality of beans produced in more humid environments such as the Petén Department in Guatemala and eastern Nicaragua. Project personnel have the expertise and experience needed to reliably phenotype the Andean and Middle American Diversity Panels for traits of economic importance. This should contribute to the identification of new sources of resistance.

There are regions and/or growing seasons in Central America, Haiti and Africa that are too hot and/or dry to produce common beans. The tepary bean (*P. acutifolius*) is a potential alternative grain legume for these stressful environments. In fact, farmers on the Pacific coast of Central America and some countries of Africa already produce tepary beans on a limited scale. In addition to heat and drought tolerance, tepary bean lines with resistance to common bacterial blight, root rots, BCMV, bruchids and other important traits, such as tolerance to low soil fertility, have been identified. Resistance to BCMV, BGYMV, larger seed size and improved agronomic traits, would increase the potential adoption of tepary bean. In addition to pyramiding these traits within tepary, interspecific crosses with common bean are being used as a long-term effort to introgress these traits into tepary bean. This effort represents the first systematic attempt to genetically improve cultivated tepary bean.

Bean breeders were early adopters of marker-assisted selection to identify lines with desired combinations of traits. This resulted in increased efficiency in the development of improved breeding lines. There are, however, molecular markers available for a limited number of traits. Others, such as the SAP6 SCAR marker, are only effective in a specific gene pool. Therefore, there is a need to develop new or more robust markers, particularly for traits of economic importance to bean breeding programs in the tropics. Recent advances by the BeanCAP project, led by North Dakota State University, in sequencing the bean genome and the development of a SNP array, will facilitate the mapping and development of molecular markers for traits of economic importance, while breeder-friendly InDel markers are a broadly applicable technology. The availability of phenotypic data in appropriate populations is a major factor limiting the development of these markers. This Legume Innovation Lab will assist this effort through the development of the populations and information needed to identify improved markers for traits such as the *Ur-11* gene for rust resistance and the *Bgp-1* gene for resistance to pod deformation in the presence of GYMV. Dr. Phil McClean and Dr. Phil Miklas have lead the collaborative effort to develop improved molecular markers.

Differences and linkages between S01.A4 and other Feed the Future (FtF) Projects

- The focus of the S01.A4 project is on biotic constraints and abiotic constraints in the tropical lowlands. Successful bean cultivars need resistance/tolerance to both types of constraints. Other FtF projects are focused on beans in the highlands of Africa and Guatemala. Given the expected trends in climate change, breeding beans for adaptation to the lowlands may help to identify bean germplasm with improved adaptation to future highland environments.
- The focus of the S01.A4 project is on Latin America/Caribbean vs. Africa. However, exchange of breeding lines among FtF projects is mutually beneficial.

- Genomic research, development of molecular markers and the sharing of breeding strategies and breeding populations are common links among projects.

III. Technical Research Progress

Objective 1. Genetic improvement of common and tepary beans for Central America and Haiti.

Development, testing and release of improved bean cultivars

Conventional plant breeding techniques and marker-assisted selection has been used by Legume Innovation Lab scientists to develop common bean cultivars and breeding lines with enhanced levels of disease resistance and greater tolerance to abiotic stresses. Plant breeders have focused on the most important biotic and abiotic constraints in lowland (< 1000 m) bean production regions in Central America and Haiti. The bean research program at Zamorano has coordinated the regional testing of small red and black bean breeding lines. The University of Puerto Rico has coordinated the development and testing of Andean beans in the Caribbean. These trials have been conducted in collaboration with national bean research programs and CIAT. Promising lines have been tested throughout Central America and the Caribbean, including countries that are not participating in this Legume Innovation Lab project. Testing lines in different countries provides more information concerning the potential performance of the lines and expands the potential impact of the research supported by the Legume Innovation Lab. In addition to yield trials, field trials have been conducted to screen bean lines for resistance to different diseases such as angular leaf spot, powdery mildew, ashy stem blight, web blight, efficiency for biological nitrogen fixation and high temperatures. Testing sites have been chosen to produce reliable results for screening for specific traits.

In collaboration with Bioversity International and CATIE, more than 2000 trials using the methodology of “massive participatory evaluation” were evaluated by farmers in the Trifinio region (borders of Guatemala, El Salvador and Honduras) which is part of the Central American “dry corridor”. Another 1000 trials were conducted in the departments of Matagalpa and Jinotepe in Nicaragua. The small red and black lines included in these trials were developed by the project in collaboration with national bean research institutions.

Table 1. Bean trials distributed to Central American and Caribbean Bean Research Network collaborators during 2015-2016.

Trial name	Small red	Small black	Countries
Regional bean adaptation nursery (VIDAC)	42 entries + 2 checks	55 entries + 2 checks	7
Regional yield and adaptation trial (ECAR)	14 entries + 2 checks	14 entries + 2 checks	7
Regional Rojo de Seda Nursery (VIROS)	22 entries + 3 checks	--	3
Bean variety validation trial (COVA)	9 entries + 1 check	9 entries + 1 check	4
Regional angular leaf spot trial (ERMAN)	10 entries + 2 checks		5
Regional web blight trial (ERMUS)	14 entries + 2 checks		5
Biofortified bean trial (AGROSALUD)	8 entries + 2 checks		6
Regional BNF trial (ERFBN)	8 entries + 2 checks		3
Regional high temperature trial (ERSAT)	20 entries + 4 checks		6
Regional drought trial (ERSEQ)	22 entries + 2 checks		6
Regional low fertility trial (ERBAF)	22 entries + 2 checks		5

Greater tolerance to abiotic stress

Although disease resistance is the primary focus of this Legume Innovation Lab project, the performance of bean breeding lines is evaluated in low fertility soils. Honduras has an ideal site for the evaluation of lines for adaptation to low P soils. Puerto Rico has good locations for screening beans for performance in a low N soil, root rot resistance and high temperature. These sites were used to evaluate the performance of bean breeding lines derived from recurrent selection for increased BNF and/or selected for greater nitrogen use efficiency. These sites were inoculated with efficient *Rhizobium* strains to allow indirect selection for enhanced BNF. Several S01.A4 Co-Principal Investigators also participate in the Feed the Future Innovation Lab for Climate Resilient Beans. This extends the range of environments that promising bean lines can be screened for adaptation to abiotic stress. Results from the BASE 120 trials conducted at Isabela, Puerto Rico identified the black bean cultivar XRAV-40-4 to have among the best root nodulation scores.

Bruchid resistance

Red kidney bean breeding lines developed by Dr. Paul Kusolwa (Sokoine University of Agriculture) and Dr. Jim Myers (Oregon State University) were screened in Puerto Rico for bruchid and virus (BCMV and BCMNV) resistance. One of the bruchid and virus resistant lines, AO-1012-29-3-3A, was formally released (Kusolwa et al., 2016). Dr. Kusolwa has used AO-1012-29-3-3A as a progenitor to transfer BCMV and BCMNV ($I + bc-1^2$) resistance into breeding lines for Tanzania. The same line has been used to introgress resistance to bruchids into different commercial seed types (black, small red, red mottled, light red kidney). A laboratory screening technique developed at the University of Puerto Rico was used to screen the RILs for bruchid resistance. A QTL analysis will be used at NDSU to explore the genetic basis of resistance to weevil derived from a cross with AO-1012-29-3. The effectiveness of using molecular markers for traits (null phaseolin, arcelin 2 and APA locus) associated with bruchid resistance is also under evaluation. During the past year, Dr. Rosas conducted a seed increase in Honduras of two black bean breeding lines that combine bruchid and virus resistance. There should be sufficient seed to conduct on-farm trials during the second growing season of 2016 to test the effectiveness of bruchid resistance with seed storage methods normally utilized by farmers.

Evaluation of bean diversity panels and identification of new sources of disease resistance

The Middle American (MDP) and Andean Diversity (ADP) panels have been screened in Central America and the Caribbean for specific traits. For example, the Andean Diversity Panel will be screened in Haiti for reaction to powdery mildew and it was screened in Honduras and Puerto Rico for angular leaf spot. Performance of the Middle American Diversity Panel has been evaluated in low N environments in Central America and in Puerto Rico in 2016. The Middle American Diversity Panel has been screened in Puerto Rico for resistance to ashy stem blight. A greenhouse

trial was planted in Juana Diaz, PR, to evaluate the reaction of the trial BASE 120 to the *Macrophomina phaseolina* isolate Mph-JD2. A suspension of inoculum of the pathogen was applied at the base of the bean plants. Disease severity was assessed at maturity based on the CIAT (1-9) scale and stem microsclerotia colonization. Lines that showed resistance to Mph-JD2 were: BAT 477, BIOF 2-106, SB-754, SER 78, SXB-405, SJC 730-79.

Genetic Improvement of Tepary Beans

Although tepary bean has high levels of abiotic stress tolerance it is susceptible to viruses such as BGYMV, BCMV, and BCMNV. In order to expand the potential use of tepary bean in abiotic stress prone regions, a primary focus of this project has been to incorporate newly identified resistance in tepary accessions into the ARS-TARS tepary breeding program, and to initiate the introgression of virus resistance from common bean into tepary bean. Advanced breeding lines developed from these and previous breeding efforts have been increased and shared with the collaborators for testing in Tepary Adaptation Trials (TAT). New tepary breeding lines have been generated from crosses between promising large and round seeded genotypes from the Tepary Diversity Panel (TDP) and breeding lines selected for disease and abiotic stress tolerance. These breeding lines were initially tested through a shuttle breeding program with M. Brick at Colorado State University. This effort has focused on seed size/shape, drought and heat tolerance, and CBB and bruchid resistance in PR; and on photoperiod insensitivity, broad adaptation, rust resistance, and yield in Colorado. TDP accessions with higher efficiency for biological nitrogen fixation (BNF) were also identified in the thesis work of Ana Vargas. In 2016, over 10 accessions were identified in the Tepary Diversity Panel (TDP) with resistance to the NL3 strain of BCMNV. These 10 accessions are being tested against strains representing different pathogroups of BCMV and BCMNV at Prosser, WA to evaluate for broad resistance. Previously, TDP lines with tolerance to BGYMV and to ALS were identified in Honduras. Resistance to these diseases and BNF efficiency is being pyramided in the breeding program through a separate approach involving the generation of bulk breeding populations (bulked up to the F4 generation). These bulk breeding populations are currently undergoing selection of individual plants under both biotic and abiotic stress in Honduras and Puerto Rico in 2016. By project end (FY17) we expect to have tepary breeding lines with disease resistance loci for further pyramiding in future efforts. Superior lines have been included in the TAT and tested in the host countries including Burkina Faso, Tanzania, Angola, Honduras, Haiti, and the U.S. for potential future release. Through collaboration with the FtF-ARS Legumes Project, the TDP has been genotyped using GBS, the diversity of tepary analyzed, and the nutritional composition of tepary compared to common bean in a recent publication, thus providing valuable information for current and future breeding efforts.

Objective 2. Develop and implement robust molecular markers for disease resistance genes

This project has leveraged results from the USDA Common Bean Agricultural Project and the USDA/DOE/JGI common bean sequencing project. The BeanCAP project developed a suite of ~3000 InDel markers distributed across all common bean chromosomes. These markers are codominant and designed to be functional in labs with a simple set of equipment and reagents (Thermal Cycler, gel chambers, and UV lamp).

The power of these markers is that they are simple to implement and thus completely portable in all laboratories and are amenable to multiplexing with suites of markers. Multiplexing reduces the cost of genotyping an individual line. The release of the common bean whole genome assembled sequence allows for precise localization of each of these markers. The final key element that facilitates this project is the development, over the last fifteen years, of markers (mostly SCARS) that are linked, from 0-5 cM, to important target disease genes. While useful, there has been some difficulty in the portability of these markers from one laboratory to another. SCAR markers all have unique experimental conditions that preclude multiplexing, and > 5% recombination reduces effectiveness due to recombination between marker and target gene. In addition, these SCAR markers don't work across different market classes or genetic backgrounds. Contrastingly, most InDel markers developed at NDSU are market class specific, which will facilitate their use and increase their reliability.

Identify genetic materials for marker evaluation

Potential targets for improved marker development include:

- Bean golden yellow mosaic virus resistance genes and QTL (*bgm*, SW12, *Bgp*)
- Bruchid resistance genes (*Arc2*, *Arl3*, *PHA* and *aAl3*)
- BCMV and BCMNV (*I*, *bc-3*, *bc-1²*)
- Bean rust (*Ur-3*, *Ur-4*, *Ur-5*, *Ur-11*)
- Common bacterial blight (*SAP-6*, Xa11.4, Pv07-QTL)

For each of these targets, we will adopt the same procedure. First, we will search the published literature and communicate personally with breeders, geneticists, and pathologists in both Legume Innovation Lab projects to identify genetic materials with contrasting phenotypes (resistance, susceptibility) for the specific disease. These could be genetic populations or a collection of lines with known phenotype that can then be used for the identification of closely linked indel markers. Project personnel have already participated in the evaluation of the Middle American and Andean Diversity Panels for reaction to several different diseases.

Development of InDel markers

- DNA will be isolated from genetic populations or collections of lines with known phenotypes.
- The physical locations of target genes or markers will be identified using sequence information. If the sequence information is poor or unavailable, the specific marker will be cloned and sequenced.

- InDel marker selection: Once the location of the marker is determined, it will then be compared to the InDel database to discover InDel markers that straddle the physical location of the marker. Those InDel markers will be used in PCR amplification to determine which one acts as a definitive marker that is unambiguous in its predictive power. If several markers have equal predictive power, then the one that will best work as a multiplexing marker will be selected. Legume Innovation Lab bean breeding programs in Guatemala, Honduras, Ecuador, Tanzania and Uganda have the facilities and technical expertise needed to immediately adopt the use of InDels for marker-assisted selection.

Objective 3. Institutional capacity building

Formal and informal training activities have been conducted to enhance the capacity of host country bean research programs to develop and release superior-performing bean cultivars that increase production or reduce losses in the target countries. At the end of this project, these bean research programs should have the capacity to utilize the newly-developed suite of InDel markers for marker-assisted selection. The Ph.D. and M.S. degree students will be provided a broad range of training in conventional and molecular plant breeding techniques so that they can assume roles of leadership in bean research programs in the target countries. Informal training of technicians should improve the reliability and quality of bean research conducted in host countries.

Informal training

- In-service training was provided during FY16 at NDSU for Legume Innovation Laboratory scientists and graduate students to review recent advances in sequencing the bean genome and the utilization of SNP arrays to develop InDel markers for traits of economic importance.
- A workshop was held in Puerto Rico in August 2016 to train technical personnel from Central America and the Caribbean concerning bean research related to abiotic stress. Topics included research related to breeding for greater resistance to drought and high temperature and greater tolerance to soils having low soil fertility. Participants had the opportunity to observe research plots in Isabela and Juana Diaz Substations and evaluate genotypes grown in sterile sand for nodulation in selected for early nodulation. This workshop was conducted in collaboration with the Feed the Future (FtF) Innovation Lab for Climate Resilient Beans and the FtF USDA-ARS Bean Research Team. The workshop presentations will be posted on the FtF USDA-ARS Bean Research Team Web Site.
- A significant amount of information concerning bean research techniques is already available on the Bean Improvement Cooperative (BIC) web site <http://bic.css.msu.edu/ResearchTechniques.cfm>. This Legume Innovation Lab project will collaborate with the BIC in developing modules for the BIC web site that will describe research techniques for additional traits such as bruchid resistance.

IV. Major Achievements

Development, testing and release of improved bean cultivars

- The BGYMV, BCMV and BCMNV resistant black bean line MEN2201-64ML was released in Honduras as 'Lenca Precoz'. This early maturity line has also performed well in drought-prone regions of Haiti. During the upcoming year, seed of MEN2201-64ML will be distributed to farmers in Central America and Haiti.
- XRAV-40-4, a multiple disease resistant black bean adapted to the humid tropics, was developed and released cooperatively by the Puerto Rico (UPR) and Nebraska (UNL) Agricultural Experiment Stations, the USDA-ARS, the Instituto Dominicano de Investigaciones Agropecuarias y Forestales (IDIAF), the Escuela Agrícola Panamericana, Zamorano, Honduras, and the National Seed Service of the Ministry of Agriculture of the Republic of Haiti. 'XRAV-40-4' combines resistance to BGYMV, BCMV, and BCMNV. When planted at higher altitudes, XRAV-40-4 has earlier maturity than the black bean cultivar 'DPC-40'. A description of the release of XRAV-40 was published in the *J. of Agric. of the Univ. of Puerto Rico* (Beaver *et al.*, 2014. *JAUPR*. 98:83-87). XRAV-40-4 was released in Central America as 'Azabache 40' and in Haiti as 'Sankara'. XRAV-40-4 performed well in on-farm trials conducted by the NSS with support from FAO. Phil Miklas found XRAV-40-4 to be well adapted to the Western U.S. bean production region and may be a candidate for release as an early maturity cultivar. There is interest among Western U.S. seed producers to market high-quality bean seed in Central America and the Caribbean.
- The small red line MIB 397-72 was released in Honduras as the biofortified bean cultivar "Honduras Nutritivo". The original MIB 397 line was developed at CIAT and tested in Central America as part of the regional project Agrosalud. The line MIB 397-72 is a selection for resistance to BGYMV using field testing and SCAR markers. This cultivar contains 25% more iron than traditional small red cultivars.
- The small red line RS 901-6 developed by Zamorano was released in collaboration with INTA in Nicaragua as the cultivar "INTA Jinotega". This cultivar was selected for resistance to BGYMV, earliness and "Rojo de Seda" commercial grain type.
- White bean lines were released that combine the *bgm-1* gene and the SW12 QTL for resistance to BGYMV, the *I* and *bc-3* genes for resistance to BCMV and BCMNV and resistance to a wide range of rust races. Results from inoculations with specific races of rust conducted by Dr. Pastor-Corrales, USDA-ARS-Beltsville suggest that the white bean lines have a unique combination of the *Ur-4*, *Ur-5* and *Ur-11* rust resistance genes. A manuscript describing the release of these bean breeding lines as germplasm releases was published in the *J. Plant Registrations* (Beaver *et al.*, 2015. *J. Plant Reg.* 9:208-211). This was the first release of improved bean germplasm that combines multiple virus (BGYMV, BCMNV and BCMV) and rust resistance. An elite white bean regional nursery will be distributed by Dr. Juan Carlos Rosas to collaborators in Central America and the Caribbean.
- Rust resistant white bean breeding lines were used as parents to introgress high levels of rust resistance into black beans. DPC-40 and XRAV-40-4 were used as parents to ensure that progeny from these crosses will also have multiple virus resistance. F₅ black bean lines with good agronomic type were selected from these populations. These lines were screened during second growing season of 2015 at Zamorano for resistance to rust. Previous research has found rust races in Honduras to have high levels of virulence. Dr. Pastor-Corrales (USDA FtF project

collaborator) will conduct greenhouse evaluations of lines that are rust resistant in field trials in Honduras. This effort should lead to the development of black bean lines that combine multiple virus resistance and the *Ur-4*, *Ur-5* and *Ur-11* rust resistance genes. The most promising lines will be included as entries in regional performance trials for Central America and the Caribbean.

- Yellow bean lines that combine the *bgm-1* gene for resistance to BGYMV and the *I* gene for resistance to BCMV were developed and tested in Puerto Rico, Angola and Haiti. PR1146-138, also expressed tolerance to leafhoppers in a trial planted in Damien, Haiti in 2014 and produced a mean seed yield of 1,884 kg/ha over seven environments. A manuscript describing the release of PR1146-138 was published in the *J. Plant Reg* (Beaver et al., 2016). Yellow bean breeding lines that combine resistance to BGYMV, BCMV and BCMNV have been developed
- An advanced generation yellow bean breeding line, PR1501-162 with good agronomic traits and commercial seed was screened using molecular markers and found to combine the *bgm-1* allele and the SW12 QTL for resistance to BGYMV, the *I* allele that confers resistance to BCMV and the SAP6 QTL for resistance to common bacterial blight. The performance of this line will be evaluated in Haiti and Puerto Rico during the upcoming year.
- Pinto beans gained popularity in Haiti after this market class was imported as food aid. Consumers note that pinto beans have a shorter cooking time than other seed types used in Haiti. During the past year, we multiplied seed of advanced generation lines that have the *bgm-1* allele and the SW12 QTL for BGYMV resistance and the *I* and *bc-3* alleles for resistance to BCMV and BCMNV. All of the lines have commercial pinto seed type and many have an erect growth habit. These lines were evaluated in trials in Haiti, Honduras and Puerto Rico. These pinto lines should segregate for the *Ur-11* gene so special attention will be given to lines that do not develop rust symptoms. Because Durango race beans have performed well in Eastern Africa, the pinto lines were sent to Dr. Phil Miklas for evaluation in Tanzania. The pinto lines had high levels of disease resistance and good seed yield potential when tested in Mbeya, Tanzania.
- The performance of red mottled and cranberry bean lines with commercial seed type and resistance to BGYMV, BCMV and BCMNV will be evaluated in Puerto Rico and Haiti during the upcoming year. Seed of these and other promising lines from the UPR were sent to Mr. Isaac Dramadri for evaluation in Uganda and Dr. Kelvin Kamfwa for evaluation in Zambia. Miklas sent a BCMNV resistant Kablanketi breeding line to Dr. Kamfwa in Zambia.
- Advanced generation black bean lines from Puerto Rico that were identified to possess the *bgm-1* gene for resistance to BGYMV and the *I* and *bc-3* genes for resistance to BCMV and BCMNV were tested in Haiti, Guatemala and Puerto Rico. Many of these lines have progenitors with heat tolerance and resistance to common bacterial blight and web blight. These lines expressed less damage from leafhoppers in Damien, Haiti and Jutiapa, Guatemala although many bean lines in neighboring trials were severely damaged. Black bean lines with resistance to BGYMV, BCMV, BCMNV and rust were selected for in San Jerónimo, Guatemala by ICTA researchers.
- 'Beseba' is a BGYMV resistant Haitian Lima bean from a collection made by the Dry Grain Pulse CRSP and sent to CIAT to be included in the germplasm collection. Dr. Rao, CIAT Physiologist, reported at the 2014 PCCMCA meeting in Nicaragua that 'Beseba' (G 27529) produced the greatest seed yield in a high temperature trial conducted in Colombia. Emmalea Ernest reported at the 2014 BIC meeting that G 27529 had among the greatest amount of pollen shed in a high temperature trial conducted in Delaware (BIC 57:41-42). Heat tolerant Lima beans

should be tested as an alternate crop for the dry corridors of Honduras, El Salvador and Guatemala. The CIAT Lima bean germplasm collection should be screened for reaction to BGYMV to attempt to identify additional sources of resistance.

- IICA personnel reported that the black bean variety ICTAZAM, that has a shiny seed coat, is acceptable to consumers in Guatemala. ICTAZAM has resistance to BGYMV, BCMV and web blight and was identified by ICTA researchers to have superior performance in more humid regions such as the Petén region of Guatemala.
- The virulence patterns of *Pseudocercospora griseola* isolates from Honduras and Puerto Rico were studied. One isolate from Honduras was virulent to all of the ALS differential lines (race 63-63). The virulence of *Phaeoisariopsis griseola* isolate ALS-9029-JD2 from Juana Diaz, PR and isolate ALS-900-ISAD from Isabela, PR was determined by inoculating 76 bean lines of the BASE 120 trial in two different screenhouse trials. The only bean genotypes resistant to both isolates were G-21212 and SER 125 with disease severity scores below 3 and no synnemata emerging after 24 hours of leaf incubation under humid chamber conditions. Moderate resistant lines to the same isolate were ALS 0532-6, TARS HT-1, TARS MST-1, BNF 1205-31, RCB 593, SEF 14, SEF 15, PR1418-15, PR0806-81, NCB 280, SER 16, FNB 1210-48, SER 118, PR1165-3, SB 747, SB 757 and BFS 29. Important sources of resistance for the two isolates from Juana Diaz and Isabela in Puerto Rico were identified.

Greater tolerance to abiotic stress

- INTA and CENTA researchers reported that the black bean line MEN-2201-64ML from Zamorano had superior performance under drought conditions in Nicaragua and El Salvador. This line was also selected for further evaluation in Haiti based on its performance during the dry season. In addition to drought tolerance, MEN-2201-64ML was selected for resistance to BCMV, BCMNV and BGYMV. During the upcoming year, this Legume Innovation Lab project will support the on-farm testing of MEN-2201-64ML in Haiti and Guatemala and other Central American countries where drought is a frequent constraint to bean production. The NSS in Haiti multiplied seed of this line during the summer of 2015 to distribute to farmer groups in November. This line has been recently released in Honduras as the cultivar 'Lenca Precoz'.
- The small red bean breeding line IBC-301-204, selected at Zamorano for resistance to BGYMV, BCMV and tolerance to low fertility, was released in Nicaragua as 'INTA Centro Sur'.
- The small red bean breeding line RS 901-6, selected at Zamorano for earliness, resistance to BGYMV, BCMV and tolerance to drought and the highly preferred "Rojo de Seda" commercial grain type, was recently released in Nicaragua as the cultivar 'Rojo Jinotega'.
- The small red bean breeding line SJC 730-79, selected at Zamorano for resistance to BGYMV, BCMV and tolerance to drought and high temperatures was released by CENTA in El Salvador.
- Small red and black breeding lines were selected at Zamorano from the second cycle of recurrent selection having greater nodulation, plant growth, seed yield, and resistance to BCMV and BGYMV. The most promising lines were distributed to collaborators in Central America for field evaluation in diverse conditions. Greater nodulation in the low N field (0.08 % N), soil: sand benches (0.06% N) and plastic pouches (nodulation speed) was obtained with *Rhizobium tropici* (CIAT 899) and *R. etli* (CIAT 632) strains.

- Many of the best performing entries in BASE 120 trials conducted in low N soils at Isabela, Puerto Rico are breeding lines and cultivars developed by S01.A4 project breeding program. Most of these lines also possess resistance to BGYMV and BCMV.

Bruchid resistance

- Rojo' backcross lines selected in collaboration with Paul Kulsolwa at Sokoine University and Jim Myers at Oregon State University combine resistance to bruchids [*Acanthoscelides obtectus* (Say)] and the *I* and *bc-1²* genes that confer resistance to BCMV and BCMNV. AO-1012-29-3-3A yielded as well as USLK-1 and 'Badillo' in trials conducted in Puerto Rico. This breeding was released as improved germplasm (Kusolwa et al., 2016).
- The 'Rojo' backcross lines were used as parents to develop Andean and Middle American lines with bruchid resistance. Black, red and white bean lines were developed that combine resistance to bruchids, the *bgm-1* gene for resistance to BGYMV and the *I* and *bc-3* genes for resistance to BCMV and BCMNV. Seed of bruchid resistant black bean lines were sent to Honduras, Guatemala and Haiti where they have been evaluated for adaptation and for resistance to local eco-types of bruchids. A Zamorano student evaluated lines selected in Puerto Rico for bruchid resistance. The lines were infested with a Honduran ecotype of *Zabrotes subfasciatus* (the Mexican bean weevil) and found to be resistant. ICTA researchers confirmed the resistance of lines from Puerto Rico after screening with two ecotypes of *A. obtectus* and one ecotype of *Zabrotes subfasciatus* from Guatemala. These results help to confirm that the bruchid resistance will be effective over a broad geographical region and will suppress the development of both of the genera of bruchids that can cause major post-harvest losses in Central America and the Caribbean.
- Many lines selected for bruchid resistance were found to possess the molecular markers developed by Dr. Paul Kusolwa for alpha amylase and phyto-haemagglutinin. These markers, however, did not account for all of the phenotypic variability associated with the bruchid resistance. One of the parents of the 'Rojo' backcross lines has the recessive null phaseolin allele. We are exploring, in collaboration with the USDA-ARS FtF project and Dr. Jim Myers, the possibility that the null phaseolin trait may contribute to bruchid resistance.
- The low frequency of F₄ breeding lines with high levels of resistance to weevils (< 10%) in several different populations suggests that, in addition to the complex APA locus, there are other genetic factors associated with resistance to the common bean weevil. The UPR obtained from Dr. Kelvin Kamfwa a recombinant inbred line (RIL) population that segregated for resistance to bruchids. The RILs are derived from the cross between 'Solwezi', a bean landrace variety from Zambia, and AO-1012-29-3, a breeding line with resistance to common bean weevil. During the past year, we phenotyped the RILs for reaction to bruchids. Dr. Kamfwa conducted a QTL analysis for bruchid resistance and found significant QTL peaks on chromosomes Pv04 and Pv10.
- The ICTA bean research program has generated several populations from crosses between sources of resistance to bruchid and major diseases. Seed of two bruchid resistant black beans has been increased at Zamorano. This seed will be used to evaluate the bruchid resistance of these lines using seed storage practices of Honduran farmers.

Evaluation of bean diversity panels and identification of new sources of disease resistance

- Project personnel were co-authors in a paper published in *Crop Science* entitled “A *Phaseolus vulgaris* Diversity Panel for Andean Bean Improvement” (Cichy et al. 2015, *Crop Sci.* 55: 2149-2160). Legume Innovation Lab project S01.A4 contributed lines to the ADP and collaborated in the evaluation of the ADP for several traits of economic importance.
- The Andean Diversity Panel was screened in the greenhouse at the UPR for resistance to powdery mildew. Although none of the Andean lines were immune to powdery mildew, we did observe differences among lines in severity of infection. We made individual plant selections at the Isabela Substation in an Andean bean population that appeared to segregate for resistance to powdery mildew. The lines will be screened in the greenhouse during the upcoming year for reaction to the disease.
- The virulence patterns of *Pseudocercospora griseola* isolates from Honduras and Puerto Rico were studied. One isolate from Honduras was virulent to all of the ALS differential lines (race 63-63). The most virulent isolate from Juana Díaz Puerto Rico was race 63:39. This high level of virulence points to the need to pyramid genes for ALS resistance. Molecular markers SH-13 (*Phg-1*), SN02 (*Phg-2*) and E-ACA/M-CTT330 (G10474 dominant gene) were used at Zamorano for marker-assisted selection. Resistance in the field was confirmed using highly virulent races of the ALS pathogen. During the upcoming year, a small red bean line (ALS 0532-6) and a black bean line (ALS 0546-60) that combine resistance to multiple viruses (BGYMV and BCMV), high yield potential and commercially acceptable seed type will be evaluated in validation trials in Central America. ALS resistant white bean breeding lines were developed for Puerto Rico using sources of resistance from Zamorano.
- The National Seed Service in Haiti plans to screen in the field the Andean Diversity Panel for reaction to powdery mildew. Results will be used to conduct an associated mapping analysis for reaction to *Erysiphe polygoni* in the Andean gene pool.
- In collaboration with the USDA-ARS FtF project, association mapping of the response to *Macrophomina phaseolina* in the Andean Diversity Panel was conducted. Results from field screening identified a small group of lines in the ADP with resistance to ashy stem blight. Regions on Pv03, Pv09, and Pv11 were significant for charcoal rot resistance in the association mapping analysis.
- Isolates from a root rot nursery in Isabela were identified as *Rhizoctonia solani* (Rs), *Fusarium solani* (Fs) and *Pythium ultimum* (Pu). Inoculation tests were conducted with each fungal pathogen. Interspecific line INB 835 and TARS-LFR1 were found to be resistant to Rs. ADP 518, ADP 508 and ADP 475 were the Andean bean lines with the highest levels of resistance to Rs. Tepary beans inoculated with Fs did not develop symptoms. In contrast, common beans showed reddish lesions on the hypocotyl and browning of the tap root. The lines ADP 475, ADP 518, ADP 269 and LFR-1 had only small reddish lesions on the hypocotyl (disease scores ≤ 3). These results suggest that Rs and Fs independently produced hypocotyl and root rots.
- The Mesoamerican Diversity Panel (MDP) and a set of the Andean Diversity Panel (ADP), resistant to ALS in South Africa, were evaluated for their response to ALS in a trial at Zamorano, HN in collaboration with the FtF-USDA project. Resistant genotypes were identified for breeding efforts in both panels.

- In an attempt to identify a set of differential genotypes for *Macrophomina phaseolina* (Mp), a screenhouse trial was established to evaluate the response of common bean, tepary and interspecific (common bean x tepary bean) bean lines to Mp isolate Mph-01-JD. TARS-MST1, DOR 364 and BAT 477 were resistant to the isolate.
- The Haitian landrace lima bean variety 'Beseba' expressed a high level of resistance to BGYMV in trials conducted at Zamorano. This allows this lima bean to be planted near common bean without risking the spread of BGYMV.

Genetic Improvement of Tepary Beans

- Tepary bean breeding lines are being developed that should combine virus resistance with superior agronomic and seed traits and resistance to other diseases such as common bacterial blight and rust.
- In collaboration with the USDA-ARS FtF project, UPR graduate student Ana Vargas identified tepary bean accessions from the TDP that show necrotic and resistant reactions when inoculated with the NL-3 isolate of BCMNV. This resistant reaction was confirmed with ELISA for over 10 genotypes. BCMV and BCMNV are currently major constraints to tepary bean production.
- Recombinant Inbred Line (RIL) populations were developed and evaluated to identify genes and molecular markers for BCMV resistance. One of these RIL populations showing a necrotic response to NL3 inoculation is being genotyped using Genotyping-by-sequencing (GBS), phenotyped using visual scoring and ELISA, and QTL analysis will subsequently be completed.
- Putative early generation interspecific lines for combining BGYMV and BCMNV resistance from common bean with tepary bean have been developed and are being advanced for the evaluation of effective hybridization and for virus resistance.
- Tepary adaptation trials have been conducted in Honduras, Nicaragua, El Salvador, Tanzania and Burkina Faso. A set of eight tepary lines will be evaluated in Guatemala "dry corridor" by 100 farmers in collaboration with Bioversity International and CATIE under the methodology massive participatory evaluation.
- Through the USDA-FtF project and a USDA Postdoc, the Tepary Diversity Panel (TDP) composed of 314 accessions was developed and genotyped with SNP markers using GBS. This represents the first comprehensive genotyping of all currently available tepary accessions between the USDA and CIAT collections. This panel has been evaluated for a number of different traits including agronomic traits in the field under abiotic stress, and CBB, BNF, and response to NL3 inoculation. QTL on chromosomes 1 and 11 of tepary bean (using common bean as the reference genome) were found for resistance to BCMNV.
- In collaboration with USDA-ARS FtF project, 12 Interspecific hybrid *Phaseolus acutifolius/Phaseolus vulgaris* INB lines were evaluated in a replicated field trial with inoculation of *Bradyrhizobium* USDA 3254. A non-inoculated control and Nitrogen treatments were included. In addition to that a local check "Verano", TARS-LFR1 and a non-nodulator line 'G51496A' were inoculated with *Rhizobium tropici* CIAT 899 strain. An average of 25 nodules were recorded for line INB 835 and six nodules for line INB 826. The average for line Tepary 1 was 1.5 nodules and for TARS-LFR1 26 nodules.

- Interspecific hybrids between the common bean (*Phaseolus vulgaris* L.) and the tepary bean (*Phaseolus acutifolius* A. Gray) were nodulated by different rhizobia; the slow growing *Bradyrhizobium* sp. and the fast growing *Rhizobium* spp. Elite strains of *Bradyrhizobium* (USDA 3254) and *Rhizobium tropici* (CIAT 899) were studied in their ability to nodulate effectively in interspecific hybrids. The experiment was arranged in a split plot design with inoculation of the combination of both strains, a control without inoculation and a NPK treatment in the main plot. The small plot consisted of common beans: 'Verano', LFR-1, tepary bean Tep 23, Tep 32 and the Interspecific hybrids: INB-817, INB-848, INB 817 and INB-835. The experiment was established in Juana Diaz with a population of 1×10^2 *Bradyrhizobium* and 1×10^4 *Rhizobium* per gram of soil. The experiment was replicated four times. Six weeks after sowing nodulation and plant biomass were evaluated and at maturity seed grain yield was measured. Ten nodules were isolated in Yeast-Mannitol-Agar with bromotymol blue from each treatment to differentiate bradyrhizobia and rhizobia based on growth rate and acid production. The hybrids and the common beans differed in nodule numbers from the tepary beans. More than 90 percent of the nodules that were isolated from the hybrids resulted in a fast growing rhizobia. In contrast from the tepary bean nodules the isolations were from a slow-growing bradyrhizobia. The lines LFR-1, INB-809 and INB-826 were outstanding in nodule numbers. All genotypes were different from the tepary beans in nodulation. INB-809 was superior in nodulation followed by LFR-1, Verano and INB-826, INB-835, INB-848 and INB-817. The inoculated treatment was different from the NPK and the control in nodule numbers independently of the presence of soil rhizobia. Root dry weight was higher for INB-809 and LFR-1 compared to the other genotypes. Grain weight differences among treatments suggested that inoculation increased seed yield and INB-817 was superior to the other genotypes. The nodules isolated in media produced fast growing rhizobia and produced acidity in the media that was consistent with the cultural characteristics of *Rhizobium tropici*.
- A collaborative tepary bean genome sequencing effort is being formed with HudsonAlpha, NDSU, Michigan State Univ., and USDA-ARS. The goal of this effort would be to produce the first reference genome of *Phaseolus acutifolius*. This should lead to a better understanding of the genetic basis of factors that allow tepary beans to be more tolerant to abiotic stresses such as heat and drought.

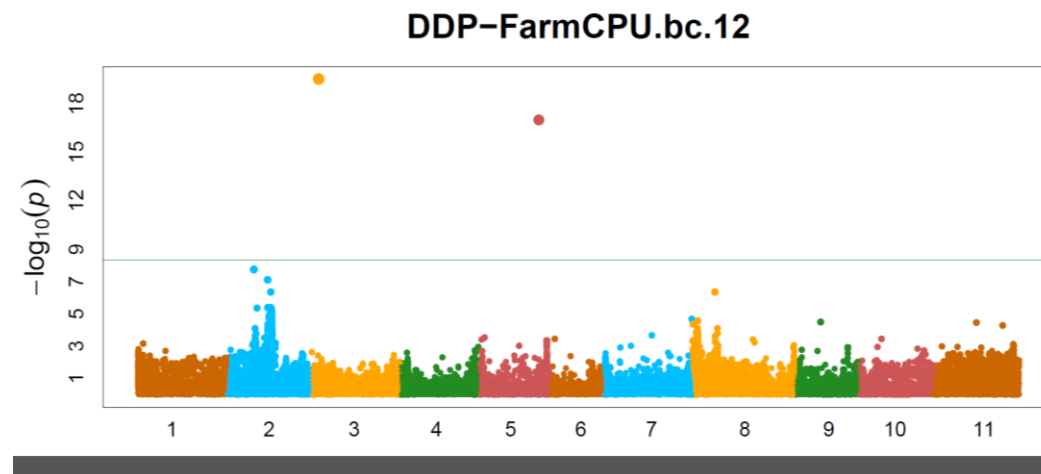
Development of molecular markers for traits of economic importance

BCMV, halo blight and BGYMV resistance

New SNP-based markers for MAS of the *I* gene that confers resistance to BCMV have been developed and are being used for indirect selection for this resistance in our breeding program and by CIAT. Meanwhile a candidate gene for *I* has been identified. Primers spanning the genomic region containing this gene are being used to sequence the gene in resistance and susceptible lines in order to develop gene-based markers and to provide the molecular tools for eventual cloning of the gene. For GWAS, more than 1000 lines representing three diversity panels – Andean, Durango, and Snap bean, were phenotyped for reaction to NL-3 strain to detect presence of the *I* gene and were genotyped with available SNP data sets possessing approximately 25,000 (Porch lab), two million (McClellan lab), and 30,000 (Porch/Hart lab) SNPs, respectively. The Durango

and Snap bean diversity panels were also screened with NL-8 strain. The GWAS for 200 Durango lines with reaction to both NL-3 and NL-8 strains of BCMNV with 2 million SNPs enabled fine mapping of the recessive *bc-1²* gene and discovery of the genomic location for the *bc-u* gene. The GWAS peak for *bc-1²* is located between two candidate genes which are being sequenced for more in depth analysis. This finding of the *bc-u* gene was unexpected and supports the power that GWAS analyses can have for detecting and fine mapping epistatic genes. SNP-based markers for detection of *bc-u* have been developed but await generation of F₂ populations and F₃ progeny tests for validation. The *bc-u* gene can be critical because it is required for expression of *bc-1²*, *bc-2²*, and *bc-3* genes in the absence of the *I* gene. Being able to detect the *bc-u* gene with markers enables more precise characterization and genotyping of resistance to BCMV and BCMNV and will facilitate mapping and development of markers for the elusive *bc-2²* locus. For example presence of *bc-u* as detected by these SNP-based markers seems to enhance the resistance response of *I + bc-1²* materials to the NL-3 strain of BCMNV.

Figure 1. Manhattan plot detecting *bc-1²* and *bc-u* genes.



A global collaboration (S01.A4; ARC-South Africa; Warwick University, UK; and Starke Ayres Seed Company) was sparked by a presentation by Andy Tock, a Ph.D. candidate from University of Warwick, Wellesbourne, UK, at the PanAfrican Legume conference in Zambia. His presented

results on mapping halo bacterial blight resistance to Race 6 complimented our unpublished results. Together these collaborative results encompassing linkage mapping in four RIL populations and GWAS of 400 ADP lines in the field have revealed new genes and QTL for halo blight resistance on chromosomes Pv04, Pv05 and Pv10. SNP-based markers are in development and have been requested by CIAT recently to address a critical need for deploying halo blight resistance in Ethiopia.

The presence of the 5398 SNP chip enables rapid development of linkage maps with dense marker coverage for QTL discovery. We are revisiting an old RIL population Dorado/XAN 176 from UPR-breeding program (1992) that was sparsely populated with RAPD markers but nonetheless was used to discover and generate markers for the SAP6 QTL for common bacterial blight resistance and the SW12 QTL for resistance to BGYMV resistance. Both resistance-linked RAPD markers were converted to SCAR markers and have been widely used for MAS. The SW12 marker has been used widely by CIAT, the UPR, Zamorano and other programs for breeding beans resistant to BGYMV. The Dorado/XAN 176 RIL population has been submitted for SNP genotyping in order to develop a denser linkage map to contribute to ongoing efforts by S01.A4 and CIAT researchers to develop more tightly-linked breeder-friendly markers for these two QTL. A better marker for SW12 QTL is considered a critical need for breeding for high levels of resistance to BGYMV because of recent severe outbreaks of the disease in Central America. Populations are under development at Zamorano that segregate for the dominant gene *Bgp-1* that confers resistance to pod deformation in the presence of BGYMV. Individual F₅ plants were selected at Zamorano for normal and deformed pods. F_{5:6} plant rows will be screened for BGYMV resistance during the upcoming growing season. The data will be used by Dr. Phil McClean at NDSU to identify a molecular marker for this important gene for resistance to BGYMV.

Bruchid resistance

A collaborative breeding effort among Paul Kulsolwa (SUA, Tanzania), Dr. Jim Myers (Oregon State University) and Dr. Jim Beaver (University of Puerto Rico) resulted in the development of breeding lines in which the arcelin (ARC or ARL)-phytohemagglutinin (PHA)-alpha-amylase inhibitor (AAI) locus (collectively known as the APA locus) from tepary accession G40199 was introduced. This locus is presumed to be the source of bruchid resistance and results in significantly reduced seed storage damage by the common and Mexican bean weevil. A molecular analysis of these lines and the tepary source of resistance was initiated by Ms. Lucy Lund, a graduate student of Dr. Phil McClean (North Dakota State University). A series of primers were designed across the three genes within the APA locus. The goal was to search for any molecular difference that distinguished the resistant from susceptible lines. Seed of the RIL population from Dr. Kelvin Kamfwa that was screened in Puerto Rico for resistance to bruchids was sent to Dr. Phil McClean to identify candidate markers for resistance genes.

When data from the AAI amplification was analyzed, it was discovered that the products differed by 45bp, and that the polymorphism was completely diagnostic between the resistance source, the resistant lines, the two susceptible recipient lines and two susceptible checks. That difference is depicted in Figure 1. Sequence analysis of the PCR products revealed that the 45bp difference resulted in the deletion of the lectin domain, a domain that is common to many LegB genes.



Figure 1. Amplification of common bean genotypes with primers designed to the alpha-amylase gene from the APA locus. The first four lanes are the Middle American resistant lines PR1464-1 and PR1464-6 and the Andean resistant lines AO1012-29-3-1A and AO1012-29-3A. The next four susceptible lines are: XRAV-40-4 and Rojo, the recipient parents, and Verano, and Badillo, two standard susceptible checks. The last lane is line G19833, the genotype used to develop the common bean reference genome sequence. The upper band is 275bp and the lower band is 230bp.

Multiple clones from the fragment generated by amplifying each of the resistant lines were sequenced to determine if the original source of resistance contained a single or multiple copies of the AAI gene. From that analysis it was determined that at least two copies of the gene existed for each line, and that each copy had unique sequence signatures. Additionally, the two copies were separated (at the amino acid sequence) using neighbor-joining phylogenetic tree analysis. That tree is shown in Figure 2.

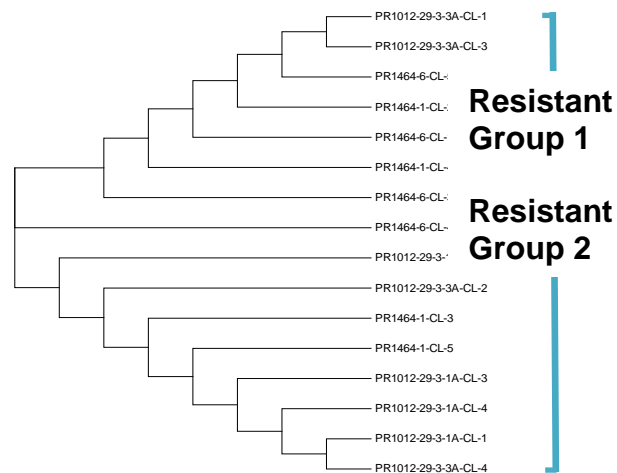


Figure 2. Neighbor-joining tree based on the amino acid sequence data for the four bruchid resistant lines developed by introgressing the resistant phenotype from tepary bean G40199. Two distinct groups of sequences are noted.

Given that the arcelin, and not the alpha-amylase locus, it is thought to be the causative protein associated with resistance, a sequence analysis of multiple clones of arcelin-specific amplification products was performed. This study focused on the four resistant lines, the tepary source, and other tepary lines. To date all of the sequence data has been collected and is now being analyzed. A few early analyses have been performed. First, the common bean genome consists of multiple LegB genes, genes with the lectin domain such as the members of the APA locus. One question of concern was the APA genes distinct from the other LegB proteins, and the answer from the neighbor-joining phylogenetic analysis is yes, they form a unique cluster distinct from the members of the APA locus. That is noted in Figure 3. Also observed was that the arcelin sequences were distinctly different from the phytohemagglutinin (PHA) and alpha-amylase inhibitor (AAI) proteins, which together formed a cluster among themselves. Furthermore, the arcelin sequence data formed two distinct clusters. Sequence analysis demonstrated that the tepary donor G40199 contains two distinct classes of arcelin sequences, two sequences that are indeed related (because of their relationship to only each other, and not to the other lectin containing genes). What is yet to be determined is if these two clusters actually map in the same vicinity in the genome of the tepary source, and thus also in the recipients from the introgression effort.

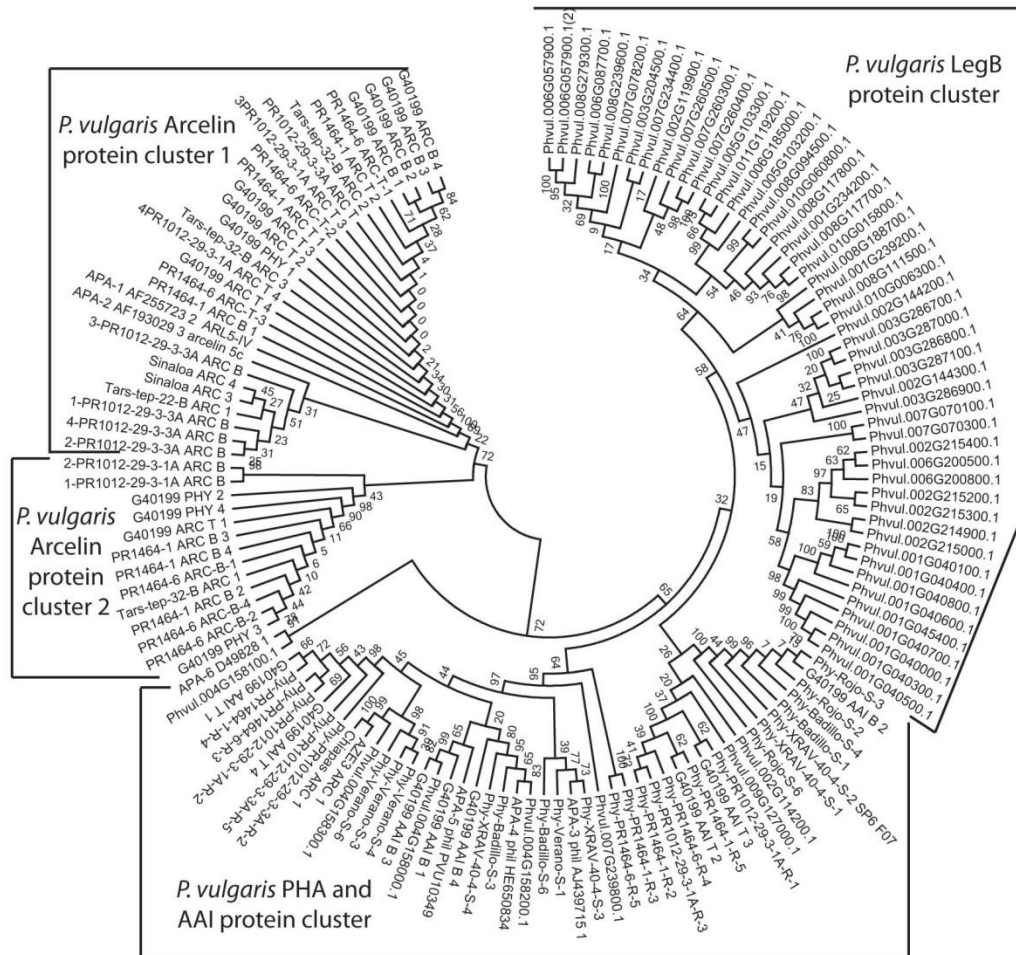


Figure 3. Neighbor-joining tree of *P. vulgaris* arcelin, PHA, AAI, and other lectin containing proteins, and *P. acutifolius* arcelin proteins

Bean rust resistance

At the distal end of chromosome Pv11 of common bean, a large cluster of coiled-coil, nucleotide-binding site, leucine-rich (CNL) repeat genes ($n \sim 50$) is located. CNL regions have been discovered to act as dominant resistance genes in many species. In addition, many resistance specificities are located at this locus. Principal among those are the bean rust resistance gene *Ur-3*, *Ur-6*, and *Ur-11*. Because of their importance, developing highly functionally molecular markers linked to these loci has been a goal for years. Dr. McClean's group, along with Dr. Phil Miklas (USDA/ARS, Prosser WA) and Dr. Talo Pastor Corrales (USDA/ARS, Beltsville) have collaborated on a molecular marker development effort.

Dr. Pastor Corrales screened 301 members of the Middle American Diversity Panel (MDP) to assess their response against bean rust races that can discover *Ur-3* and *Ur-11* specificity. Previously, Dr. McClean's group had placed the *Ur-3* resistance gene in the vicinity of 47 Mb on Pv11. In addition, a group of 5 indel markers were noted to co-segregate with *Ur-3* resistance in 24 genotypes. This year the MDP was scored for those markers. That data is found in Table 1.

Table 1. Genetic analysis of the MDP and UI 114 x C-20 F₂ population with indel markers associated with the *Ur-3* resistance locus of common bean. The MDP data represents the number of mismatches out of 301 lines with percentage mismatches in parentheses. The F₂ data represents the number of recombinants in a population of 811 individuals.

Indel marker position (bp) on Pv11	# MDP mismatches	# F ₂ recombinants
46,961,210	18 (6%)	0
46,981,156	10 (3%)	0
46,985,504	10 (3%)	unknown
47,016,881	13 (4%)	2
47,768,400	31 (10%)	9

The data clearly places *Ur-3* at the proximal end of these markers. Importantly, two markers at 46,981,156 bp and 46,985,504 bp have a very high (but not perfect) diagnostic ability. They are certainly worthy of implementation in breeding programs once, the allelic state of the parents in a cross have been determined. Additional work is underway at this time to further map additional indel markers that are proximal of the marker at 46,961,210. The goal is to discover a marker that shows recombinants in the F₂ population. This result will place *Ur-3* in a definitive interval from which a candidate gene can be discovered.

Similar efforts are underway, but not as advanced, for *Ur-6* and *Ur-11*. For *Ur-6*, a F₂ population of ~2000 individual is ready for screening with race 47 (uncovers the *Ur-6* resistance specificity). Additionally, the MDP will be screened also in the next year and a similar analysis will be performed. For *Ur-11*, 30 F₁ plants are being grown and F₂ seeds will be harvested. That F₂ population will be challenged with the appropriate

bean rust race to score for the *Ur-11* resistance specificity. That data will be coupled with a molecular screening for the 5 indel markers discovered this year to be closely associated with *Ur-11*. These markers were discovered by scoring the MDP and looking for markers with only a few mismatches. That data is presented in Table 2. The table shows that we have two perfect markers that can be immediately implemented for the screening for the *Ur-11* resistance locus in common bean.

Table 2. Genetic analysis of the MDP with indel markers associated with the *Ur-11* resistance locus of common bean. The MDP data represents the number of mismatches out of 301 lines with % mismatches in parentheses.

Indel marker position (bp) on Pv11	# MDP mismatches	# F ₂ recombinants
47,956,000	4 (1%)	0
48,242,933	0 (0%)	0
48,356,332	2 (0.6%)	unknown
48,414,723	0 (0%)	2
48,459,800	3 (1%)	9

Importantly, we have now placed to very important resistance specificities in very close proximity of each other. The *Ur-3* locus maps to the 11b cluster of CNL genes on Pv11, while the *Ur-11* locus maps to the 11e cluster. With further mapping of this locus, using the same approach described here, other resistance specificities can be fine-mapped and very functional markers with a high-degree of reliability can be developed.

The better more tightly linked markers for the *Ur-3* and *Ur-11* rust resistance genes developed to date by S01.A4 (NDSU) and ARS (Beltsville) are being used for MAS and characterization of advanced breeding lines. These markers are easier to assay and more diagnostic than the previous RAPD based markers, and are expected to have worldwide utility for detection of the genes across market classes and breeding programs. New dry bean cultivar releases for University Idaho and Colorado State University, and some multiple disease resistant germplasm within S01.A4 breeding materials were more effectively characterized for rust resistance using these new markers.

V. Research Capacity Strengthening

Legume Innovation Lab plant breeders assist bean research programs in Guatemala and Haiti to develop the capacity to develop populations and test breeding lines that will lead to the release of improved bean cultivars. This should contribute to the long-term sustainability of bean breeding activities in the region. The ICTA bean research team has developed numerous populations having different breeding objectives and is evaluating bean breeding lines in the field and using marker-assisted selection to identify lines that possess the *Co-4*² allele for resistance to

anthracnose. Dr. Porch provided bulk populations of black beans to Haiti to provide National Seed Service researchers with experience making field selections and managing breeding lines.

The project used funds to support the bean research network in Central America and the Caribbean. These funds permit bean researchers in the region to attend the annual meeting of the PCCMCA where research results can be shared. The PCCMCA meeting also provides an opportunity for Legume Innovation Lab and CIAT scientists to meet with bean researchers to plan collaboration for the upcoming year. During the past year, the project collaborated with INTA to commemorate the International Year of the Pulse at the PCCMCA meeting in Costa Rica. Dr. Porch made an invited presentation entitled “Técnicas para desarrollar frijol con mayor resistencia al cambio climático” at a plenary session of the PCCMCA.

This Legume Innovation Lab project continues to collaborate with many CRSP alumni institutions. This collaboration extends the potential impact of Legume Innovation Lab research and generates information that is valuable to the global bean research community. A few of the collaborative research activities are listed below:

- Andean bean lines with multiple virus resistance sent to Kelvin Kamfwa in Zambia and Mr. Isaac Dramadri in Uganda.
- Bruchid resistance research with Paul Kusolwa at Sokoine Agricultural Univ. in Tanzania.
- Evaluation of red mottled and black bean breeding lines by IDIAF collaborators in the Dominican Republic.
- Regional performance trials (SISTEVER) in Nicaragua, El Salvador and Costa Rica.
- Evaluation of Andean and pinto bean lines and bulked breeding populations in Angola and Tanzania.
- Rhizobium Inoculant production in Haiti.

VI. Human Resource and Institution Capacity Development

1. Short-Term Training

Table 2. Summary of short-term training of Legume Innovation Lab project S01.A4 during FY15.

Purpose of Training	Abiotic stress research techniques ¹
Type of training	Workshop
Countries benefiting	Costa Rica, El Salvador, Nicaragua, Honduras, Guatemala, Haiti
Location and dates of training	Puerto Rico August 2016
Number receiving training (by gender)	3 F, 14 M
Host institution(s)	University of Puerto Rico
Institution providing training	Zamorano, UPR, USDA-ARS

¹ Workshop co-sponsored by the USAID Climate Resilient Beans project.

2. Degree Training

Table 3. Summary of graduate degree training of Legume Innovation Lab project S01.A4 during FY16.

Name of trainee	Héctor Martínez	Iveth Rodríguez	Diego Rodríguez	Didier Joseph	Ana Vargas	Bernardo Mateo	Carlos Maldonado	Lucy Lund
Country of citizenship	Guatemala	Honduras	Ecuador	Haiti	Nicaragua	Dom. Rep.	Guatemala	U.S.
Gender	M	F	M	M	F	M	M	F
H.C. institution	ICTA	Zamorano	INIAP	NSS	None	None	ICTA	None
Training institution	UPR	UPR	UPR	UPR	UPR	UPR	NDSU	NDSU
Supervising CRSP PI	J.S. Beaver	T.G. Porch	C. Estevez	J.S. Beaver	T.G. Porch	J.S. Beaver	Juan Osorno	Phil McClean
Degree program	M.S.	M.S.	M.S.	M.S.	M.S.	M.S.	M.S.	M.S.
Field or discipline	Plant breeding	Plant breeding	Plant breeding	Plant Pathology	Plant breeding	Plant breeding	Plant breeding	Plant genomics
Research project title	Web blight resistance	Drought-heat tolerance	ALS resistance	Bean seed quality	Tepary bean breeding	Bruchid resistance	Resistance to anthracnose	Mol. genetics res. to bruchids
Start date	Aug. 2015	Aug. 2015	Aug. 2015	Jan. 2016	Aug. 2014	Aug. 2015	Aug. 2015	July 2015
Completion date	Jul. 2017	Jul. 2017	Jul. 2017	Dec. 2017	May 2016	May 2016	Dec. 2017	June 2017
Participant trainee and registered on TraitNet?	Yes	Yes	No	Yes	No	No	Yes	No

Table 4. Summary of undergraduate degree training of Legume Innovation Lab project S01.A4 during FY16.

Name of trainee	Sara Salgado	Priscila Campos	Jorge Chanaluisa	Klever Arroba	Segundo Gavilanes	Belky Cabana	Enrique Zevallos	Elisa Solis	María Besilla	Katya Rivera	Daniel Daza	Andrés Rosas
Country of citizenship	Ecuador		Ecuador	Ecuador	Ecuador	Peru	Peru	Guatemala	Ecuador	Honduras	Ecuador	Ecuador
Gender	F	F	M	M	M	M	M	F	M	F	M	
H.C. institution	None	None	None	None	None	None	None	None	None	None	None	None
Training institution	Zamorano	Zamorano	Zamorano	Zamorano	Zamorano	Zamorano	Zamorano	Zamorano	Zamorano	Zamorano	Zamorano	Zamorano
Supervising CRSP PI	J.C. Rosas	J.C. Rosas	J.C. Rosas	J.C. Rosas	J.C. Rosas	J.C. Rosas	J.C. Rosas	J.C. Rosas	J.C. Rosas	J.C. Rosas	J.C. Rosas	J.C. Rosas
Degree program	B.S.	B.S.	B.S.	B.S.	B.S.	B.S.	B.S.	B.S.	B.S.	B.S.	B.S.	B.S.
Field or discipline	Agronomy	Agronomy	Agronomy	Agronomy	Agronomy	Agronomy	Agronomy	Agronomy	Agronomy	Agronomy	Agronomy	Agronomy
Research project title	ALS resistance	Web blight Resistance	Web blight resistance	BGYMV resistance	BGYMV resistance	Low fertility tolerance	Low fertility tolerance	Low fertility tolerance	Low fertility tolerance	Heat tolerance	Rhizobium	Rhizobium
Start date	Jan. 2016	Jan. 2016	Jan. 2016	Jan. 2016	Jan. 2016	Jan. 2016	Jan. 2016	Jan. 2016	Jan. 2016	Jan. 2016	Jan. 2016	Jan. 2016
Completion date	Nov. 2016	Nov. 2016	Nov. 2016	Nov. 2016	Nov. 2016	Nov. 2016	Nov. 2016	Nov. 2016	Nov. 2016	Nov. 2016	Nov. 2016	Nov. 2016
Participant trainee and registered on TrainNet?	No	No	No	No	No	No	No	No	No	No	No	No

Name of trainee	Ivan Alarcon	Kevin Burgos	Fatima Avaroma	Fatima Arteaga	Edhinson Espinoza
Country of citizenship	Ecuador	Ecuador	Bolivia	El Salvador	Ecuador
Gender	M	M	F	F	M
H.C. institution	None	None	None	None	None
Training institution	Zamorano	Zamorano	Zamorano	Zamorano	Zamorano
Supervising CRSP PI	J.C. Rosas	J.C. Rosas	J.C. Rosas	J.C. Rosas	J.C. Rosas
Degree program	B.S.	B.S.	B.S.	B.S.	B.S.
Field or discipline	Agronomy	Agronomy	Agronomy	Agronomy	Agronomy
Research project title	Drought tolerance	Drought tolerance	Bruchid resistance in tepary	Bruchid resistance in tepary	BNF
Start date	Jan. 2016	Jan. 2016	Jan. 2016	Jan. 2016	Jan. 2016
Completion date	Nov. 2016	Nov. 2016	Nov. 2016	Nov. 2016	Nov. 2016
Participant trainee and registered on TraiNet?	No	No	No	No	No

VII. Achievement of Gender Equity Goals

The development and dissemination of improved bean cultivars using conventional techniques and marker-assisted selection should produce greater or more reliable bean yields. This should contribute to economic growth and improve the lives of the families of bean producers in Central America and Haiti. The project also supports the participation of women in formal and informal training activities.

VIII. Achievement and Progress along the Impact Pathway

Central America

Since the majority of bean improved cultivars used in Central America were developed with support from the Legume Innovation Lab, Zamorano continues to be the main source of genetic and foundation seed for the national research institutions, national seed systems, NGOs and farmer organizations. Under the Bean Dissemination Project (2010-13) nearly 27,000 farmers received high quality seed of improved bean cultivars in Honduras, and Zamorano provide foundation seed and *Rhizobium* inoculant to INTA/Nicaragua and ICTA/Guatemala, and UPR to NSS/Haiti. In Honduras, several Local Agricultural Research Committees (CIALs), which were involved in this project, continue producing seed using registered seed provided by Zamorano.

Under a similar approach, Zamorano continues to provide foundation seed of improved bean cultivars and *Rhizobium* inoculant to the MAS Frijol project coordinated by MSU in collaboration with Technoserve and DICTA in Honduras. In addition, Zamorano trained a technician and provided *Rhizobium* strains and peat to DICTA's lab supported by this project.

Haiti

During the summer of 2016, the National Seed Service produced of seed of the multiple disease resistant black bean varieties 'XRAV-40-4', 'DPC-40' and MEN2201-64ML and the red mottled bean PR0737-1. The stationary thresher, purchased with funds from the Pulse CRSP, facilitated the harvest at Savane Zombi and generated interest among bean growers. Project funds will be used to improve seed storage facilities at Savane Zombi. Dry weather during the summer reduced seed yields.

Former collaborators in the Bean Technology Dissemination project continued to produce a significant amount of seed of improved bean cultivars in Haiti during 2016. The NGOs Zanmi Agrikol and Hands Together produced seed of DPC-40 during 2016. This seed was sold to small-scale farmers in the Central Plateau and Gonaives.

IX. Explanation for Changes

- A second year of drought during the first growing season in Central America and the Caribbean significantly reduced bean yield and caused a severe shortage of seed for the second growing season. During the past year, Zamorano and other bean research programs in Central America needed to increase the production of basic seed to replenish stocks for the 2016 growing seasons.
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X. Self-Evaluation and Lessons-Learned

- Bean cultivars initially selected for specific traits using marker-assisted selection need to be monitored for genetic purity. It has been necessary to re-select seed of Aifi Wuriti and PR1146-138 to insure the presence of the *bgm-1* allele for BGYMV resistance.
- The bruchid collection at the Isabela Substation was infested with what appears to be mites. In addition to reducing the ability of the bruchids to reproduce, the mites cause technical personnel working with the bruchids to have an unpleasant itching sensation. We plan to establish a new colony of bruchids.
- During the past year, leafhopper (*Empoasca krameri*) infestations were observed in black bean trials at Jutiapa and San Jerónimo, Guatemala and Damien, Haiti. Resistance to this pest needs to be included as a breeding objective, especially for beans produced under dry conditions.

XI. Scholarly Accomplishments

Beaver, J.S., J.C. Rosas, T.G. Porch, M.A. Pastor-Corrales, G. Godoy-Lutz and E.H. Prophete. 2015. Registration of PR0806-80 and PR0806-81 white bean germplasm with resistance to BGYMV, BCMV, BCMNV and rust. *J. Plant Reg.* 9:208-211.

Beaver, J.S., E. Prophete, G. Démosthène, and T.G. Porch. 2016. Registration of PR1146-138 Yellow Bean Germplasm Line. *J. Plant Registrations.* 10:145-148.

Beaver J.S., González, A., Mateo, B., Rosas, J.C., Trukhina, Y., and Porch T.G. 2016. Development of black bean lines that combine bruchid and multiple virus resistance. Poster presented at the 2016 PanAfrican Grain Legume and World Cowpea Conference held in Livingstone, Zambia from 28 February to 4 March 2016.

Cichy, K.A., T.G. Porch, J.S. Beaver, P. Cregan, D. Fourie, R. Glahn, M.A. Grusak, K. Kamfwa, D.N. Katuramu, P. McClean, E. Mndolwa, S. Nchimbi-Msolla, M.A. Pastor-Corrales and P.N. Miklas. 2015. A *Phaseolus vulgaris* diversity panel for Andean bean improvement. *Crop Sci.* 55:2149-2160.

De Ron, A.M., Papa, R., Bitocchi, E., González, A.M., Debouck, D.G., Brick, M.A., Fourie, D., Marsolais, F., Beaver, J., Geffroy, V., McClean, P., Santalla, M., Lozano, R. Yuste-Lisbona, F.J. and P.A. Casquero. 2015. Common bean. P. 1-36. *In Handbook of Plant Breeding: Grain Legumes.* Springer-Verlag, New York.

Estevez de Jensen, C., T.G. Porch, J. Colley, O. Gonzalez, Timothy Porch and J.S Beaver. 2016. Virulence of *Macrophomina phaseolina* isolates in common bean (*Phaseolus vulgaris*) genotypes. Poster presented at the 2016 PanAfrican Grain Legume and World Cowpea Conference held in Livingstone, Zambia from 28 February to 4 March 2016.

Estevez de Jensen. 2016. Reacción de las líneas del BASE 120 a la mancha angular en Puerto Rico. Poster presented at the 2016 Annual Meeting of the PCCMCA held in San José, Costa Rica from 5 to 7 May 2016.

Estevez de Jensen, C., Rodriguez, D., T.G. Porch and J.S. Beaver. 2016. Reaction of the BASE 120 Lines to angular leaf spot in Puerto Rico. Poster presented at the 2016 PanAfrican Grain Legume

and World Cowpea Conference held in Livingstone, Zambia from 28 February to 4 March 2016

Humphries, S., J.C. Rosas, M. Cerna, J. Jiménez, F. Sierra, O. Gallardo, C. Avila y M. Barahona. 2015. Synergies at the interface of farmer-scientific partnerships: Agricultural innovation through participatory research and plant breeding in Honduras. *Agriculture and Food Security* 4:27. 17p.

Humphries, S., J.C. Rosas y M. Gomez. 2016. A farmer-NGO-scientist synergy. *Farming Matters, The Netherlands*, 32(1):14-16.

Hart, J., A.G Vargas, M.A. Brick, J. Burrridge, J.S. Beaver and T.G. Porch .2016. Genome-wide association studies of morphological and agronomical traits in cultivated tepary beans (*Phaseolus acutifolius*). Poster presented at the 2016 PanAfrican Grain Legume and World Cowpea Conference held in Livingstone, Zambia from 28 February to 4 March 2016.

Kusolwa P.M, J.R. Myers, T.G. Porch, Y. Trukhina, A. González-Vélez and J.S. Beaver. 2016 Registration of AO-1012-29-3-3A red kidney bean germplasm line with bean weevil, BCMV, and BCMNV Resistance. *Journal of Plant Registrations* 10:149-153.

Lorenzo Vázquez, G., A. González, C. Estévez de Jensen, T.G. Porch and J.S. Beaver. 2016. Evaluación en Puerto Rico líneas del ensayo BASE 120 para adaptación al estrés abiótico. Paper presented at the 2016 Annual Meeting of the PCCMCA held in San José, Costa Rica from 5 to 7 May 2016.

Mateo, B. 2016. Selección de líneas de frijol que combinan resistencia al gorgojo común con resistencia a los virus BGYMV, BCMV y BCMNV. M.S. Thesis, University of Puerto Rico, Mayaguez, Puerto Rico.

Mateo, B., Beaver J.S., González A., Trukhina Y., and T.G. Porch. 2016. Desarrollo de líneas de frijol que combinan resistencia a los gorgojos y múltiples virus. Poster presented at the 2016 Annual Meeting of the PCCMCA held in San José, Costa Rica from 5 to 7 May 2016.

Rodriguez, D., T.G. Porch and J.S. Beaver. 2016. Reaction of the BASE 120 Lines to angular leaf spot in Puerto Rico. Poster presented at the 2016 PanAfrican Grain Legume and World Cowpea Conference held in Livingstone, Zambia from 28 February to 4 March 2016.

Porch, T.G. 2016. Técnicas para desarrollar frijol con mayor resistencia al cambio climático. Paper presented at the 2016 Annual Meeting of the PCCMCA held in San José, Costa Rica from 5 to 7 May 2016.

Porch, T. G., K. Cichy, W. Wang, D., M. Brick, J. S. Beaver, D. Santana-Morant, and M. Grusak. 2016. Nutritional composition and cooking characteristics of tepary bean (*Phaseolus acutifolius* Gray) in comparison with common bean (*Phaseolus vulgaris* L.). *Genetic Resources and Crop Evolution* doi:10.1007/s10722-016-0413-0

Porch T.G., Cichy K., Pastor-Corrales M.A., Grusak M.A., Beaver J.S., Hart J., Fourie D., Estevez de Jensen C., Nchimbi S., Miklas P.N. Characterization and application of the Andean Diversity Panel for the improvement of common bean productivity in Sub-Saharan Africa. Poster presented at the 2016 PanAfrican Grain Legume and World Cowpea Conference held in

Livingstone, Zambia from 28 February to 4 March 2016.

Prophete, E.H., G. Demosthene, J.S. Beaver, J.C. Rosas and T.G. Poch 2016. Breeding black beans for Haiti with multiple virus resistance. Poster presented at the 2016 PanAfrican Grain Legume and World Cowpea Conference held in Livingstone, Zambia from 28 February to 4 March 2016.

Rosas, J.C., J.S. Beaver, T.G. Poch, S.E. Beebe, J.D. Burrige and J.P. Lynch. 2016. Evaluation of common bean lines for adaptation to high temperatures in Honduras. Poster presented at the 2016 PanAfrican Grain Legume and World Cowpea Conference held in Livingstone, Zambia from 28 February to 4 March 2016.

Rosas, J.C. 2016. Resultados del SISTIVER 2015 de la red de frijol de Centroamérica y el Caribe. Paper presented at the 2016 Annual Meeting of the PCCMCA held in San José, Costa Rica from 5 to 7 May 2016.

Rosas, J.C. 2016. Evaluaciones de líneas de frijol tolerantes a las altas temperaturas en Honduras. Paper presented at the 2016 Annual Meeting of the PCCMCA held in San José, Costa Rica from 5 to 7 May 2016.

Rosas, J.C. 2016. Adaptación de frijol a suelos deficientes en nitrógeno mediante el mejoramiento de la nodulación. Paper presented at the 2016 Annual Meeting of the PCCMCA held in San José, Costa Rica from 5 to 7 May 2016.

Winham, D.M., A.E. Bries, M.B. Reddy, K.A. Cichy, T.G. Poch and M.A. Brick. 2016. White tepary bean shows higher in vitro iron bioavailability than brown tepary or common bean. Paper presented at the 2016 PanAfrican Grain Legume and World Cowpea Conference held in Livingstone, Zambia from 28 February to 4 March 2016.

Valentín Torres, S., M.M. Vargas, G. Godoy-Lutz, T.G. Poch, and J.S. Beaver. 2016. Isolates of *Rhizoctonia solani* can produce both Web Blight and Root Rot Symptoms in Common Bean (*Phaseolus vulgaris* L.). Plant Disease 100:1351-1357.

Vargas, A.G. 2016. Estudio de la reacción al Virus del Mosaico Necrótico Común del Frijol (BCNMV) y la habilidad de fijación biológica del nitrógeno (FBN) en frijol tépari (*Phaseolus acutifolius* A. Gray) e introgresión de la FBN al frijol común (*Phaseolus vulgaris* L.). M.S. Thesis. University of Puerto Rico, Mayaguez, Puerto Rico.

Vargas, A.G., J. Hart, C. Estevez de Jensen, J. Beaver. 2016 Evaluation of the tepary bean (*Phaseolus acutifolius*) diversity panel for response to the NL 3 Strain of Bean Common Mosaic Necrosis Virus (BCMNV) and for biological nitrogen fixation with *Bradyrhizobium* strains. Presentation made at the 2016 PanAfrican Grain Legume and World Cowpea Conference held in Livingstone, Zambia from 28 February to 4 March 2016.

Professional Recognition

James Beaver received a certificate of recognition from the House of Representatives of the Commonwealth of Puerto Rico for contributions to local agriculture.

XII. Data Management

A revised data management plan was submitted to the Legume Innovation Laboratory Management Office in July 2016. Scientists interested in using a data set generated with support from the Legume Innovation Lab should contact the PI or Co-PI responsible for generating the data set to confirm how and for what purpose the data was collected. The PI or Co-PI responsible for maintaining the data set will deposit the information in the USAID Development Data Library (DDL).

ANNEXES

Annex 1. Tables, Figures, and Photos Cited in the Report

See below

Annex 2. Literature Cited

None