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

FY 2017 Annual Project Technical Progress Report
(October 1, 2016 – September 29, 2017)

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

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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 farmers in Central America and Haiti. A BGYMV resistant black bean will be released by ICTA in Guatemala as ‘Patriarca’. The BGYMV and BCMNV resistant and drought tolerant black bean line MEN-2201-64ML from Zamorano was released in Honduras as ‘Lenca Precóz’. Seed of Lenca Precoz, BGYMV and BCMV resistant red mottled bean breeding line PR0737-1 and BGYMV, BCMV and leafhopper resistant yellow bean line PR1146-138 were multiplied in Haiti. Red mottled, cranberry and pinto bean lines with BGYMV and BCMNV resistance were tested in field trials. Black bean breeding lines that combine resistance to BGYMV, BCMNV and bruchids are also ready for field testing. The ICTA bean program has begun to test breeding lines developed in Guatemala demonstrating a greater capacity to conduct research. Angular leaf spot isolates from Honduras and Puerto Rico were found to have high levels of virulence. Populations were developed to identify a molecular marker for the *Bgp-1* gene that confers resistance to pod deformation in the presence of BGYMV. Novel QTLs for resistance to angular leaf spot, halo bacterial blight and the common bean weevil were identified.

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.

A bean research workshop was held in Honduras. Bean researchers from Central America and Haiti described research achievements and discussed future research and training needs to promote the production and consumption of beans in CA/C. Héctor Martínez from Guatemala, Iveth Rodríguez from Honduras and Diego Rodríguez completed M.S. degree training at the UPR. Carl Didier Joseph from Haiti plans to complete M.S. degree training at the UPR in June, 2018. 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). The greater heat tolerance and BGYMV resistance of the small red cultivar CENTA EAC contributed to 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 addressed 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, Sankara and MEN-2201-64ML that combine resistance to BCMNV and BGYMV. Small red bean breeding lines with the same combination of resistances were developed at Zamorano. These BGYMV and BCMNV resistant black and small red bean lines are 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 permits 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 five 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 have been released in Central America and the Caribbean. This Legume Innovation Laboratory project continued, in collaboration with CIAT, to support the bean research network activities in Central America and the Caribbean. Collaborative activities such as the regional performance nurseries helped to extend the impact of this project through the release of improved cultivars throughout the region.

In Haiti, this project tested and released red mottled, yellow and black bean cultivars with enhanced levels of disease resistance. 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. Yellow, red mottled and white bean breeding lines having resistance to BCMNV and other diseases are available for Legume Innovation Lab or Feed the Future projects, CIAT and National Bean Research Programs 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 environments such as the dry corridor in Central America and southwestern Haiti. 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 and RIL populations for traits of economic importance. This has contributed to the identification and genetic mapping of new sources of disease resistance and tolerance to abiotic stress.

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 viable 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 beans. In addition to pyramiding these traits within tepary, interspecific crosses with common bean are being used as a long-term effort to introgress useful traits between common and 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. Advances in sequencing the bean genome and the use of GWAS facilitated the mapping and development of molecular markers for traits of economic importance, while breeder-friendly InDel and KASPar markers are a broadly applicable technology. This Legume Innovation Lab assisted in the development of the populations and the collection of phenotypic data needed to identify improved markers for traits such as the *Ur-11* gene for rust resistance *I* gene for resistance to BCMV and the *Bgp-1* gene for resistance to pod deformation in the presence of BGYMV. 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 Central America/Caribbean vs. Africa. It should be noted that the exchange of breeding lines among FtF projects has been 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 common bean cultivars

Conventional plant breeding techniques and marker-assisted selection were used by Legume Innovation Lab scientists to develop common bean cultivars and breeding lines with enhanced levels of disease resistance and with 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 coordinated the regional testing of small red and black bean breeding lines. The University of Puerto Rico coordinated the development and testing of Andean beans in the Caribbean. These trials were conducted in collaboration with national bean research programs and CIAT. Promising lines were tested throughout Central America and the Caribbean, including countries that did not participate in this Legume Innovation Lab project. Testing lines in different countries provided more information concerning the potential performance of the lines and expanded 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 specific 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.

Table 1. Bean trials distributed to Central American and Caribbean Bean Research Network collaborators during 2016-2017.

Trial name	Small red	Small black	Countries
Regional bean adaptation nursery (VIDAC)	42 entries + 2 checks	55 entries + 2 checks	Costa Rica, El Salvador, Guatemala, Honduras, Haiti, Nicaragua, Panama, Puerto Rico_
Regional yield and adaptation trial (ECAR)	14 entries + 2 checks	14 entries + 2 checks	Costa Rica, El Salvador, Guatemala, Honduras, Haiti, Nicaragua, Panama

Bean variety validation trial (COVA)	8 entries + 2 checks	8 entries + 2 checks	Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua_
Regional angular leaf spot trial (ERMAN)	14 entries + 2 checks		Costa Rica El Salvador, Guatemala, Honduras,
Regional web blight trial (ERMUS)	14 entries + 2 checks		Costa Rica, El Salvador, Guatemala, Honduras,
Biofortified bean trial (AGROSALUD)	8 entries + 2 checks		Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, Panama
Regional high temperature trial (ERSAT)	22 entries + 2 checks		Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, P Rico___
Regional drought trial (ERSEQ)	22 entries + 2 checks		Costa Rica, El Salvador, Guatemala, Honduras, ___
Regional low fertility trial (ERBAF)	22 entries + 2 checks		Costa Rica, El Salvador, Guatemala, Honduras, Puerto Rico

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 sites for screening beans for performance in a low N soil, root rot resistance and different levels of drought and high temperature stress. 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 helped to identify bean breeding lines with superior nodulation scores and root rot resistance.

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 used AO-1012-29-3-3A as a progenitor to transfer BCMV and BCMNV (I + $bc\text{-}1^2$) 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. QTL analyses were used at NDSU and at the University of Zambia to explore the genetic basis of resistance to the common bean weevil derived from a cross with AO-1012-29-3. During the past year, seed increases of black bean breeding lines that combine bruchid and multiple virus resistance were conducted in Honduras. On-farm trials will be conducted during the second growing season of 2017 to test the effectiveness of bruchid resistance with seed storage methods normally utilized by farmers in Honduras. Another black bean line with bruchid and multiple virus resistance was selected by ICTA bean researchers for evaluation in multi-location performance trials in Guatemala.

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

The Middle American (MDP) and the Andean Diversity (ADP) panels were screened in Central America and the Caribbean for specific traits. Performance of the Middle American Diversity Panel was 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 screenhouse trial was planted in Juana Diaz, PR, to evaluate the reaction of the trial BASE 120 to *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 and to other diseases. 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 were increased and shared with the collaborators for testing in Tepary Adaptation Trials (TAT). New tepary breeding lines were 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 are 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. The superior lines were considered for entry in the TAT. 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 were tested against strains representing different pathogroups of BCMV and BCMNV at Prosser, WA to evaluate for broad resistance, and one wild accession was found to have resistance to more than one strain. 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 F_4 generation) and testing in Puerto Rico and Honduras. Individual plant selections from these bulk breeding populations were completed under abiotic stress in Puerto Rico in 2016 and under high levels of BGYMV pressure in Honduras in 2017. The tepary breeding lines with tolerance to BGYMV and with resistance to BCMV will be pyramided in subsequent efforts. Superior lines from the breeding program have been included in the TAT trials and tested in the host countries including Burkina Faso, Tanzania, Angola, Honduras, Haiti, and the U.S. for potential future release. Lines in these adaptation trials have been identified with tolerance to low fertility, leaf hopper, and CBB, and with broad adaptation. Through collaboration with the FtF-ARS Legumes Project and the Climate Resilient Bean Project, a RIL population, from the cross G40001/G40022, was genotyped and evaluated for a number of seed quality, agronomic, disease, phenological, and physiological traits in trials conducted under heat, drought, and non-stress conditions and a number of novel QTL have been identified. In addition, 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 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 *aAI3*)
- BCMV and BCMNV (*I, bc-u, bc-3,* $bc\text{-}1^2$)
- 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.
- <u>InDel marker selection</u>: 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 were 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. The M.S. degree students received 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.

A workshop was held in Honduras in July 2017. Bean research accomplishments in Central America and the Caribbean were presented. Participants discussed future bean research and training needs in Central America and the Caribbean. This workshop was conducted in collaboration with the Feed the Future (FtF) Innovation Lab for Climate Resilient Beans and the FtF USDA-ARS Legume Project. The workshop presentations were posted on the FtF USDA-ARS Bean Research Team Web Site.

IV. Major Achievements

Development, testing and release of improved bean cultivars

- In Guatemala, the ICTA bean research program plans to release a black bean cultivar with superior yield potential and resistance to BGYMV and BCMV as ‘Patriarca’.
- The BGYMV, BCMV and BCMNV resistant black bean line MEN2201-64ML was released in Honduras as ‘Lenca Precoz’. Seed this early maturity and drought tolerant line was multiplied in Haiti in preparation for release as a cultivar.
- The black bean cultivar ‘Sankara’ combines resistance to BGYMV, BCMV, and BCMNV. When planted at higher altitudes, Sankara has earlier maturity than the black bean cultivar ‘DPC-40’. Phil Miklas found XRAV-40-4 to be well adapted to the Western U.S. bean production region. There is interest among Western U.S. seed producers to market high-quality bean seed in Central America and the Caribbean. The project collaborated with the USDA-ARS FtF project to contract the production of seed of ‘Sankara’ in Idaho during the summer of 2016. Approximately, 11,000 kg of seed were shipped from Idaho to Haiti in time for the 2016-2017 winter growing season. The National Seed Service distributed seed to bean producers with access to irrigation including long-term collaborators such as Zamni AgriKol and AKOSAA
- ‘Beseba’ is a photoperiod insensitive and heat tolerant lima bean landrace originally collected by the National Seed Service in Haiti. Beseba was observed to have BGYMV resistance in nurseries planted at Zamorano. During the past year, F_3 lines were selected at Isabela, Puerto Rico from the cross ‘Beseba x Sieva’. The goal is to develop a determinate, white-seeded lima bean cultivar adapted for production in the Carribean. Seed of the F_3 lines will be sent to Dr. Raphael Colbert and Mr. Gasner Demosthene for further evaluation and selection in Haiti.

- Recombinant inbred lines from the crosses 'A-429 x Morales' and 'A-429 x Tío Canela 75' were phenotyped in Honduras for degree of pod deformation in the presence of BGYMV. The seed of the phenotyped RILs will be sent to Dr. Phil Miklas to identify a molecular marker for the dominant gene *Bgp-1* that confers resistance to pod deformation in the presence of BGYMV.
- Determinate black bean lines with *bgm*-1, *I* and *bc-3* virus resistance genes were developed at the UPR. These early maturity lines may be useful for avoiding terminal drought or for production at higher altitudes.
- Several of the most recent cultivar and breeding line releases (Sankara, MEN 2201-64 ML, PR0806-80, AO-1012-29-3-3A) possess combinations of genes (*I* + *bc-3*) o (*I* + $bc\text{-}1^2$) that confer resistance to BCMV y BCMNV.
- Web blight continues to be a serious bean disease in the humid tropics such as the Petén region of Guatemala. A total of 644 lines from different bean research programs were screened over a period of two years (2015 and 2016) at Isabela, PR for reaction to web blight. Thirty-seven lines were identified that had mean web blight scores over years ranging from 2.4 to 4.5 based on the CIAT 1-9 evaluation scale. Some of the web blight resistant lines produced commercial seed yields > 1,500 kg/ha. Some lines such as Amadeus 77 have susceptible leaf reaction but yielded well with a low % of damaged seed. These results suggest the desirability of evaluating leaf reaction, seed yield and % damaged seed.
- During the summer of 2017, these heat tolerant and web blight resistant lines and 450 F_7 lines from the 3rd cycle of recurrent selection for web blight resistance were evaluated in field trials planted at Isabela, Puerto Rico and Zamorano. Breeding lines were identified that had low levels of web blight infection at both locations and good yield potential under moderately hot conditions. These lines were included as entries in a ERMUS trial that will be distributed to collaborators in CA/C.
- Small red and black bean breeding lines were developed at Zamorano (ALS 0532-6, ALS 0531-41, ALS 0532-38 y NIC 604-29) with durable resistance to ALS. These lines, which combine Mesoamerican (*Phg-2*) and Andean (*Phg-1*) genes for ALS resistance and genes for resistance to BGYMV and BCMV, were evaluated in the regional ALS trial (ERMAN). These sources of resistance were used in Puerto Rico to develop white bean breeding lines with enhanced levels of resistance to ALS.
- Three QTL for ALS resistance were identified in Puerto Rico in the Andean RIL population 'CAL 143 x Rojo'. Two of the three QTL (ALS2RC y ALS11RC) had not been reported in previous studies.
- Sources of different genes for resistance to ALS were sent to NDSU to conduct 10X sequencing of genomic libraries. The goal is to develop breeder-friendly molecular markers linked to important clusters of resistance genes of common bean.
- Sister lines from the cross 'PR1212-5 x PR0737-1' were screened at Isabela, PR for powdery mildew resistance over two growing seasons. Bulk segregant analysis will be used to identify QTL in this Andean bean population associated with powdery mildew resistance.
- Ashy stem blight caused by *Macrophomina phaseolina* (Mp) is a serious bean disease, especially in hot and dry environments. Bean lines were screened for reaction to ashy stem blight using a virulent isolate of MP from Juana Díaz, PR. The screenings, which were conducted twice, identified the following lines to have

intermediate levels of resistance: BAT 477, MER 2212-28, PR 1147-3, TARS-MST1 and TARS-LFR-1. No lines were identified to have high levels of resistance to this isolate of Mp.

- Evaluations were conducted in the field and greenhouse to screen F_2 and $F_{2:3}$ lines from the cross 'PR0313-58 / VAX 6' for reaction to the common bacterial blight (CBB) strain *Xap* UPR 3353. Segregation patterns suggest that in this population two dominant genes confer resistance to CBB. Seed of the lines phenotyped for reaction to CBB will be sent to Dr. Phil Miklas to screen for putative markers associated with resistance.
- During the past few years severe damage caused by leafhoppers (*Empoasca kraemeri*) has been observed on beans in Honduras, Guatemala, Haití and Puerto Rico. The damage is generally more severe in hot and dry environments. The yellow bean breeding line, PR1146-138 released in 2016 (Beaver et al., 2016. J. Plant Reg. 10: 145-148), has resistance to BGYMV, BCMV and leafhoppers. Black bean lines were identified in Damien, Haiti that expressed good levels of resistance to leafhoppers. PR1146-138, Morales, EMP 299 have been used as parents to introgress leafhopper resistance into common bean breeding populations for Central America and the Caribbean.
- Dr. Porch conducted trials in Puerto Rico in collaboration with project S01.A3 to screen bean populations for reaction to leafhoppers. These trials led to the identification of QTLs associated with leafhopper resistance in common bean (Brisco et al., 2014. Crop. Sci. 54: 2509-2519). Several pinto bean lines from these genetic studies were confirmed to have leafhopper resistance in multiple trials in the Dominican Republic, Puerto Rico, and Haiti, with resistance sourced from EMP 507.
- Several tepary lines from the TAT also showed resistance to leaf hoppers, QTL were identified for leafhopper resistance in the G40001/G40022 RIL population in a single trial, and lines from the RIL were identified with high levels of leafhopper resistance and multiple disease resistance.
- 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. F_5 black bean lines with good agronomic type were selected from these populations. These lines were screened during the 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. 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.
- The 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 BCMV resistance and the SAP6 QTL for resistance to common bacterial blight. Other yellow bean breeding populations were developed that should combine genes for resistance to BGYMV, BCMV and BCMNV. The performance of these lines 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. The lines have commercial pinto seed type and many have an erect growth habit. These lines were evaluated in trials in Haiti and Puerto Rico. These pinto lines should segregate for the *Ur-11* gene so special attention was given to select lines that did not develop rust symptoms. Because Durango race beans have performed well in Eastern Africa, the performance of the pinto lines were evaluated in Tanzania in collaboration with the USDA-ARS FtF bean research project. A few of the pinto lines expressed 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 were evaluated in Puerto Rico and Haiti. 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. Dr. 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. A few of 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. Dr. Raphael Colbert and Mr. Gasner Demosthene selected lines for further evaluation in Haiti.
- IICA personnel reported that the black bean variety ICTA ZAM, that has a shiny seed coat, is acceptable to consumers in Guatemala. ICTA ZAM 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. ICTA ZAM is expected to be released in Guatemala during the upcoming year.
- 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 *Phaeoisariopis 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. Lines with moderate resistance 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. This Legume Innovation Lab project supported on-farm testing and seed multiplication 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 2017 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 as the cultivar ´CENTA EAC´.
- 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.
- Mesoamerican bean breeding lines were identified that combine good yield potential with superior nodule numbers in low N soils. ICA Pijao, IBC-301-204, FBN-1203-47 had superior nodule numbers when inoculated in sand benches with a mixture of *R. tropici* strain CIAT 899 and *R. etli* strain CIAT 632 and in BASE 120 field trials conducted in Puerto Rico over a period of three years.
- 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.
- The UPR cooperated with project S01.A3 in the evaluation of a RIL population for biological nitrogen fixation (BNF) at Isabela Puerto Rico. Breeding lines with superior BNF and a QTL associated with seed yield at Isabela, Puerto Rico were identified (Heilig et al. 2017. Crop Sci. 57: 1: 118-129).
- Six Andean and six Mesoamerican bean lines were identified at Zamorano that demonstrated a differential reaction when inoculated with different strains of *Rhizobium*. These lines were used to develop a set of differentials for biological nitrogen fixation in common bean that is being tested. The differentials can be used

to monitor *Rhizobium* populations in the field and can lead to the identification of strains with superior biological nitrogen fixation.

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-1*^{2} 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 F_4 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 'Solwezi x AO1012-29-3-3A'', 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 were 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

- 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. The small red bean line ALS 0532-6 and a black bean line ALS 0546-60 combine resistance to multiple viruses (BGYMV and BCMV), high yield potential and commercially acceptable seed type were evaluated in validation trials in Central America. ALS resistant white bean breeding lines were developed for Puerto Rico using sources of resistance from Zamorano.
- 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.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 and GWAS has identified QTL of key traits of interest.
- 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. These accessions were tested at ARS-Prosser with multiple strains and one wild accession was found to have resistance to more than one strain. BCMV and BCMNV are currently major constraints to tepary bean production.
- GWAS analysis of the TDP identified two loci for resistance to the NL3 strain in tepary bean. Efforts to develop KASPar markers for these loci are underway.
- 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.
- A second RIL population, G4001/G40022, was evaluated in multiple trials for a number of abiotic, biotic, agronomic and seed quality characteristics. Iveth Rodriguez, a Masters student with the LIL project, completed QTL analyses identifying the first QTL for a number agriculturally important traits in tepary bean consistent over multiple trials.
- Single plant selections for tolerance to BGYMV and resistance to BCMV are being advanced with the goal of pyramiding this resistance to both pathogens in future efforts. .
- Tepary adaptation trials have been conducted in Honduras, Guatemala, Nicaragua, El Salvador, Tanzania and Burkina Faso. A set of eight tepary lines were evaluated in the Guatemalan “dry corridor” by 100 farmers in collaboration with Bioversity International and CATIE under the methodology massive participatory evaluation. The project has recently requested black seeded tepary lines.
- 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.

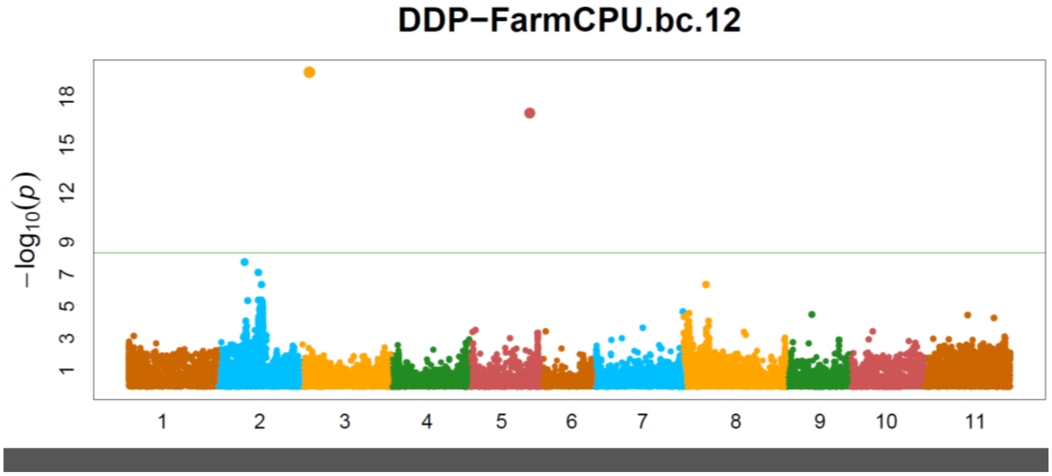
- Interspecific hybrids between the common bean (*Phaseolus vulgaris* L.) and the tepary bean (*Phaseolus acutifolius* A. Gray) were nodulated by different rhizobia; a slow growing *Bradyrhizobium* sp. and a fast growing *Rhizobium* spp. Elite strains of *Bradyrhizobium* (USDA 3254) and *Rhizobium tropici* (CIAT 899) were studied for 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, TARS-Tep 23, TARS-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 x 10^2 *Bradyrhizobium* and 1 x 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, the isolations from the tepary bean nodules were from a slow-growing bradyrhizobia. The lines LFR-1, INB-809 and INB-826 were outstanding in nodule numbers. All non-tepary bean genotypes had higher values than the tepary beans for nodulation. INB-809 was superior in nodulation followed by LFR-1, Verano, INB-826, INB-835, INB-848 and INB-817. The inoculated treatment was different from the NPK treatment and the control in terms of nodule numbers, independent 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 between 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 consortium is being formed with Hudson Alpha, NDSU, Michigan State Univ., and USDA-ARS. The goal of this effort will be to produce the first reference genome of *Phaseolus acutifolius* using the parent of the RIL population, G40001, as the reference genotype. The tepary genome will lead to the identification, characterization of the genetic basis of factors that allow tepary beans to be more tolerant to abiotic stresses such as heat and drought for the improvement of both tepary bean and common bean.

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 (McClean 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-1*^{2} gene and discovery of the genomic location for the *bc-u* gene (Fig. 1). The GWAS peak for *bc-1*^{2} 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 and are in the process of validation in $F_{2:3}$ progenies which thus far have yielded promising results. The *bc-u* gene can be critical because it is required for expression of *bc-1*^{2}, *bc-2*^{2}, 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-2*^{2} locus. For example presence of *bc-u* as detected by these SNP-based markers seems to enhance the resistance response of *I* + *bc-1*^{2} materials to the NL-3 strain of BCMNV.

Figure 1. Manhattan plot detecting *bc-1*^{2} (on chromosome Pv03) and *bc-u* (on Pv05) 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 Pan African 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 on Pv04 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 was re-genotyped with the 5398 SNP chip 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 QTL conditioning resistance to BGYMV. Better markers for MAS of resistance 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. The new Dorado/XAN 176 linkage map revealed that a QTL on Pv07 had greater effect on resistance to BGYMV than the previous QTL on Pv04 flanked by the SW12 marker (Fig. 2). This Pv07 QTL was validated in a CIAT RIL population DOR 476/Sel1309 which we re-genotyped with the 5398 SNP chip as well. This effort also revealed a novel QTL on Pv08 that interacted with the *bgm-1* gene to provide a greater level of resistance to BGYMV. The Pv07 QTL cosegregates with the *Bct-1* gene which confers resistance to *Beet curly top virus*.

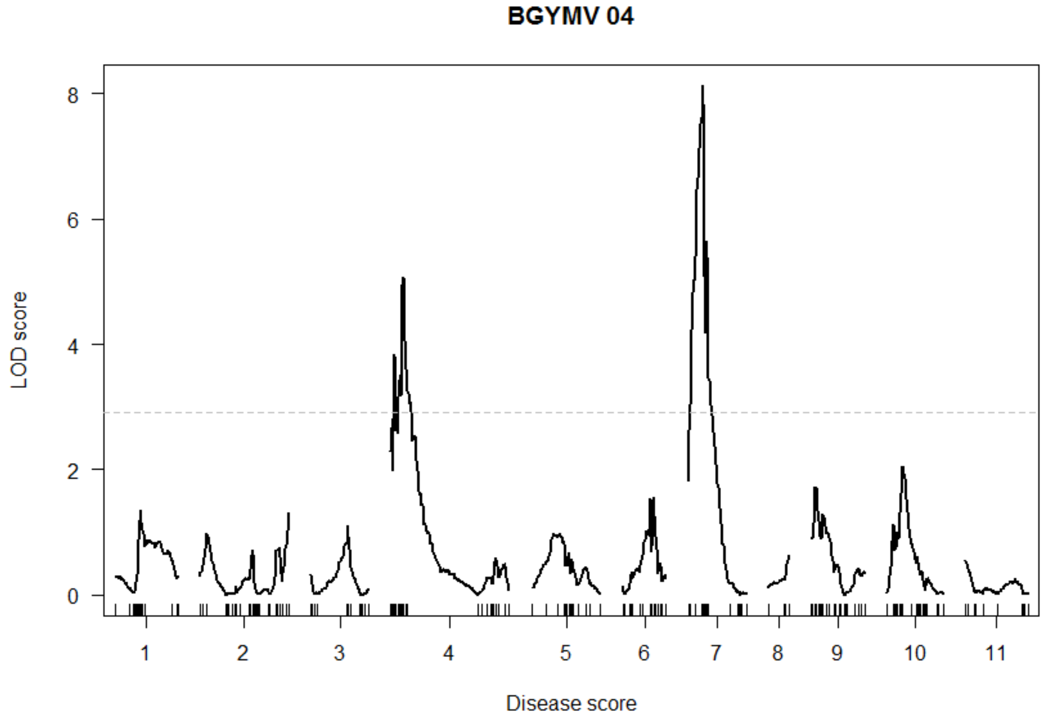


Figure 2. QTL for resistance to BGYMV detected on Pv04 (associated with SW12 marker) and Pv07 (associated with *Bct-1* gene) in the Dorado/XAN 176 RIL population re-genotyped with 5398 SNPs.

. 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 F_5 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)-phytohemagluttin (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 resistant and susceptible lines. Seed of the RIL population from the cross ‘AO1012-29-3-3 x Solwezi’ 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 alpha-amylase inhibitor (α-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 the two susceptible checks. That difference is depicted shown in Figure 3. 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 3. 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 show in Figure 4.

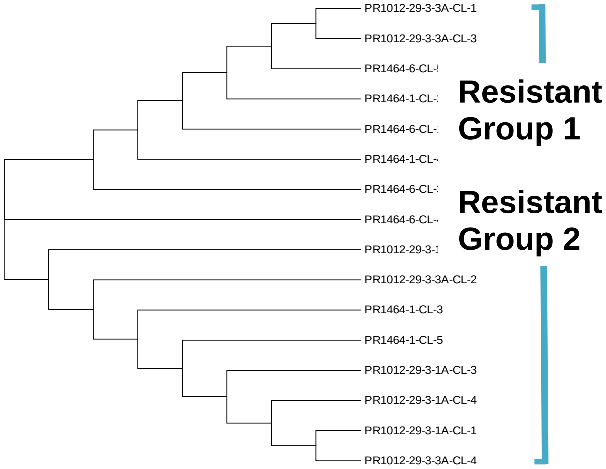


Figure 4. Neighbor-joining tree based on the amino acid sequence data for the four

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, 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 analyzed. We now know that 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 if the APA locus genes were distinct from the other LegB proteins. 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 5. Also observed was that the arcelin sequences were distinctly different from the phytohemagluttin (PHA) and alpha-amylase inhibitor (AAI) proteins. These later two proteins 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 was 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. To address this question, a RIL population derived from the cross ‘Solwezi x AO-1012-29-3-3’ provided by Dr. Kelvin Kamfwa. AO-1012-29-3-3 contains the tepary introgression for bruchid resistance. The primer pair that defined the 45bp size difference was used. The correlation between the resistance and polymorphism was not definitive, although mapping data by Dr. Kamfwa showed a resistance peak on Pv04, the chromosome where the APA locus is located.

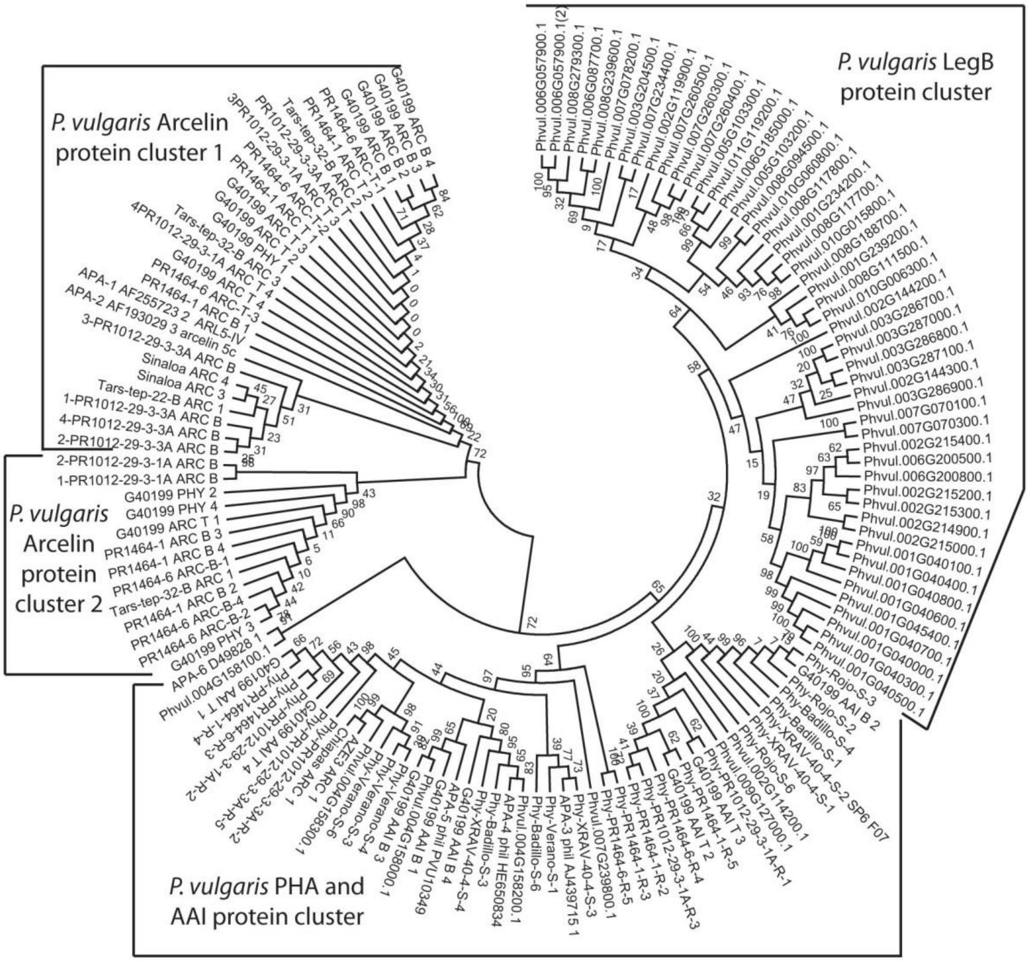


Figure 5. Neighbor-joining tree of *P. vulgaris* arcelin (ARC), PHA (phy; phytophaemaglutin), α-AI (AAI), and other *P. vulgaris* leg B domain containing proteins identified by a Pfam analysis of the amino acid sequences of the protein. The tepary (*P. acutifolius*) arcelin proteins are designated as "Tars-tep" sequence.

Bean rust resistance

At the distal end of chromosome Pv11 of common bean is located a large cluster of coiled-coil, nucleotide-binding site, leucine-rich (CNL) repeat genes (n~50). CNL genes have been shown to act as dominant resistance genes in many other host-pathosystems. In addition, many resistance specificities are located at this locus. Principal among those are the bean rust resistance genes *Ur-3*, *Ur-6*, and *Ur-11*. Because of their importance, developing highly functional 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 in 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 interval 50.63 – 50.68 Mb on Pv11. 811 F2 plants were scored, and mapped with several SNP markers selected from the 50.63 – 50.68 Mb interval defined by the GWAS analysis.

Table 1. Genetic analysis of the UI 114 x C-20 F_2 population with indel and SNP markers associated with the *Ur-3* resistance locus of common bean. The F_2 data represents the number of recombinants in a population of 811 individuals.

Ur3 Marker V2.0 Pv11 Positions	# Recombinants
50,549,756	3
50,557,492	3
50,567,470	2
50,612,732	0
50,615,957	0
50,616,063	0
50,631,529	0
50,634,865	0
50,638,985	0
50,657,864	0
50,684,143	0
50,711,016	2
50,755,552	4
50,766,742	4
50,791,702	5
51,499,870	9

The data clearly places *Ur-3* between 50,567,470 and 50,711,016 Mb on Pv11. This is the most accurate positioning currently available for the position. Importantly, there are eight markers that co-segregate with the Ur-3 locus that are ready for implementation in breeding programs.

Similar efforts are underway, but not as advanced, for *Ur-6* and *Ur-11*. For *Ur-6,* a F_2 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 F_1 plants were grown, and ~ 2000 F2 seeds are available. 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. Two indel markers located with 160 kb of each other cosegregate with the *Ur-11* locus.

Importantly, we have now placed two very important resistance specificities in very close proximity of each other. The *Ur-3* locus maps to the 11C cluster of CNL (coiled-coil NBS-LRR) 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 *Ur-3* and *Ur-11* rust resistance genes markers described here have already been used by Dr. Phil Miklas and Dr. Tim Porch in their breeding programs to screen 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.

V. Research Capacity Strengthening

A Central America / Caribbean (CA/C) bean research workshop supported by the Legume Innovation Laboratory project S01.A4 was held in Tegucigalpa, Honduras from 25-27 July 2017. Leaders of bean research programs from Panama, Costa Rica, El Salvador, Honduras, Guatemala, Mexico, Haiti, Dominican Republic and Puerto Rico participated in the workshop. Each research leader made two presentations. The first presentation described bean research achievements in their country during the past 10 years. The presentations provided several examples where the Legume Innovation Laboratory has made contributions in the development, testing and release of improved cultivars in the region. A keystone to collaboration has been the distribution of regional yield trials and other cooperative nurseries more focused on specific abiotic and biotic constraints. The second round of presentations made by the investigators described research and training needs for the next 10 years to promote the production and consumption of beans. During group discussions, the participants identified areas of research that would benefit from continued collaboration in the CA/C region. The presentations of the workshop were posted on the USDA-ARS FtF Legume Project web site (FtF USDA-ARS Bean Research Team).

Funding was leveraged from the USDA-PASA program in Haiti to support the purchase and transportation of seed of the improved black bean cultivar Sankara from Idaho to Haiti. The National Seed Service (NSS) in Haiti distributed seed to producers for multiplication in irrigated fields during the winter months. During a visit to Haiti in February 2017, Dr. Porch and Dr. Beaver observed that most of the production fields had excellent emergence, vigorous plant development and little or no disease. Although the NSS had contracts to re-purchase the seed for distribution to farmers for production on the hillsides during the summer months, several of these agreements were not honored and the seed was sold in the market. This effort, nevertheless, demonstrated that bean seed produced in the Western U.S. could play a role in supplying countries in CA/C with a dependable supply of disease free, genetically pure basic seed stocks.

VI. Human Resource and Institution Capacity Strengthening

1. Degree Training in the US or elsewhere

Name of trainee	Héctor Martínez	Iveth Rodríguez	Diego Rodríguez	Didier Joseph	Carlos Maldonado	Lucy Lund
Country of citizenship	Guatemala	Honduras	Ecuador	Haiti	Guatemala	U.S.
Gender	M	F	M	M	M	F
H.C. institution	ICTA	Zamorano	INIAP	NSS	ICTA	None
Training institution	UPR	UPR	UPR	UPR	NDSU	NDSU
Supervising CRSP PI	J.S. Beaver	T.G. Porch	C. Estevez	J.S. Beaver	Juan Osorno	Phil McClean
Degree program	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 genomics
Research project title	Web blight resistance	Drought & heat tolerance	ALS resistance	Haitian bean seed quality	Resistance to anthracnose	Mol. genetics res. to bruchids
Start date	Aug. 2015	Aug. 2015	Aug. 2015	Jan. 2016	Aug. 2015	July 2015
Completion date	Jul. 2017	Sept. 2017	Jul. 2017	May 2018	Dec. 2017	June 2017
Participant trainee and registered on TraiNet?	Yes	Yes	No	No	Yes	No
Training status (Active, Completed, Pending, Discontinued, or Delayed)	Completed	Completed	Completed	Pending		

Name of trainee	Priscila Campos	Jorge Chanaluisa	Klever Arroba	Segundo Gavilanes	Belky Cabana
Country of citizenship	El Salvador	Ecuador	Ecuador	Ecuador	Peru
Gender	F	M	M	M	M
H.C. institution	None	None	None	None	None
Training institution	Zamorano	Zamorano	Zamorano	Zamorano	Zamorano
Supervising LIL 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	Web blight resistance	Web blight resistance	BGYMV resistance	BGYMV resistance	Low fertility
Start date	Jan 2017	Jan 2017	Jan 2017	Jan 2017	Jan 2017
Completion date	Nov 2017	Nov 2017	Nov 2017	Nov 2017	Nov 2017
Participant trainee and registered on TraiNet?	No	No	No	No	No
Training status (Active, Completed, Pending, Discontinued, or Delayed)	To be completed	To be completed	To be completed	To be completed	To be completed

Name of trainee	Enrique Zevallos	Elisa Solis	Maria Besilla	Katya Rivera	Andres Rosas
Country of citizenship	Peru	Guatemala	Ecuador	Honduras	Ecuador
Gender	M	F	F	F	M
H.C. institution	None	None	None	None	None
Training institution	Zamorano	Zamorano	Zamorano	Zamorano	Zamorano
Supervising LIL 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	Low fertility	Low fertility	Low fertility	Heat tolerance	BNF
Start date	Jan 2017	Jan 2017	Jan 2017	Jan 2017	Jan 2017
Completion date	Nov 2017	Nov 2017	Nov 2017	Nov 2017	Nov 2017
Participant trainee and registered on TraiNet?	No	No	No	No	No
Training status (Active, Completed, Pending, Discontinued, or Delayed)	To be completed	To be completed	To be completed	To be completed	To be completed

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. Co-PIs from Puerto Rico and Guatemala are women. Project S01.A4 also supports the participation of women in formal and informal training activities.

VIII. Implementation of Data Management Plan

A data management plan was prepared and submitted to the Management Office of the Legume Innovation Laboratory. A portion of the data collected by project personnel concerning the Andean and Middle American Diversity Panels, and the SNP data from the ADP, is available on the web site of the USDA-ARS FtF project (www.arsftfbean.uprm.edu). Other data sets will be uploaded into the USAID DDL.

IX. Scholarly Accomplishments

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.

Colbert, R. 2017. Selection of common bean to broad environmental adaptation in Haiti. Paper presented at the Grain Legume Research Conference, Burkina Faso, 13-18 August 2017.

Estevez de Jensen, C., T.G. Porch and J.S. Beaver. 2017. Nodulation ability of the common bean genotypes composing the BASE 120 trial after inoculation with *Rhizobium tropici* and *R. etli*. Poster presented at the Grain Legume Research Conference, Burkina Faso, 13-18 August 2017.

Heilig, J.A., J.S. Beaver, E.M. Wright, Q. Song, and J.D. Kelly. 2017. QTL Analysis of Symbiotic Nitrogen Fixation in a Black Bean Population. Crop Sci. 57:118–129.

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

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.

Maredia, M.K.,; D. DeYoung, D.; E. Prophete, E.; C.D. Joseph, C.D.; Beaver, J.; T. Porch, T. 2017. Adoption of improved bean varieties in Haiti: An assessment using farm surveys, bean seed supply chain analysis, and DNA fingerprinting. Paper presented at the Grain Legume Research Conference, Burkina Faso, 13-18 August 2017.

Martínez Figueroa, H.D. 2017. Desarrollo de líneas de frijol (*Phaseolus vulgaris* L.) que combinen resistencia a mustia hilachosa [*Thanatephorus cucumeris* Frank (Donk)], bacteriosis común (*Xanthomonas axonopodis pv. phaseoli*) y los virus BGYMV, BCMV y BCMNV. M.S. Thesis. Univ. of Puerto Rico. Mayaguez, Puerto Rico. Mayaguez, Puerto Rico. 102 p.

Miranda, A. 2017. Screening bean lines for resistance to the common and Mexican bean weevil in Guatemala. Paper presented at the Grain Legume Research Conference, Burkina Faso, 13-18 August 2017.

Martínez Figueroa, H.D., J.C. Rosas, C. Estévez de Jensen, T.G. Porch, J.S. Beaver. 2017. Selection of bean lines that combine resistance to web blight and common bacterial blight. Poster presented at the Grain Legume Research Conference, Burkina Faso, 13-18 August 2017.

Miklas, P.N, D. Fourie, B. Chaves, and C. Chirembe. 2017. Common bacterial blight resistance QTL BC420 and SU91 effect on seed yield, seed weight, and canning quality in dry bean. Crop Sci. 57:802-811.

Porch, T., I. Rodriguez, J. Hart, A. Vargas, J.C. Rosas, M.Brick, J.S. Beaver. 2017. Advances in tepary bean (*Phaseolus acutifolius* A. Gray) genetics and breeding. Paper presented at the Grain Legume Research Conference, Burkina Faso, 13-18 August 2017.

Prophete, E., G. Démosthène, J.C. Rosas, P.N. Miklas, T.G. Porch and J.S. Beaver. 2017. Development, release and dissemination of Sankara black bean in Haiti. Poster presented at the Grain Legume Research Conference, Burkina Faso, 13-18 August 2017.

Rodriguez, I.Y. 2017. Análisis fenotípico y genético de frijol tépari (*Phaseolus acutifolius* A. Gray) para tolerancia a factores bióticos y abióticos. M.S. Thesis. Univ. of Puerto Rico. Mayaguez, Puerto Rico. 82 p.

Rodriguez, I.Y., J. Hart, J.S. Beaver y T.G. Porch. 2017. Identificación de QTLs en frijol tépari bajo estrés abiótico, usando una población RIL. Paper presented at the 2017 meeting of the PCCMCA, San Salvador, El Salvador.

Rodríguez Ortega, D.G. 2017. Análisis fenotípico y genético de la resistencia a mancha angular (*Pseudocercospora griseola*) en el cultivo de frijol común (*Phaseolus vulgaris* L.). M.S. Thesis. Univ. of Puerto Rico. 67 p.

Rosas, J.C., C. Estevez de Jensen, C., J.S. Beaver and T.G. Porch. 2017. A differential nursery for testing nodulation and effectiveness of Rhizobium strains in common beans. Poster presented at the Grain Legume Research Conference, Burkina Faso, 13-18 August 2017.

Rosas, J.C., J.S. Beaver, T.G. Porch, S.E. Beebe, J.S. Burridge and J.P. Lynch. 2017. Progress in the selection of common bean lines with adaptation to high temperatures. Poster presented at the Grain Legume Research Conference, Burkina Faso, 13-18 August 2017.

Tock, A.J., D. Fourie, P.G. Walley, E.B. Holub, A. Soler, K.A. Cichy, M.A. Pastor-Corrales, Q. Song, T.G. Porch, J.P. Hart, R.C.C. Vasconcellos, J.G. Vicente, G.C. Barker and P.N. Miklas. 2017. Genome-wide linkage and association mapping of halo blight resistance in common bean to race 6 of the globally important bacterial pathogen. Front. Plant Sci. 8:1170. doi: 10.3389/fpls.2017.01170

Valentín Torres, S., M.M. Vargas, G. Godoy-Lutz, T.G. Porch, 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.

X. Achievement of Impact Pathway Action Plan

- Many of the bean improved cultivars used in Central America and Haiti were developed with support from the Legume Innovation Laboratory.
- Zamorano continues to be an important source of genetic and foundation seed for the national research institutions, national seed systems in CA/C, NGOs and farmer organizations in Honduras.
- Zamorano also provided foundation seed to the dissemination project coordinated by MSU in collaboration with Technoserve and DICTA in Honduras.
- Supplemental funding provided by the Legume Innovation Laboratory enhanced the capability of the NSS to produce and store bean seed at the research station in Savane Zombi.
- It is encouraging that the bean research programs in Guatemala and Haiti have begun to develop bean breeding populations. This reflects an enhanced capacity to conduct independent research. Within the CA/C region, it is reassuring that bean research in the Dominican Republic and Costa Rica has continued after support from USAID discontinued several years ago. It should be noted, however, that the modest level of funding provided by S01.A4 to support collaboration and training among CA/C bean programs has allowed a regional network to continue to exist.