

**LEGUME INNOVATION LAB FOR COLLABORATIVE RESEARCH
ON GRAIN LEGUMES**

FY 2017 WORKPLAN FORMAT

Project Code and Title: SO1.A1- Genetic Improvement of Middle-American Climbing Beans for Guatemala

Lead U.S. Principal Investigator (PI) and affiliated Lead U.S. University:

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Host Country and U.S. Co-PIs and Institutions:

Phil McClean, Dept. of Plant Sciences, North Dakota State University. Fargo-ND 58108

Julio C. Villatoro, ICTA-Guatemala

Angela Miranda, ICTA-Guatemala

Jessica Moscoso, ICTA-Guatemala

I. Project Problem Statement and Justification:

II. Planned Project Activities for the Workplan Period (October 1, 2016 – September 30, 2017)

Objective 1: Development of germplasm with improved disease resistance and agronomic performance.

Collaborators:

NDSU: Juan M. Osorno and Phil McClean.

ICTA: Julio Cesar Villatoro, Angela Miranda, Jessica Moscoso.

Approaches and Methods:

1.1: Validation plots and release of at least 1 climbing bean cultivar (ICTA): After 3 years of farmer's field testing (see previous annual reports), a selected group of 2-3 breeding lines will be grown in what ICTA calls validation plots across 10-15 locations in the western highlands of Guatemala. Each field consist of ~400 m² planted with one of the selected lines and growth next to or near the variety or varieties the grower normally uses. Some lines such as Bolonillo-Texel have been already in validation plots during the 2015 and 2016 growing seasons. Seed yield and other traits will be compared with common varieties and landraces grown in the vicinity of the testing fields (similar to sentinel plots).

The resulting information coming from multiple locations will allow understanding if only 1 line should be released or recommended for the entire highland ecosystem or if specific lines seem to be more adapted to specific regions and/or ecosystems better than others. This validation step (locally known as "Ensayos de Validacion"), is crucial for the approval of a new variety under the ICTA-Guatemala seed system. The releasing of an improved variety of climbing bean in 4 years of this project

is possible thanks to the previous efforts made by the ICTA bean breeding project. In addition, we'll keep monitoring the phenotypic heterogeneity of the lines tested (objective 1.3).

1.2: Breeding pipeline (ICTA/NDSU): Approximately 97 advanced lines obtained from individual plant selections and purifications made during the last 2 years (objective 1.3) will be evaluated in replicated field trials at the ICTA stations in Chimaltenango and Quetzaltenango. This number includes individual plant selections made within heterogeneous lines (based on overall disease resistance/tolerance, pod load, color, and seed type). An average of 3 individual plants was selected within lines of interest. This material was planted again in 2016 at ICTA-Quetzaltenango for field evaluation under trellis conditions (Monoculture instead of intercropping to facilitate visual selection). Phenotypic selection will be practiced again. Selected advanced lines will be given to ICTA to continue field testing in subsequent years beyond FY2017 if additional funding is available after this 4-year cycle.

1.3: Genetic purification of selected advanced lines (ICTA/NDSU): A final effort to purify the advanced lines will be made if genetic heterogeneity is visually detected. During the 2016 growing season, plants with apparent superior performance (e.g. disease resistance, seed yield, pod distribution, seed quality, climbing aggressiveness, earliness, etc.) were tagged and individually harvested as a single plant selections. The seed from each individual plant selected will be in the off-season nursery at San Jeronimo in FY2017 for: i) seed increase, and ii) further evaluations.

1.4: Third crossing block (ICTA/NDSU): Another set of crosses will be attempted in FY2017 at the ICTA-Chimaltenango station in order to keep feeding the breeding pipeline (Objective 1.2). At least 40 new parental combinations will be made in order to create new segregant populations that can be used for selection in later generations. All this material contributes to the breeding pipeline (objective 1.2) that will help to establish a long-term breeding program that will continue developing improved climbing beans adapted to the region in the future. It is important to note that besides the breeding efforts made by CIAT on climbing beans, this is the second breeding program focused on climbing beans in the western hemisphere.

Objective 2: Characterization of the genetic diversity of this unique set of germplasm.

Collaborators:

NDSU: Juan M. Osorno and Phil McClean.

ICTA: Julio Cesar Villatoro.

Approaches and Methods:

2.1 Evaluation of core collection with the 6k SNP chip (NDSU): Genotyping of the climbing bean germplasm collection has been completed by running the 6k chip in ~400 accessions. SNP data was recently received from Hudson Alpha Institute and it is currently being processed for analysis as it is usually done in Phil McClean's lab. Gene call and identification of heterozygotes is one of the first main steps in order to control for data quality. We are nearing the completion of the SNP calling step. This has required us to evaluate several SNP calling software for our purposes. These include GATK and VarScan. That final SNP data set will be used for multiple GWAS analysis using data collected at ICTA and data collected by LIL project participants.

Monomorphic markers as well as markers with more than 50% of missing information will be discarded. Several parameters of population diversity and structure will be used to assess the organization of the genetic diversity in this group of germplasm. An attempt to do comparisons with other genetic groups/races previously analyzed by the BeanCAP project and others, will also allow having a better understanding of where this group of germplasm could fit into what is known about bean genetic diversity (gene pools and race organization). As suggested in several previous studies, the climbing beans from Guatemala tend to cluster as a separate race (labeled as "Guatemala race") within the Mesoamerican gene pool.

The genotyping and analyses will be part of the research topic for one of the M.S. students coming to NDSU from ICTA. The NDSU bean genomics lab under the direction of Phil McClean has a lot of expertise in this area and will be in charge of these analyses. Some of the results found in this study will aid in the planning and designing of the crossing block during FY2017. This core 300 collection could be used as a diversity panel that could be used for Genome Wide Association Studies (GWAS). This will allow identifying genomic regions associated with traits of agronomic/economic importance within this unique group of germplasm. This approach has been successful already in common bean, identifying regions associated with growth habit, seed color, seed size, days to flowering, among others. A similar approach is being used as a thesis topic for two more M.S. students from ICTA to identify candidate regions associated with disease resistance genes (rust and anthracnose). GWAS analysis will be made by using the GAPIT software/pipeline commonly used in the lab. The analysis will consist of four models: naïve (no population structure or relatedness control); general linear model (structure control); mixed linear model (relatedness control); and mixed linear model (population and relatedness control). The output from the analyses will be compared, and the model that best fit the expected distribution will be selected. The p-value distribution will be bootstrapped, and the SNPs at the 0.1% and 0.01% lower tail of the distribution will be considered significant. This project has pre-release access to version 2 of the annotation of common bean genome. The release will be used to select candidate genes. Genes within 50kb up- and down-stream of the most significant SNP peak will be selected as potential candidate genes that control a specific trait.

Other members of the project will also use the SNP data during the upcoming year. The original ICTA population is being screened for response to local bean rust and anthracnose isolates collected in Guatemala as part of this project. That data will be subjected to GWAS will use the same protocol just

described above. This approach will provide a unique opportunity to confirm the results obtained by the evaluation of the bean rust and anthracnose isolates. Disease resistance genes are well-known to cluster in common bean. The specific disease resistance specificities have been localized within these clusters. Therefore, the location of the GWAS peak will correspond to a unique resistance specificity. If the peak does not fall within the location of a known resistance specificity, then we will know that we are observing a new race of the pathogen and a new resistance locus. Those lines expressing resistance will then be useful parents for further genetic studies to identify the resistance gene and to introgress that resistance into the Guatemalan bean improvement program.

2.2 SNP Evaluation of the new Guatemalan collection (NDSU): A total of 452 samples of beans were donated by local farmers as part of the grower's survey made during FY2015 (Objective 3). This came in the form of donations from 1-2 seeds to several dozen seeds. These seeds represent the beans that are currently being grown throughout the highlands of Guatemala, the target region of the project. This is a major new resource that needs full characterization both phenotypically and genotypically. In addition, an additional 48 samples were provided by an additional ICTA collection made during 2014. Of the 500 samples, 420 contained only a single seedtype (black, red, white). Sequence-based SNP data from this new collection will be compared with the SNP data from the original collection housed at ICTA. A major advantage of this newer collection is that passport data (GPS coordinates, location, altitude, etc.) is available while this was lost for the original collection as mentioned in previous documents. We will attempt to do a geographical correlation between both collections using the genotypic data obtained from both groups.

This 500-entry collection consists of black, red, and white seeded beans, and 420 of the donations contain only a single seed type. Those donations with mixed seed types were split into two subsamples based on seed type. All totaled, 580 samples will be characterized. Plants for all of the samples will be grown in the greenhouse until the initial trifoliolate leaves emerge. Leaf samples will be collected on a single plant. DNA will then be isolated from all of the samples. We will then develop five or six (depending on final number of lines) pooled, low-pass sequencing libraries, and DNA sequence data will be collected from 230 base pair reads. The reads will be processed using the GATK software from The Broad Institute, MIT. The final SNP collection will be imputed to develop the final SNP set to be used for analysis.

The SNP data from this population will be merged with the SNP data for ICTA population. A STRUCTURE analysis will be performed that will provide a characterization of the subpopulation structure of the original and new populations. We will be looking to determine if members of the new population are distributed across the subpopulations of the original populations, or whether they represent a new distinct population. The results of this analysis will inform choices for parents in new crosses for Guatemalan bean improvement.

2.3 Field evaluation of the newer ICTA collection of climbing beans (ICTA/NDSU): A final evaluation of the new germplasm collection obtained from the grower survey (see objective 3.2) will be made at the ICTA station in Chimaltenango during the growing season of 2017 to allow a re-evaluation of the material and also the production of a newer batch of seed. Approximately 490 accessions were obtained during the grower survey. In addition, 100 accessions have been obtained from the genetic resources

group at ICTA which were collected during 2014. Each accession is planted in short rows (~2 m) in a trellis system mostly for phenotypic observation. The following traits are recorded: disease reaction under natural conditions (Ascochyta, Rust, Anthracnose), earliness, biomass/climbing aggressiveness, seed yield potential, and pod distribution (upper vs. homogeneous distribution). Selected germplasm will be used in future crosses.

Objective 3: A better understanding of the current socio-economic status and needs of bean production within the context of intercropping systems in the region.

Collaborators:

NDSU: Juan M. Osorno.

ICTA: Julio Cesar Villatoro, Jessica Moscoso, Angela Miranda.

MSU: Mywish Maredia and David DeYoung.

Approaches and Methods:

3.1 Final statistical analyses of survey data and publication of results (ICTA/MSU/NDSU):

Final statistical analyses and publication of results are the remaining activities of this objective. The survey activity was very successful thanks to a great collaboration established with the project lead by Mywish Maredia (SO4.1). They have far more experience with surveys than any person in our team, so we appreciate their willingness to help. In the same way Gustavo Mejia, a social economist at ICTA-Quetzaltenango was of key importance in coordinating this activity. Results of this survey will be shared not only within the project but with other projects currently working in Guatemala (e.g. Masfrijol) and government agencies interested. No field activities are planned for this objective during FY17.

3.2 Conditioning and storage of seed samples collected during the survey (ICTA): During the deployment of the survey during FY2015, growers were asked to provide a small seed sample of the variety or varieties they commonly grow in their farms. Once the seed increase of these germplasm is completed during FY2016, seed will be conditioned and stored at the cold seed room available at the ICTA-Chimaltenango station.

Objective 4: Capacity building: training the next generation of plant breeders for Guatemala and establishing a long-term breeding plan to increase the productivity of climbing bean in the region.

4.1. Graduate Students (NDSU): The 2 students recruited from ICTA for M.S. degrees at NDSU continue to make progress towards their degree. We expect they will be able to complete their programs by the end of FY2017. Funds are needed to pay for their assistantship as well as research supplies so they can successfully complete their degrees.

4.2. Long-term breeding plan (ICTA/NDSU): A final document describing a long term plan to continue breeding activities for climbing beans will be developed. This document will help to be a future roadmap regardless of the availability of funds in the future. If future funding opportunities arise, this document will facilitate the writing of a new project that will keep the momentum gained during these 4 year cycle.

4.3. Plant Breeding workshop (ICTA/NDSU): A 2-day workshop will be held during the summer 2017 at ICTA-Guatemala focused on plant breeding theory and techniques. All ICTA personnel currently doing work related to plant breeding in any crop will be invited. In addition, faculty/students from local universities as well as national programs from the Central American region will be invited to participate but they will have to cover their travel expenses.

III. Contribution of Project to USAID Feed the Future Performance Indicators:

See attached table with Future Performance Indicators.

IV. Outputs:

1. Objective 1:

- 1.1. Validation plots and release of at least 1 climbing bean cultivar (ICTA)**
- 1.2. Breeding pipeline (ICTA/NDSU)**
- 1.3. Genetic purification of selected advanced lines (ICTA/NDSU)**
- 1.4. Third crossing block (ICTA/NDSU)**

2. Objective 2:

- 2.1. SNP evaluation of core collection with the 6k SNP chip (NDSU).**
- 2.2. SNP evaluation of the new Guatemalan collection (NDSU).**
- 2.3. Field evaluation of the new collection of climbing beans (ICTA/NDSU)**

3. Objective 3:

- 3.1. Final statistical analyses of survey data and publication of results (ICTA/MSU/NDSU).**
- 3.2. Conditioning and storage of seed samples collected during the survey (ICTA)**

4. Objective 4:

- 4.1. Two graduate students at NDSU (ICTA/NDSU).**
- 4.2. Long-term breeding plan (ICTA/NDSU).**
- 4.3. Plant Breeding workshop (ICTA/NDSU).**

V. Engagement of USAID Field Mission(s)

Local USAID Mission in Guatemala is always contacted when U.S. scientists visit. In most cases, briefing meetings are held in their offices. Host country scientists are also responsible of informing local USAID Missions about progress of the Legume Innovation Lab project toward research and training objectives. Opportunities will be sought to obtain USAID Mission support to expand activities in host countries.

VI. Partnering and Networking Activities:

The NDSU scientists responsible for this project (Osorno and McClean) are also involved in other projects from the Legume Innovation Lab (e.g. S0.A4). Therefore, some collaboration among projects is expected. The personnel from EAP-Honduras (J.C. Rosas) have also shared germplasm and expertise to help in any way possible. Efforts will be made to travel around the same dates to the region in order to discuss the project's evolution. Close collaboration with project SO4.1 (Mywish Maredia) has been crucial for the success of the survey activities. In addition, P. McClean is directly involved with the project lead by Penn State (J. Lynch) on climate-resilient beans and also funded by USAID.

Several Legume Innovation Laboratory scientists participate in Regional Hatch Project W-3150 which is a multi-disciplinary network of U.S. bean researchers. The NDSU dry bean breeding program at NDSU conducts winter nurseries at Puerto Rico and this will allow for further discussion of the projects on a person-to-person base. In addition, most scientists involved in the project will meet every other year at the Bean Improvement Cooperative (BIC) meetings and other scientific meetings. Researchers in Central America and the Caribbean often make scientific presentations at the annual meeting of the PCCMCA. The meeting provides an opportunity for the Central American/Caribbean research network which includes national programs, CIAT and the Legume Innovation Laboratory scientists to meet to exchange results from research and plan activities for the upcoming year. Efforts will be made to participate at these meetings in Central America and share the project developments.

Last but not least, efforts will be made to have close collaboration with the Masfrijol project funded by the USAID Guatemala mission and lead by Luis Flores from Michigan State Univ. Advanced genetic material developed by our project will be shared with them for field testing and studies on consumer preferences. Efforts will be made to meet with members of this group whenever possible to keep both project updated on the current activities.

VII. Leveraging of CRSP Resources:

Germplasm exchange is still a common activity among dry bean breeders and even boosted up by some of the networks previously mentioned. The germplasm developed in this project could be useful in other regions growing climbing beans. In addition, the genetic material could have unique genes/sources of resistance/tolerance to production problems also present in the United States.

Some of the genomic resources and tools developed by the BeanCAP project funded by USDA-NIFA will be of great help to start these breeding platforms in Guatemala and other developing countries.

Legume Innovation Lab breeders and pathologists (Kelly, Steadman, Urrea, Osorno, Beaver, Estevez, and Porch) have an opportunity to meet at least once a year in Puerto Rico. This facilitates communication between the Legume Innovation Lab bean breeding projects. In addition, close collaboration with CIAT breeders will allow germplasm exchange and sharing of the scientific knowledge.

VIII. Timeline for Achievement of Milestones of Technical Progress:

See attached file with project Milestones.

XI. Training/Capacity Building Workplan for FY 2016 – 2017 (use format below)

Degree Training:

Degree Training:

First and Other Given Names: Maria Gabriela

Last Name: Tobar Piñon

Citizenship: Guatemalan

Gender: Female

Training Institution: NDSU

Supervising CRSP PI: Phil McClean

Degree Program for training: M.S. in Plant Sciences

Program Areas or Discipline: Plant breeding/genomics

If enrolled at a US university, will Trainee be a "Participant Trainee" as defined by USAID? Yes

Host Country Institution to Benefit from Training: ICTA

Thesis Title/Research Area: Molecular characterization of germplasm collection of Guatemalan climbing beans.

Start Date: August 2015

Projected Completion Date: December 2017

Training status (Active, completed, pending, discontinued or delayed): Active

Type of CRSP Support (full, partial or indirect) g for training activity: Full

Degree Training:

First and Other Given Names: Luz de Maria

Last Name: Montejo

Citizenship: Guatemalan

Gender: Female

Training Institution: NDSU

Supervising CRSP PI: Juan M. Osorno

Degree Program for training: M.S. in Plant Sciences

Program Areas or Discipline: Plant breeding/genomics

If enrolled at a US university, will Trainee be a "Participant Trainee" as defined by USAID? Yes

Host Country Institution to Benefit from Training: ICTA

Thesis Title/Research Area: Disease resistance (rust) in Guatemalan climbing bean germplasm collection.

Start Date: August 2015

Projected Completion Date: December 2017

Training status (Active, completed, pending, discontinued or delayed): Active

Type of CRSP Support (full, partial or indirect) g for training activity: Full

Short term training:

Type of training: Short term in-service training

Description of training activity: A 2-day workshop focused on plant breeding theory and techniques for all ICTA personnel doing work related to plant breeding in any crop will be invited. In addition, faculty/students from local universities as well as national programs from the Central American region will be invited to participate but they will have to cover their travel expenses.

Location: ICTA/Barcenas

Duration: 2-3 days

When will it occur? - 2017

Participants/Beneficiaries of Training Activity: 12

Anticipated numbers of Beneficiaries (male and female): 7M, 5F

PI/Collaborator responsible for this training activity: Juan Osorno

List other funding sources that will be sought (if any): Some participants from the region will be funded for travel by their institution

Training justification: This training is needed to permit host country scientists to take advantage of the recent advances in crop breeding.

Equipment (costing >\$5,000):

None.