

# LEGUME INNOVATION LAB FOR COLLABORATIVE RESEARCH ON GRAIN LEGUMES

## FY 2015 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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### I. Project Problem Statement and Justification:

With approximately 11 million habitants, Guatemala is mostly a rural country, with 60% of the population living in farms and 50% of the population being indigenous. Maize and beans are the main staple food in most households with a per capita consumption of 9.4 kg per year. Since few other sources of protein are available, this amount is not enough to ensure an acceptable nutritional quality, especially within poor households. As expected, the lack of protein intake has reduced the nutritional quality in many households, significantly affecting children.

Beans are grown on 31% of the agricultural land and mostly in the low to mid-altitude regions (0-1500 masl) in a monoculture system. Contrastingly, intercropping (locally known as Milpa) is the main production system in the highlands, where maize-bean is the most common crop association. The system uses climbing beans that grow around the corn stalks. Two main methods are used: direct planting, in which both maize and beans are planted simultaneously, and relay, in which the maize is planted first and the beans are planted at a later date in order to avoid strong competition between the two crops. Unfortunately, on-farm productivity of these climbing beans is approximately one third of their genetic yield potential mostly due to the lack of improved cultivars that are able to withstand biotic and abiotic stresses. Fungal and bacterial diseases as well as pests are the main cause for yield reductions. In addition, production is made with almost no inputs of fertilizers and/or other chemicals. Historically, climbing beans worldwide have received less attention and breeding efforts in comparison with the bush-type beans commonly grown in the lowlands, as shown by the significant yield gap between regions. In addition, there are genetic and environmental interactions among species (maize, bean, squash, etc.) not well understood within the intercropping system that affect crop performance and hence, seed yield. The legume Innovation Lab has been involved in collaborative bean

breeding research targeting lowland agro-ecologies in Central America, but research for the highland bean production systems is still lacking. A significant seed yield differential between the lowlands and the highlands can be observed, especially in Guatemala.

There is an existing collection of approximately 600 accessions of climbing beans collected across all bean production regions in Guatemala. This collection is kept by ICTA and has been characterized morphologically and with few molecular markers (6 SSR primers). In addition, some field notes concerning disease resistance (natural pressure) and other agronomic traits of economic importance have been collected as well. Initial results suggest that ½ of the collection consist of duplicates. In addition, some initial crosses among climbing beans and selections have been made by Dr. Fernando Aldana (ICTA-Quetzaltenango) and the rest of the ICTA group. These lines will be used intensively in this study.

## **II. Planned Project Activities for the Workplan Period (October 1, 2014 – September 30, 2015)**

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

#### **Collaborators:**

NDSU: Juan M. Osorno and Phil McClean.

ICTA: Julio Cesar Villatoro, Fernando Aldana, Edgardo Carrillo.

#### **Approaches and Methods:**

**1.1: Field testing of 10 selected accessions (ICTA) and other early-generation genetic material:** The bean breeding program at ICTA has planted a selected a group of 10 genotypes that include accessions from the germplasm collection and crosses made by Dr. Fernando Aldana that offer agronomic traits of interest such as plant growth type, seed yield, disease resistance, earliness, and seed quality, among others. Field trials were planted in April and May 2014 at 10 locations representing five departments. Most locations will be tested under the intercropping system and few under monoculture. The accessions were planted using a Randomized Complete Block Design (RCBD) with 2 or 3 replications depending on space and resources at each location.

An effort to collect all the following agronomic data will be made within each plot at all locations:

- Days to emergence: Bean seedlings counted 20 days after planting
- Vigor: Visual scale of 1-9 where 1 is best and 9 is worst.
- Early disease symptoms: CIAT scale 1-9 will be used for any disease naturally occurring at any of the locations.

- Days to Flowering: Number of days after planting in which 50% of the plants in a plot have at least 1 flower.
- Pod distribution: Classified either as columnar (even pod distribution across all plant) or pyramidal (uneven pod distribution with most pods around the higher part of the plant).
- Climbing aggressiveness: Classified as low, medium, or high depending on a visual estimate of plant biomass (leaf, stems, and pods)
- Disease symptoms (natural pressure): CIAT scale 1-9 will be used for any disease naturally occurring at any of the locations.
- Days to maturity: Days after planting when at least 50% of the plants within a plot are ready for harvest.
- Seed yield: Weight in g of seeds after threshing and converted to kg/ha.
- 100-seed weight: the weight in g of 100 seeds collected randomly for the seeds obtained from each plot.

In addition to these 10 selected accessions, a group of 23 bolonillo advanced lines and 28 early-generation ( $F_3$ ) bolonillo lines were planted at the ICTA station in Quetzaltenango in FY2014 and will be harvested at the beginning of FY2015. Also, 23 lines of different classes of climbing beans (bolonillo, piloy, etc.), were planted at the ICTA station in Chimaltenango in FY2014. Agronomic evaluation of this material during the last phenological stages (early FY2015) will include several of the traits mentioned previously.

Once all the data is collected at the end of the year, decisions will be made about selecting a smaller number of lines for testing in FY2015 again across 10 locations. The number of entries to be tested in the following year will depend on this year's results. The same field testing protocol will be applied for this new cycle of field testing. In addition, we'll keep monitoring the phenotypic heterogeneity of the lines tested (objective 1.2).

**1.2: Genetic purification of selected material (ICTA):** As explained Technical Project Description, phenotypic variation has been detected within accessions. Some individual plant selections were made last year and will be tested again this year for homogeneity at the Chimaltenango station. In addition, visual evaluation for phenotypic heterogeneity will be made across all field testing locations in order to have a better estimate of genetic variability within each line tested. If genetic heterogeneity is visually detected, plants with apparent superior performance (e.g. disease resistance, seed yield, pod distribution, climbing aggressiveness, earliness, etc.) will be tagged to be harvested as a single plant selection. The seed from each individual plant selected will be used in the future for: i) seed increase, and ii) further evaluations.

**1.3: Field evaluation of Bolonillo-TEXEL (ICTA):** One of the improved lines selected by Dr. Fernando Aldana at advanced breeding stages (known as Bolonillo-Textel) was planted in 20 grower's fields during FY2014. Each field consist of 400 m<sup>2</sup> planted with Bolonillo-Textel and surrounded by the variety or varieties the grower normally uses. An attempt to measure the same traits mentioned above will be measured across all locations or at least, a subset of them. 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).

In order to obtain additional data about the agronomic performance of Bolonillo-Textel in the target regions, a similar approach will be implemented in FY2015. The resulting information coming from multiple locations will allow understanding if Bolonillo-Textel should be released or recommended for all the highland ecosystem or if the new variety seems to be more adapted to specific regions and/or ecosystems better than others. If Bolonillo-Textel has good acceptability it could be released sooner, which would allow for a significant impact of this project earlier than planned by releasing an improved variety of climbing bean thanks to the previous efforts made by the ICTA bean breeding project.

**1.4: First crossing block:** With the results obtained from the field testing and the evaluation of the germplasm collection, a first set of potential parents will be planted in the greenhouse at the ICTA station in Chimaltenango during the 2015 growing season. The use of an offseason growing cycle in the lowlands (e.g. San Jeronimo) will be tested to see if it would be possible to advance 2 generations per year. The first generation of single crosses will be designed and will serve as the first batch of genetic material towards the creation of breeding pipeline that will help to establish a long-term breeding program that will continue developing improved climbing beans adapted to the region in the future.

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

### **Collaborators:**

NDSU: Juan M. Osorno and Phil McClean.

ICTA: Karla Ponciano, Julio Cesar Villatoro, Fernando Aldana, Edgardo Carrillo.

### **Approaches and Methods:**

**2.1: Evaluation of core collection with the 6k SNP chip (NDSU):** The core collection of approximately 300 accessions will be re-evaluated with a larger number of markers at NDSU. A new set of 6000 Single Nucleotide Polymorphism (SNP) markers is available as product from the BeanCAP project ([www.beancap.org](http://www.beancap.org)) funded by USDA-NIFA. This set of markers is highly precise, reliable, and allow higher resolution and differentiation among genotypes compared to SSR markers. With the goal of having a better understanding of the organization of the genetic diversity of this group, we will extract DNA of the core 300 accessions and screen them with the 6k beancap chip and do a genetic diversity study of possible genetic relationships among the accessions. The big issue to accomplish this objective has been the processing of a phytosanitary

certificate by the Guatemalan Ministry of Agriculture in order to send the seed to NDSU for DNA extraction and molecular analysis. Therefore, we are behind our proposed timeline in this regard. Once a phytosanitary certificate is obtained from the Guatemalan Ministry of Agriculture (expected in the next two months), seed from each accession of the climbing bean collection will be sent to NDSU for DNA extraction and SNP genotyping.

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 it 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 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. In addition, a random group of 20 accessions previously identified as duplicates based on the SSR data will be also screened in order to confirm these results or evaluate the need to include more of these duplicates in the screening. Some of the results found in this study will aid in the planning and designing of the crossing block during FY2015.

**2.2: Assessment of the intra-accession variability (NDSU):** As mentioned before, once a phytosanitary certificate is obtained from the Guatemalan Ministry of Agriculture, seed from each accession of the climbing bean collection will be sent to NDSU for DNA extraction and SNP genotyping. Genetic assessment of variation within the 10 selected lines used in objective 1A will be made in order to account for the heterogeneity at the molecular level not only among but within accessions and possibly, extrapolate that information to the rest of accessions. In addition, this information will be compared with the data obtained from visual evaluation of the phenotypic heterogeneity in the field.

Preliminary phenotypic observations in the field suggest that there is a high amount of genetic heterogeneity (heterozygosity) within accessions. Therefore, 20 plants from each accession will be planted in the greenhouse at NDSU and DNA will be extracted, for a total of 200 DNA samples/individuals. These genotypes will be also screened with a subset of INDEL markers developed in the NDSU bean molecular genetics lab (Moghaddam et al., 2013). The INDEL markers were developed from polymorphic SNPs, but their advantage is that they can be easily reproduced by PCR and visualized in an agarose gel. Since the main goal is to assess intra-accession variability, this will be easily detected by looking at the bands in the gels. Polymorphic Information Content (PIC) and other genetic parameters will be estimated.

**2.3 Field evaluation of the ICTA collection of climbing beans (ICTA-NDSU):** The entire collection of climbing beans from ICTA has been planted in FY2014 at the ICTA station in Chimaltenango to allow a re-evaluation of the material and also the production of a newer batch of seed. Each accession has been planted in short rows (~2 m) mostly for phenotypic

observation. The project director will spend time taking notes and evaluating this collection in order to make sure accessions with potential interest are not missed. Some of these evaluations will overlap with FY2015.

All this information will allow a better understanding of the organization of the genetic diversity within this core collection for future use and research. The results obtained in this first phase will allow making informed decisions about the potential parents for the first set of 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 Martinez, Julio Cesar Villatoro, Fernando Aldana, Edgardo Carrillo.

**Approaches and Methods (Julio Martinez-ICTA):**

A grower survey will be deployed in the main regions where climbing beans are produced. We will focus on the following departments: Quiche, Huehuetenango, San Marcos, Totonicapán, and Quetzaltenango which represent most of the climbing bean production areas. A proper sample size for accurate statistical analyses and estimates will be decided based on the previous information collected. The survey will include questions about cultivation methods, preferred seed types, household consumption, and marketing of harvested beans, among other things.

The survey is currently under development (FY2014) in collaboration with Legume Innovation Lab. project SO5.A1 (Dr. Mywish Maredia). In addition, IRB approval of the survey protocol by NDSU will be needed before the survey can be actually executed. Julio Martinez is the social economist at ICTA and will be leading the execution of these surveys in the field once approved. Once information is collected, data will be tabulated and analyzed by Julio in collaboration with project SO5.A1. 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.

**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.**

Recruiting efforts during FY2014 at ICTA have allowed the identification of one potential candidate for M.S. at NDSU. Gabriela Tobar Piñon is an ICTA employee initially identified through the CAPA project, which is an early career program at ICTA to identify outstanding individuals for future employment and ICTA. Gabriela has expressed interest and hopes to start graduate studies at NDSU in the fall semester of 2014. She is currently in the application process, with TOEFL and GRE requirements already met. The project director interviewed four

more candidates during FY2014 and will keep interviewing potential candidates during his visits to Guatemala during FY2015 in order to identify a second candidate. The main issue with the candidates is the low level of English skills found among most of them. If suitable candidates cannot be found in Guatemala, good candidates from neighboring countries could be considered. We expect to have a second individual to come and do graduate studies at NDSU (Plant Sciences) starting in 2015 with the goal that they will be incorporated into agricultural research back into the region in the future.

Research topics will be directly related to the research objectives described above. We foresee research projects focused on the analyses of genetic diversity, genetic resistance to diseases, and production systems, among others. The graduate students will be provided a broad range of training in conventional and molecular plant breeding techniques so that they can assume leadership roles in bean research programs in the target countries.

### **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. Field testing of 10 selected accessions and other early-generation genetic material (ICTA).
- 1.2. Genetic purification of selected material (ICTA).
- 1.3. Field evaluation of Bolonillo-TEXEL (ICTA).
- 1.4. First crossing block (ICTA)

#### **2. Objective 2:**

- 2.1. Evaluation of core collection with the 6k SNP chip (NDSU).
- 2.2. Assessment of the intra-accession variability (NDSU).
- 2.3. Field evaluation of the ICTA collection of climbing beans

#### **3. Objective 3:**

- 3.1. Grower surveys at 5 departments (ICTA).
- 3.2. Data tabulation and analysis (ICTA).

#### **4. Objective 4:**

- 4.1. Identification/recruitment of potential graduate students (ICTA-NDSU).
- 4.2. First 2 graduate students at NDSU (ICTA-NDSU).

### **V. Engagement of USAID Field Mission(s)**

Host country scientists will be 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. Local USAID Missions will be contacted when U.S. scientists visit 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. S01.A4). Therefore, some collaboration among projects is expected. The personnel from EAP-Honduras (J.C. Rosas) have also expressed their willingness 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. In addition, P. McClean will be 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-2150 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. Advance 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.

**Training/Capacity Building Workplan for FY 2013 – 2014** (use format below)

**Degree Training:**

1 graduate student are expected to start M.S. at NDSU in the fall 2014.  
Recruitment efforts are currently underway to identify a second candidate.

**Degree Training:**

First and Other Given Names

Last Name

Citizenship

Gender

Training Institution

Supervising CRSP PI

Degree Program for training

Program Areas or Discipline

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

Host Country Institution to Benefit from Training

Thesis Title/Research Area

Start Date

Projected Completion Date

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

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

**Short-term Training:**

None for this year. Informal training will be given to the ICTA personnel when the NDSU scientists visit the country.

**Equipment** (costing > \$5,000):

None.