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

FY 2014–2015 Annual Project Technical Progress Report
(April 1, 2015 – September 30, 2016)

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

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I. Abstract of Research and Capacity Strengthening Achievements

The project continues to make progress towards the testing and release of improved climbing beans for the highlands if Guatemala. A total of 10 lines have been tested across more than 15 locations at farmer's fields in order to obtain information about their agronomic performance as well as grower's feedback. Results from framer field trials during the 2016 growing season will be needed before making final decisions regarding release of varieties. Three lines are showing the best combination of seed yield and reduced aggressiveness that would allow high productivity in the maize under the Milpa system. On-farm testing of Bolonillo Texel, Labor Ovalle, and Utatlan continued during FY2016 in order to ensure adaptation and acceptability by growers. Genetic purification of Bolonillo-Texel is at its last stages in order to guarantee a homogeneous variety released. Even though this is not a big issue for growers, this will facilitate seed production of this variety in the future. The climbing bean collection was finally received at NDSU and genotyping of the collection has been done as planned. Approximately 150k SNP markers were used to characterize a non-duplicated subset 420 accessions from this germplasm collection. Preliminary results show that this population is structured as an admixture of genotypes with no clear separation into subgroups. Genetic relationships with other races, gene pools, and species are currently under study. Approximately 95% of the seed samples obtained from the grower survey were successfully increased at the ICTA greenhouses and are now increased and characterized in the field during the 2016 growing season. This is a great opportunity to assess the current genetic

diversity being used by growers as well as to compare with the original climbing bean germplasm collection collected 30 years ago. Preliminary results from the growers survey allowed a better understanding if the current situation of climbing bean production and consumption in this region. The 3 students (2 female, 1 male) recruited to do their M.S. training in plant breeding and genomics at NDSU continue to make progress and programs should be completed by FY2017. This will ensure the next generation of bean scientists for Guatemala. Personnel from ICTA and Zamorano had the opportunity to do a site visit to NDSU dry bean breeding program during July 2016. In addition, new collaborations have been established with project SO4-1 and MASFRIJOL to augment the success of the breeding efforts of this project.

II. 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 bean 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. Chronic malnutrition is frequent among children under 5 years old in the western highlands, with 67% of children affected, making Guatemala the country with the highest malnutrition level in the western hemisphere. One out of every three children from ages six to 59 months in the western highlands shows some degree of anemia. Approximately 18% of reproductive-age women exhibit anemia, with 29% prevalence among pregnant women and 23% prevalence among breastfeeding women.

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. 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 may 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.

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, agronomically, and with few molecular markers (6 SSR primers). 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 the ICTA group. These lines will be used intensively in this project.

III. Technical Research Progress

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, Edgardo Carrillo.

1.1: Farmer's field testing of 10 selected lines (ICTA): A total of 10 climbing bean breeding lines that are at advanced breeding stages were selected to be part of field trials:

1. Bolonillo Altense
2. Bolonillo Hunapu
3. Bolonillo Texel
4. Bolonillo Anita
5. Bolonillo Labor Ovalle
6. Bolonillo San Martin
7. Bolonillo ICTA Santa Lucia
8. Voluble GUATE 1120
9. Voluble GUATE 1026
10. Local check from the grower (different among farms).

Most of these breeding lines are the product of initial crosses made 5-6 years ago and subsequent composite mass-selection and testing made by Dr. Fernando Aldana at the ICTA-Quetzaltenango station (Dr. Aldana just retired from ICTA in July 2015 after more than 40 years of service). Any superior line or lines could be released as varieties in the near future while a breeding pipeline is established. The trials were planted around May and grown both at the ICTA-Quetzaltenango station and ICTA-Chimaltenango. Results from these trials from last year (FY2015) are available as Appendix 1. In addition, an evaluation for natural infestation of *Asphondylia* sp. was also made at ICTA-Chimaltenango and results are shown in Appendix 3. In general, all genotypes were affected by this insect; however Texel was the least affected and the highest yielding genotypes, confirming its yield and agronomic potential already seen across years and locations.

1.2: Breeding pipeline: With the results obtained from the field testing and the evaluation of the germplasm collection during the 2014 growing season (objective 2.3), a first set of 23 potential parents were selected by Osorno, Villatoro, McClean, and Aldana, and planted in the greenhouse at the ICTA station in Chimaltenango during the 2015 growing season. Parental accessions were selected mainly based on uniform pod distribution, potential yield, and disease resistance.

Unfortunately, the first generation of single crosses during FY15 has encountered some difficulties in regards to flowering synchronicity in spite of planting the material at staggered planting dates. Anecdotal results suggest that this germplasm is highly sensitive to daylenght (photoperiod), so the long days during the summer would not trigger flowering in these materials. Therefore, some crosses planned initially won't be accomplished and will have to be attempted again during FY16. Since the collection was planted again in the trellis system available at ICTA-Chimaltenango, we took advantage of this opportunity and attempted to do crosses in the field rather than in the greenhouse in order to save time. By doing this, we successfully obtained 36 F1 populations (Appendix 1) instead of having to wait an extra year for a second attempt. All the F1 seed was then planted in the field at ICTA-Quetzaltenango during the 2016 growing season for evaluation and generation advancement. Additional 71 crosses (Appendix 1) were also made during the 2016 growing season at ICTA-Chimaltenango and are currently being harvested at the time of this report in order to have a continuous breeding pipeline for the future (see objective 1.2).

1.3: Genetic purification of selected material (ICTA/NDSU): As explained in the Technical Project Description, phenotypic variation has been detected not only within accessions but also within the improved lines selected by Dr. Fernando Aldana at ICTA-Quetzaltenango. The main reason for this is that Dr. Aldana kept these lines as bulked lines during multiple generations and therefore, no individual plant selections have been done during the breeding process. Therefore, individual plant selections have been made within the breeding lines since the 2013 growing season. An initial set of 101 F7 and 29 F5 individual plant selections have been made based on potential yield and quality, absence of disease symptoms, pod distribution and color, and other agronomic traits. These individual selections were sent to the ICTA-San Jeronimo station for winter increase (2015-2016) and each selection was planted as individual rows for further evaluation/selection. This allowed for detection of additional genetic heterogeneity within lines while increasing seed. Since phenotypic heterogeneity was still detected, 97 individual plants from different F8 lines as well as 19 F6 lines were selected and sent to ICTA-Quetzaltenango for evaluations during the 2016 growing season. All these lines have been selected based on the characteristics mentioned above and harvest of this material is almost complete at the time of this report.

1.4: Field evaluation of the 3 most promising genotypes (Bolonillo-TEXEL, Utatlan, and Labor Ovalle (ICTA): Validation plots were also made at farmer's fields for the 3 genotypes that have shown the best potential to be released as new improved cultivars: Bolonillo-Texel, Utatlan, and Labor Ovalle. These validation plots are a side-by-side comparison of one of these genotypes against the local landrace normally grown by the farmer. Plot size is usually a "cuerda" (~410 m^2) for each variety grown in a milpa system using the local maize variety as well. Each genotype was tested across 15 locations distributed in 3 departments (Quetzaltenango, San Marcos, and Totonicapan). Combined across locations, the highest difference between the improved genotype and the local check was for Labor Ovalle (Table 1), followed by Texel. There were no significant differences between Utatlan and the local check. However, this was somewhat expected because the interesting attribute of Utatlan is not its high seed yield but its early maturity compared with the rest of genotypes. Many farmers have said this would be highly preferred. All detailed information about these trials can be found in Appendix 2. Once results from the 2016 growing season are available (last year of field testing), final decisions will be made within ICTA in order to decide which genotypes will be officially released as new climbing bean varieties for the Guatemalan highlands.

Table 1. Seed yield of 3 improved genotypes of the ICTA-climbing bean project across 15 locations during the 2015 growing season:

Genotype	**Seed Yield (Kg/Ha)**	**Seed Yield of Local Check (Kg/Ha)**	**Yield Difference (Kg/Ha)**
Utatlan	262	221	41^{NS}
Labor Ovalle	407	235	172*
Texel	506	359	148*
Mean – All Genotypes	391	271	120*

*. Significant differences ($P<0.05$) based on paired t-test.

Since these trials are mostly managed by growers, data collection is mostly focused on seed yield, agronomic performance, and personal feedback from each grower. Technical assistance from ICTA agronomists (special tanks to Eng. Elmer Estrada) and crop extension personnel from the Ministry of Agriculture have been crucial for finding these growers and locations. Results from 2016 will allow the collection of enough data across years and locations for the validation group to present this as official release at ICTA.

Differences in pod color have been noticed in these trials, which confirm the genetic heterogeneity still present in Bolonillo Texel, even though this is not a big issue for local growers since they already grow heterogeneous material in their farms. However, it is a concern for this breeding project and we are doing all necessary activities (see objective 1.2) that will allow obtaining a uniform variety at the end of this project. The MASFRIJOL project is highly interested in obtaining a new climbing bean variety for his disseminations program, so efforts

are in coordination with them in order to speed up this process as much as possible.

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, Jessica Moscoso, Angela Miranda and Maria G. Tobar-Piñon.

2.1: Evaluation of core collection with the 6k SNP chip (NDSU): Finally after several difficulties in obtaining seed of good quality and the proper phytosanitary permits to import the seeds to the U.S. (see previous reports for details), we were able to start working on this objective with very promising results so far. This is the main research topic of one of our trainees (Maria Gabriela Tobar-Piñon). The original Guatemalan climbing bean collection has 594 accessions. After selecting the accessions with seeds with uniform color, uniform shape and uniform size, the number of accessions was reduced to 377. This step was important because accessions very different within them can represent segregation and the results will not be consistent in the population structure analysis and the association mapping study. The intra-accession diversity of the collection will be evaluated in a different step of this study (Objective 2.2).

Methods

- ✓ 377 accessions of *P. vulgaris* collected in the highlands of Guatemala 50 years ago were analyzed.
- ✓ Two seeds of each landrace were planted, tissue was collected 15 days after planting.
- ✓ DNA extraction was made using the Genomic DNA Mini Kit (Plant), from IBI SCIENTIFIC.
- ✓ The libraries were created using Genotype by Sequencing and then sequenced using Illumina platform.
- ✓ Reads were mapped to the Version 2 of the G19833 reference genome sequence using BWA-MEM.
- ✓ SNPs were called using the Genome Analysis toolkit – GATK.
- ✓ A total of 102,822 SNPs were obtained.
- ✓ Using the markers, a maximum-likelihood tree was developed using SNPhylo. Markers were selected by using a Linkage Disequilibrium (LD) cut-off value of 0.1.
- ✓ Population structure was analysed using fastSTRUCTURE, with K=1 to 10. The best K was determined using the model complexity that maximizes marginal likelihood and Distruct was used to generate the plots.
- ✓ For association mapping SNPs markers with minor allele frequency ≥ 5%

(78,754 SNPs) were used and Principal Component Analysis (PCA) was used to control for population structure.
    - Two PCs cumulatively explain 12% of the variation.
- ✓ A kinship matrix, generated by EMMA algorithm (Kang et al., 2008) was used to control for individual relatedness.
- ✓ The traits evaluated for GWAS were 26, using the morphological characterization performed to this collection with GAPIT (Lipka et al., 2012). Multiple models were tested for each trait: ***Naive*** model, ***Linear*** model with fixed effect (account for population structure), ***Mixed linear*** model (Yu et al., 2006) to control for relatedness or both relatedness and population structure.
- ✓ The best model was determined using the Mean Square Differences (MSD) (Mamidi et al., 2011) and markers with a bootstrapped (1000 replications) p-value falling in the top 0.01 percentile were considered as significant levels.

Preliminary Results

The estimated number of sub-populations was nine based on the model complexity that maximizes marginal likelihood. Figure 2 shows the individuals distribution for each population. However, based on the ML tree (figure 1) and the PCA analysis (figure 3) the population structure is low. A total of 101 accessions had a value of membership higher than 0.8 in the nine populations. The analysis also shows a high percentage of admixture in the accessions. It was concluded that the high number of markers used for this analysis allowed a false determination of K using STRUCTURE. It is suggested that Guatemalan climbing beans belong to race Guatemala, and for this reason the accessions are very similar between them. A comparison with accessions of Mesoamerican and Andean races will allow the confirmation of this hypothesis and will be our next step in this study. Additionally, a comparison with a new set of Guatemalan climbing bean accessions (500) collected in 2015 will be compared to this group to determine their geographical location.

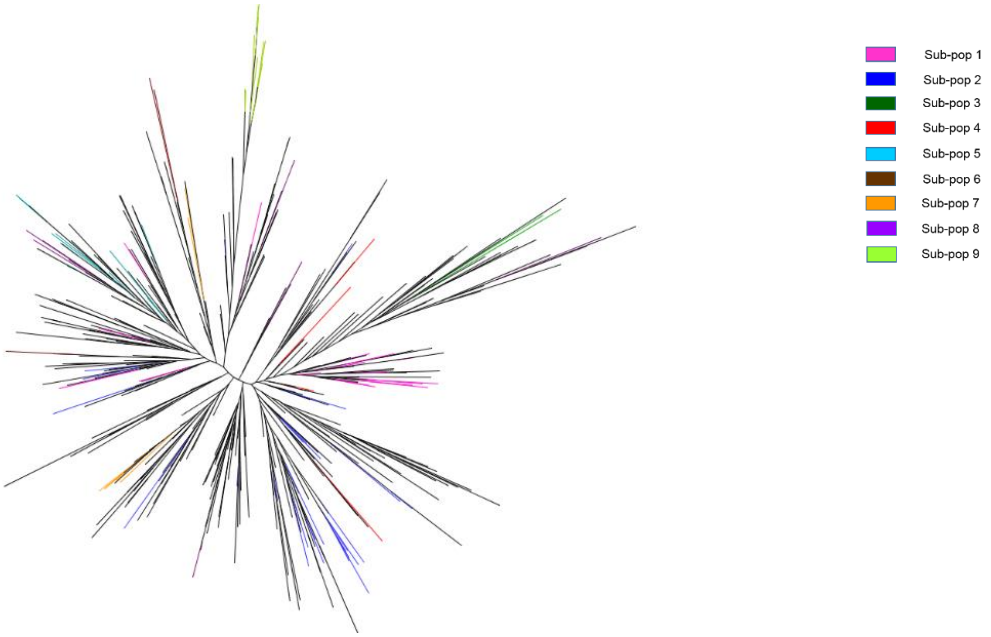

Figure 1. Maximum-likelihood tree of 369 climbing bean accessions based on 2,732 SNPs (LD=0.1). Color in the branches shows the sub-population where they belong based on the Structure analysis.

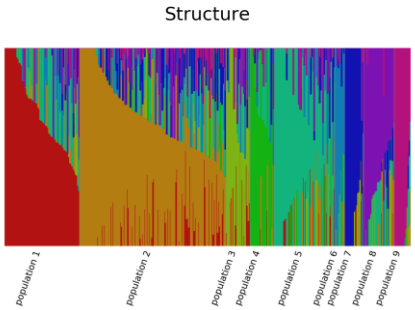

Figure 2. Hierarchical organization of the 369 climbing bean accessions based on 2732 SNPs (LD=0.1) for K=9.

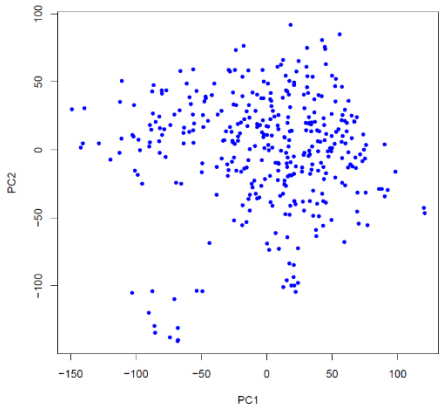

Figure 3. Principal Component Analysis (PCA) of the SNPs diversity for the Guatemalan climbing bean accessions.

A GWAS analysis was performed for multiple traits (agronomic and natural disease pressure) using phenotypic data previously recorded under field conditions at ICTA-Chimaltenango. Since disease resistance is one of the mail objectives of this project, here is an example of how genomic information could help towards the development of good resistant cultivars in the future: For the trait of Rust (*Uromyces appendiculatus*) resistance, a significant peak in chromosome 2 (Figure 4) was found. This SNP is inside a gene encoding a leucine-rich repeat transmembrane protein kinase. This type of gene is generally associated with plant resistance to diseases. For the trait of downy mildew (*Phytophthora nicotianae* var. *parasitica*) resistance there is a strong peak found in chromosome 6. This marker is inside a gene encoding an ubiquitin ligase binding protein. Other studies also relate this kind of gene with some resistance to necrotrophic pathogens. These results are promising for the use of this collection as a source of alleles in common bean breeding.

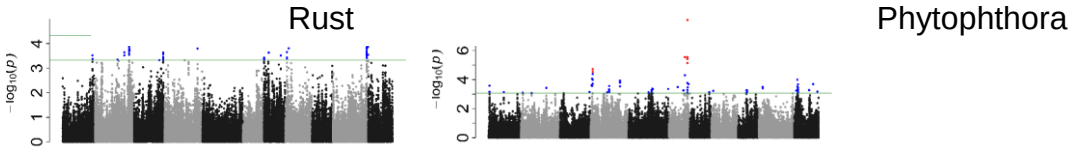

Figure 4. Manhattan plots of the best models for Rust and Phytophthora resistance. The green lines are the cut-off values to call a peak significant. The SNPs that pass the 0.01 percentile are highlighted in red, while those that pass the 0.1 percentile are highlighted in blue.

Two additional M.S. students at NDSU (ICTA trainees) are also using this germplasm collection as part of their thesis. Luz de Maria Montejo is focused on the identification of resistant accessions to bean rust, while Carlos Maldonado is evaluating the collection to find new sources of resistance to Anthracnose.

Bean rust bean samples were taken from bean producing fields at Guatemalan Highlands and two highly frequent rust races have been identified,

in collaboration with Dr. Jim Steadman from the Plant Pathology Department at University of Lincoln, Nebraska. Rust races were identified as a: 63-1 and 31-1. Samples were taken from following departments at Guatemala during the end of the 2015 growing season: Chimaltenango, Quetzaltenango, and San Marcos. Both races were found within each department and preliminary results suggest that they affect mainly Andean-origin rust genes. In addition, the entire climbing collection was screened for those two Guatemalan rust races and with race 20-3, the newest and most frequent race in North Dakota. Preliminary results are showing that 39% of the climbing bean accessions showed resistance to the 63-1 rust race and 58% showed resistance to the 31-1 rust race. The 20-3 ND race is currently being evaluated at NDSU greenhouse and final data is going to be reported soon.

In the case of Anthracnose, the climbing bean collection was evaluated with race 73 which is the most commonly found race in North Dakota. Preliminary results suggest that almost a 10% of the climbing bean germplasm accessions are resistant with no visible symptoms in the plants (disease score 1) to race 73. Same as with rust, anthracnose sampling was made at different locations from the Guatemalan highlands during the 2015 and 2016 growing seasons. Samples of climbing beans from small scale farmers from Totonicapan, Quetzaltenango, San Marcos, and Huehuetenango departments were taken from leaves, pods, or stems that showed symptoms of anthracnose. Samples will be sent to Dr. Talo Pastor-Corrales at USDA-ARS in Beltsville-MD for further race typing and characterization.

All phenotypic data collected from disease reaction to both rust and anthracnose will be used to identify genomic regions associated with genetic resistance using the same GWAS approach reported above. Results will be useful not only for Guatemala but also to find new potential sources of resistance to local races found In North Dakota, the largest producer on dry bean in the U.S. Once genomic regions can be located with accuracy, new diagnostic markers can be designed that could be used in a Marker Assisted Selection (MAS) scheme.

2.2: Assessment of the intra-accession variability (NDSU): Because of the reasons exposed in the previous section, this activity had to be postponed as well. A genetic assessment of variation within the 10 selected lines used in objective 1.1 will be made in order to account for the heterogeneity not only among but within accessions and possibly, extrapolate that information to the rest of accessions. Preliminary phenotypic observations in the field suggest that there is a high amount of genetic heterogeneity (heterozygocity) within accessions. Therefore, 20 plants from each of the 10 selected accessions 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., 2014). 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 band polymorphisms in the gels. Polymorphic Information Content (PIC) and other genetic parameters will be estimated. This information will allow a better understanding of the organization of the genetic diversity within this core collection for future use and research. This activity is currently underway and result will be reported in the next cycle.

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 FY2015 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. A first set of 23 potential parents were selected by Osorno, Villatoro, McClean, and Aldana, and planted in the greenhouse at the ICTA station in Chimaltenango during the 2014 growing season for initial crosses (Objective 1.2). A list of selected accessions can be found in Appendix 2. Parental accessions were selected mainly based on uniform pod distribution, potential yield, and disease resistance. Since the entire collection was planted again during FY2015, it gave us an opportunity to re-evaluate the 23 accessions selected the year before. The research group felt very confident that the 23 selected accessions represent the best of the collection in terms of agronomic performance. Some of these activities will overlap with FY2016. These selected accessions were used for the first set of crosses described in objective 1.2.

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: Gustavo Mejia, Julio Cesar Villatoro, Fernando Aldana.
MSU: Mywish Maredia, David DeYoung, and Byron Reyes from project SO4-1.

As described in the technical project description and FY15 work plan, a grower survey was deployed during March 2015 in the main regions where climbing beans are produced. A total of 548 farms were surveyed covering the 5 most important bean producing departments in the western highlands (details about survey protocol can be found in previous reports or in the IRB document). 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. Gustavo Mejia, social economist from ICTA-Quetzaltenango was also of key importance for the success of this activity.

Collected data has been entered into a digital format (Excel) by ICTA personnel and was revised and filtered of errors at MSU. Data was then analyzed using the proper statistical tools and preliminary results were presented at the Pan-African Grain Legume and World Cowpea Conference at Zambia in March 2016. Results of this survey will be also shared not only within the project but with other projects currently working in Guatemala (e.g. Masfrijol) and government agencies interested.

Major findings are summarized below:

Bean Consumption:

- Majority of farmers (80%) do not sell beans; only 6% sold more than 50% of their harvest.
- Own production covered less than half of annual bean consumption for 23% of farmers; For another 33% of farmers, own production covered between 50-75% of annual consumption. Once own produced beans were consumed, 50% of farmers purchased beans at least weekly.
- On average, a household cooked beans 2.5 times and consumed a total of 5 cups of (uncooked) beans in the week prior to the survey.
- Households with children (under 14) on average served beans to the children 3 days in the week preceding the interview. On days that children ate beans, more households served beans at breakfast (75%) and dinner (79%) than at lunch (53%).
- Beans are consumed throughout the year; it is highest at or after the harvest –from November to March, and lowest in July and August.

Dietary diversity, culinary preferences and farmers' perceived nutritional value of beans:

- On average a household consumed 6 out of 12 diverse food groups in the day prior to the survey; More than 85% reported consuming beans the previous day.
- '*Frijol negro', 'vulgaris*' and *'bolonillo*' were among the most preferred bean variety/type for consumption named by farmers.
- Respondents indicated that flavor (76%) was what they like most about a bean variety followed by thickness of bean broth (32%), cooking time (3.2%), expansion of size (1.5%) and color retention during cooking (0.7%).
- On a scale of 0-10, farmers rated beans 8.9 in terms of nutritional value, which was just below the score for maize (9.4), but higher than the perceived nutritional value of rice (8.1), potatoes (7.8), meat (6.9), chayote (5.9) and Coca Cola (1.4).

Bean Productivity and Production Practices:

- An average household owns 0.73 ha (median=0.27 ha) and devotes 1.3 parcels of land on the simultaneous planting of *Milpa*(direct planting) system, with an average plot size of 0.29 ha (median=0.18 ha). The average bean yield on the simultaneous planting *milpa*plots is 318 kg/ha (median=208 kg/ha).
- Farmers use the *Milpa* intercropping system with low crop rotation. The majority of farmers' plots were planted with maize in the year of the survey (94%) and also in the previous year (92%). Climbing beans were also planted on the same plot in the previous year (91%).
- Simultaneous *Milpa*(direct planting) was a more common practice (73%) than waiting several weeks to plant beans (relay) (21%); only 2.3% of plots surveyed had beans planted alone in rows or intercropped with other crops (2.0%).
- Most plots were planted with one bean variety (74%) while 18% had two varieties, and 6% had three varieties.
- Desired characteristics of a bean variety as cited by percentage of farmers are yield (49%), seed size (36%), seed color (32%), fast cooking time (32%), taste (31%), resistance to field pests (27%), resistance to lodging/weighing down maize (23%), early maturity (21%), and resistance to diseases (21%).
- Farmers are willing to pay on average $6.6 Guatemalan Quetzals per pound (US$ 0.80) for improved variety seeds that have the characteristics they desire and indicated that they would initially purchase on average 6.8 pounds of seed.

The study is one of the first representative farm surveys of climbing bean growers in the region. The analysis of this data has helped gain a better understanding of farmer characteristics, bean production practices in the *Milpa* system, and the role of beans in household food consumption. The analysis contributes towards establishing priorities for the climbing bean breeding program targeted for increasing the productivity of the *Milpa* system.

3.2 Seed increase of samples collected during the survey (ICTA/NDSU): An interesting activity performed during the survey was the collection of a seed sample (~10 seeds) that was requested to each grower surveyed. Seed samples were donated voluntarily and ~85% of the growers surveyed accepted to give us a sample of the seed they use in their farms. Therefore, now we have a newer set of ~460 (out 540 surveys) climbing bean germplasm accessions that virtually represents what climbing bean growers are using in their farms currently. Within this group, there is a set of ~100 accessions that were collected by the ICTA genetic resources unit during 2015 and were shared with us as well. Since seed amounts were limited, 4 seeds per sample were planted in the greenhouse in 2015 for increase and future evaluation during FY2016. Harvested seed from the greenhouse was then planted in the field for a second round of increase and evaluation at ICTA-Chimaltenango during the 2016 growing season and it is being harvested at the time of this report. This provided an opportunity to do a phenotypic evaluation of the germplasm collected during the survey and possibly

to identify genetic material of interest for the breeding pipeline (Objective 1.2). As a matter of fact, a group of 24 accessions from this new group have been selected in the field at ICTA-Chimaltenango for future testing and crosses.

Future activities with this set of germplasm include a comparison of the original germplasm collection from ICTA with this new collection and see what changes in genetic diversity across time could be detected. Seed samples have been received at NDSU and DNA extraction us currently underway. In addition, the specific location from where each seed sample was obtained is available and therefore, some geographical diversity analyses are possible in the near future. Even more, the new germplasm collected during the survey could be compared with the original germplasm collection via SNP analysis (Objective 2.1) and try to establish some genetic similarities and hence, try to pinpoint some possible geographical origin for the original germplasm collection since all the passport data was lost several years ago. We foresee this study as a good research topic for one of the students coming to do their M.S. training at NDSU in the future (Objective 4).

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: Recruiting efforts during FY2014 and FY2015 at ICTA have allowed the identification of three candidates for M.S. at NDSU. Gabriela Tobar Piñon and Carlos Maldonado are ICTA employees initially identified through the CAPA project, which is an early career program at ICTA to identify outstanding individuals for future employment and ICTA. The third candidate is Luz de Maria Montejo who is a graduate from the Escuela Agricola Panamericana Zamorano and was working with the Guatemalan Ministry of Agriculture. Luz was highly recommended by Juan Carlos Rosas and Jim Steadman and therefore, she was transferred to ICTA. The 3 students started their M.S. programs at NDSU in the fall of 2015. The three students are currently living in Fargo, ND and making progress towards their M.S. degree by taking classes and doing research at NDSU. Research topics are directly related to the research objectives described above. The graduate students are provided with 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.

4.2. Site visit/workshop at NDSU for ICTA personnel: As described in the technical project description, a site visit/workshop has been proposed for the third year of this project. A total of 6 host country collaborators (3 from ICTA-Guatemala and 3 from Zamorano-Honduras) spent 7 days during July 2016 at NDSU. The goal of this training/workshop was to show the visiting group how bean production is made in North Dakota (the largest producer in the U.S.) and

also to receive training on modern techniques in plant breeding, plant pathology, molecular markers, and other genomic tools that could help in the breeding process. Activities included field tours to breeding nurseries, commercial farming operations, and bean industries in the region. Visitors also received talks about molecular techniques, plant pathology, and seed production/systems, among others. Many visitors said this opportunity was an “eye opener” for them regarding how different and efficient productions systems can be and how many things could be implemented back into their countries. One of the outcomes of this sit visit was the writing of a medium and long-term plan for breeding of climbing beans in Guatemala that could be used for planning purposes if additional funding is available to continue this project in the future.

IV. Major Achievements

1- **On farm field testing and validation across 15 locations of 9 breeding lines with potential to be released in the near future.**

2- **On-farm testing and validation of Bolonillo-Texel, Utatlan, and Labor Ovalle across 15 locations.**

3- **Establishment of a breeding pipeline and second set of crosses.**

4- **Genetic purification of promising lines.**

5- **Initial molecular characterization (DNA extraction) of climbing bean collection.**

6- **Completion of grower survey and data tabulation of ~500 questionnaires.**

7- **Completion of statistical analyses of grower survey and presenting/distributing preliminary results to interested audiences.**

8- **Collection of seed samples from surveyed growers and seed increases in the greenhouses.**

9- **Selection of new genotypes from grower survey with breeding potential.**

10- **Recruitment of 2 female students and 1 male student for formal training (M.S. in Plant Sciences) at NDSU.**

11- **Site visit/workshop at NDSU made by 6 host country scientists during July 2016.**

12- **New collaborations established with project SO4-1 and MASFRIJOL will augment the success of the breeding efforts of this project.**

V. Research Capacity Strengthening

During FY2016, our project successfully obtained one of the capacity strengthening awards for host countries. We used the funds to support activities related to the PCCMCA annual meetings (Programa Cooperativo Centroamericano para el Mejoramiento de Cultivos y Animales). This is the most important scientific meeting in Central America and the Caribbean about crop and animal production research. It is an annual regional forum hosted since 1954 on a rotating basis by the National Institutes of Agricultural Research (INIA) in Central America and the Caribbean. Scientists from universities, private companies, NGOs, international organizations involved in agricultural research in the region and other countries meet to discuss and analyze current issues and perspectives of research, technology, and innovation in agriculture and to exchange experiences and promote cooperative activities. The meeting was held at San Jose, Costa Rica during April 2016. The meeting attracted approximately 400 participants from the region. The funds were used to support travel of ICTA personnel to this important event.

VI. Human Resource and Institution Capacity Development

1. Short-Term Training

i. Site visit/workshop to NDSU at Fargo, ND:

a. Purpose of Training: To train a group 6 host country scientists in modern plant breeding techniques and bean productions systems in the U.S.
b. Type of Training: 7-day visit/workshop.
c. Country Benefiting: Guatemala and Honduras.
d. Location and dates of training: NDSU, Fargo-ND, July 17-24 2016.
e. Number receiving training (by gender): 3 female, 3 male.
f. Home institution(s) ICTA-Guatemala and Zamorano-Honduras
g. Institution providing training or mechanism: NDSU (Juan M. Osorno, Phil McClean, and other NDSU personnel).

ii. Field Days – Agricultural Technology Showcase at ICTA-Quetzaltenango:

a. Purpose of Training: Area farmers are invited to the station for a day to learn about different aspects of crop production, including bean and maize.
b. Type of Training: 1-day visit/workshop.
c. Country Benefiting: Guatemala.
d. Location and dates of training: ICTA-Quetzaltenango, October 6-28, a different group each day (see table 2).
e. Number receiving training (by gender): 335 female, 533 male.
f. Home institution(s) ICTA-Guatemala
g. Institution providing training or mechanism: ICTA-Quetzaltenango (Jessica Moscoso and Karen Agreda).

Table 2. Field Days at ICTA-Quetzaltenango.

	Date	Male	Female	Total
1	10/6/2016	40	10	50
2	10/12/2016	11	5	16
3	10/13/2016	129	73	202
4	10/18/2016	52	65	117
5	10/19/2016	47	76	123
6	10/21/2016	36	4	40
7	10/23/2016	31	3	34
9	10/25/2016	123	82	205
10	10/28/2016	64	17	81
	Total	533	335	**868**

2. 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

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

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 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

First and Other Given Names: Carlos
Last Name: Maldonado-Mota
Citizenship: Guatemalan
Gender: Male
Training Institution: NDSU
Supervising CRSP PI: Juan M. Osorno
Degree Program for training: M.S. in Plant Sciences
Program Areas or Discipline: Plant breeding
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: Anthracnose resistance 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

VII. Achievement of Gender Equity Goals

The ICTA bean breeding program includes two women in their team (Angela Miranda and Jessica Moscoso) and they are in charge of the activities at San Jeronimo and Quetzaltenango. In addition, 2 women who are ICTA employees (Gabriela Tobar and Luz Montejo) have been recruited for formal training (M.S. in Plant Sciences) at NDSU.

VIII. Explanation for Changes

No changes to report.

IX. Self-Evaluation and Lessons-Learned

After 3 years working in this partnership between NDSU and ICTA-Guatemala, the project has been able to make a lot of progress, considering this is a brand new project within the Legume Innovation Lab research portfolio. The

genetic material previously developed by Dr. Aldana at ICTA-Quetzaltenango allowed having breeding material ready to be tested in farmer's fields. This will ensure this program has significant impact in the short-medium term while a new breeding pipeline is established (long term). So far we are impressed with the capabilities and passion that ICTA personnel put into this project. ICTA has proven success with the long term program supported by the Legume Innovation lab for bush type beans for the lowlands along with the University of Puerto Rico. With the results from farmer field trials from the 2016 growing season, there should be enough data across years and locations (environments) to make final decisions regarding cultivar releases. It is important to note that final decisions regarding cultivar releases are made by another group within ICTA called tha validation group. We are intensively communicating with the group leader (Mr. Julio Franco) to ensure they understand the importance of releasing genetic material and the end of the cycle of this project. This is where politics gets in the middle of science but we are trying our best to ensure this will happen. The good thing is that we have total support from ICTA director (Dr. Elias Raymundo).

We are very pleased to be able to report on the molecular characterization of the germplasm collection. Because of multiple reasons, this activity was delayed but we are happy to report we are back on track and results are being generated. In the same way, the results from the grower survey will allow a better understanding of the needs for this region as well as many bean consumption habits that were not completely clear based on previous information. In addition, we are very excited about the induction of "new" germplasm collected from this survey. This newer collection opens multiple opportunities for future research that will benefit the region directly.

After many difficulties, we were able to recruit 3 M.S. students to work in this project at NDSU. The climbing bean collection is used as part of their research requirements for completion of their degree. The training of this human resource will ensure the next generation of plant breeders for Guatemala.

X. Scholarly Accomplishments

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

DeYoung D., Reyes B., Villatoro J.C., Montejo L.M., Moscoso-Alfaro J., Osorno J.M., and Maredia M. 2016. The Role of Beans in the Milpa Production and Dietary Systems of Guatemalan Highlands: Results of a Farm Household Survey. Pan-African Grain Legume and World Cowpea Conference. Livingstone, Zambia. Feb. 28th to March 4th.

Moghaddam S.M., Mamidi S., Osorno J.M., Lee R. Brick M., Kelly J., Miklas P., Urrea C., Song Q., Cregan P., Grimwood J., Schmutz J., McClean P. 2016. Genome-wide Association Study Identifies Candidate Loci Underlying Agronomic Traits in a Middle American Diversity Panel of Common Bean (*Phaseolus vulgaris* L.). Plant Genome. doi: 10.3835/plantgenome2016.02.0012; Date posted: July 25, 2016

Montejo L.M., Dardon, D., Villatoro J.C., Aldana L.F., Osorno J.M. 2016. Phenotypic Characterization of Bean Rust Isolates from Common Bean in the Guatemalan Highlands. Pan-African Grain Legume and World Cowpea Conference. Livingstone, Zambia. Feb. 28th to March 4th.

Osorno J.M., McClean P., Villatoro J.C., Aldana L.F., Moscoso-Alfaro J., Montejo L.M., Maldonado C., Tobar M.G. 2016 Breeding Efforts for the Improvement of Climbing Beans for the Guatemala Highlands. Pan-African Grain Legume and World Cowpea Conference. Livingstone, Zambia. Feb. 28th to March 4th.

Osorno J.M., Villatoro J.C., Miranda A., Moscoso-Alfaro J. 2016. Climbing Beans Affected by *Ascochyta* spp. in the Guatemala Highlands. IV International Ascochyta Workshop. Troia, Portugal. Oct 10-11.

Soltani A., Bello M., Mndolwa E., Schroder S., Moghaddam S.M**.,** Osorno J.M., Miklas P., McClean P.E. 2016. Targeted Analysis of Dry Bean Growth Habit: Interrelationship Among Architectural, Phenological, and Yield Components. Crop Sci.
doi: 10.2135/cropsci2016.02.0119

Tobar-Piñon M.G., Moghaddam S.M., Lee R., Villatoro J.C., Osorno J.M., McClean P. 2016. Genetic diversity of the Guatemalan climbing bean collection. II International Legume Society Conference. Troia, Portugal. Oct. 12-14.

Villatoro J.C., Moscoso-Alfaro J., Agreda K.A., Osorno J.M., McClean P., Montejo L.M. 2016. Preliminary Study of the Presence, Damage Level and Population Dynamics of Mexican Pod Weevil (*Apion godmani*) in Bean Genotypes (*Phaseolus vulgaris* L.), in the Highlands of Guatemala. Pan-African Grain Legume and World Cowpea Conference. Livingstone, Zambia. Feb. 28th to March 4th.

Villatoro, J.C., Moscoso-Alfaro J., Agreda K.A., Monzon F., Osorno J.M., McClean P., Montejo L.M. 2016. Nivel de daño y dinámica poblacional de *Apion godmani* en frijol (*Phaseolus vulgaris* L.). Annu. Meet. PCCMCA. San Jose, Costa Rica. May 5-8.

Villatoro J.C., Beaver J., Porch, Rosas J.C., Miranda A., Carrillo E.E., Moscoso-Alfaro J. 2016. Evaluación de líneas de frijol para determinar resistencia al daño del gorgojo *Acanthoscelides obtectus*. Annu. Meet. PCCMCA. San Jose, Costa Rica. May 5-8.

Villatoro, J.C., Moscoso-Alfaro J., Agreda K.A., Monzon F., Osorno J.M., McClean P., Montejo L.M. 2016. Evaluación de rendimiento en 10 genotipos de frijol voluble y nivel de daño por *Asphondylia* spp. Annu. Meet. PCCMCA. San Jose, Costa Rica. May 5-8.

Villatoro, J.C., Osorno J.M., McClean P., Aldana L.F., Moscoso-Alfaro J., Miranda, A., Agreda K.A., 2016. Evaluación de genotipos de frijol voluble, densidades, y su efecto sobre el maíz. Annu. Meet. PCCMCA. San Jose, Costa Rica. May 5-8.

Professional Recognitions

Juan M. Osorno received the Distinguished Achievement Award given by the Bean Improvement Cooperative during their biennial meeting in November,

2015 in Niagara Falls, Ontario, Canada. The award is presented to a scientist who has worked in bean improvement fewer than 15 years and recognizes outstanding scientific accomplishments relating to bean improvement and education.

XI. Progress in Implementing Impact Pathway Action Plan

After three years of this new project, we are confident that our activities have been in accordance with our impact pathway plan. There are no major delays to report and all the previous delays/problems reported before have been solved. Please refer to the Impact Pathway Plan document for more details.

XII. Data Management

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

ANNEXES

Appendix 1: ICTA - Technical and financial report FY2016 (AVANCE DE INFORME TECNICO Y FINANCIERO).