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, 2013 – September 30, 2014)

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:

1A: Field testing of 10 selected accessions (ICTA): The bean breeding program at ICTA has selected a group of 10 accessions from the germplasm collection that offer agronomic traits of interest such as plant growth type, seed yield, disease resistance, earliness, and seed quality, among others. We will start field testing of these 10 accessions across 10 locations that represent the growing area of climbing bean production. Most locations will be tested under the intercropping system and few under monoculture. The accessions will be planted using a Randomized Complete Block Design (RCBD) with 2 or 3 replications depending on space and resources at each location. Plating usually occurs in April-May and harvest occurs in November to early December.

The following agronomic data will be collected across all locations:

- Days to emergence
- Vigor
- Early disease symptoms
- Days to Flowering
- Pod distribution
- Aggressiveness of growth
- Disease symptoms (natural pressure)
- Days to maturity
• Seed yield
• 100-seed weight

1B: Genetic purification of selected material (ICTA): As explained Technical Project Description, phenotypic variation has been detected within accessions. Therefore, genetic purification of selected lines will be done with the goal of isolating and homogenizing genotypes (increasing homozygocity) with traits of interest for breeding purposes. Whenever a plant within a plot is identified with a trait of interest, the plant will be tagged/labeled and individually harvested. The seed from each individual plant selected will be used for: i) seed increase, and ii) further evaluations.

1C: Field evaluation of Bolonillo-TEXEL (ICTA): One of the improved lines selected by Dr. Fernando Aldana at advanced breeding stages (known as Bolonillo-Texel) will be also tested at grower’s fields. The number and size of testing fields will be mostly dictated by the amount of seed available. The same traits mentioned above will be measured in these fields. 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). If Bolonillo-Texel 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.

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:
2A: 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) 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.

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). 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.

2B: Assessment of the intra-accession variability (NDSU): A genetic assessment of variation within the 10 selected lines used in objective 1A 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 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., in press). 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. 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):
First, an effort to find previous grower surveys made by governmental and non-governmental institutions will be made. Second, 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. Results of this survey will be shared not only within the project but with other projects currently working in Guatemala (e.g. Nutrifrijol) 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.

The first step will be to identify/recruit potential students that could start graduate studies at NDSU as soon as possible, hopefully in fall 2014. The project director will interview potential candidates during his visits to Guatemala during the spring, summer, and fall. If suitable candidates cannot be found in Guatemala, good candidates from neighboring countries could be considered. We expect to have two individuals to come and do graduate studies at NDSU (Plant Sciences) with the goal that those individuals will be incorporated into agricultural research back into the region. 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:

Objective 1:
A. Field testing of 10 selected accessions (ICTA).
B. Genetic purification of selected material (ICTA).
C. Field evaluation of Bolonillo-TEXEL (ICTA).

Objective 2:
A. Evaluation of core collection with the 6k SNP chip (NDSU).
B. Assessment of the intra-accession variability (NDSU).

Objective 3:
A. Collection of previous surveys/databases.
B. Grower surveys at 5 departments.

Objective 4:
A. Identification/recruitment of potential graduate students.
B. First 2 graduate students in graduate studies at 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 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 J. Lynch 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 person-to-person. In addition, most scientists involved in the project will meet every other year at the Bean Improvement Cooperative (BIC) 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 Nutrifijol project funded by the USAID Guatemala mission and lead by Luis Flores from Michigan State Univ. Preliminary conversations suggest that some of the genetic material could be shared with them for field testing and consumer preference. 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:**
2 graduate students are expected to start M.S. at NDSU in the fall 2014. Recruitment efforts are currently underway.

**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.