Feed the Future Innovation Lab for Collaborative Research on Grain Legumes

PROJECT TECHNICAL DESCRIPTION

COVER PAGE

Code and Title of Legume Innovation Lab Project: S01.A3 Improving Genetic Yield Potential of Andean Beans with Increased Resistances to Drought and Major Foliar Diseases and Enhanced Biological Nitrogen Fixation (BNF)

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Project Period:	Total Funding for 4.5 year Project	Total non-federal cost share commitment by U.S. institution(s)
April 1, 2013 – September 29, 2017	$1,999,928.00	18.1%

HCs where project activities will be implemented:	HC institutions to be sub-contracted (abbreviated names):	Percent of total project funding budgeted for each HC institution to be subcontracted
Ecuador	INIAP	12.3%
Uganda	NaCCRI	12.3%
Zambia	ZARI	12.3%

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SUMMARY PAGE

Code and Title of Legume Innovation Lab Project: S01.A3 Improving Genetic Yield Potential of Andean Beans with Increased Resistances to Drought and Major Foliar Diseases and Enhanced Biological Nitrogen Fixation (BNF)
Name and Institutional Affiliation of the U.S. Lead Principal Investigator: James D. Kelly, Michigan State University
Abstract : Common bean (*Phaseolus vulgaris* L.) is the most important grain legume consumed in Ecuador, Uganda and Zambia. Improved bean genotypes from Ecuador have a potentially significant spinoff in terms of the high potential for adaptation to upland farming systems in East Africa. Building on international bean germplasm, but particularly on the Ecuador germplasm, an opportunity exists to develop and deploy improved bean varieties, using a combination of traditional and the latest molecular plant improvement techniques. An improved understanding of plant traits and genotypes with resistance to multiple stresses from abiotic (drought) and biotic (root rot and foliar pathogens) sources will provide unique genetic materials for enhanced plant breeding methods and sources to study plant tolerance mechanisms. Improvements in current understanding of the physiology of drought and evapo-transpiration and the genetics of drought tolerance in common bean and the development of effective molecular and quantitative methods for the selection of drought tolerance are needed. The development of improved bean varieties and germplasm with high yield potential, healthy root systems, improved biological nitrogen fixation (BNF) with resistance to multiple diseases, and sustained or improved water use efficiency under limited soil water conditions are needed to increase profit margins, and lower production costs. The project will use QTL analysis and SNP-based genome-wide association mapping to uncover regions associated with drought tolerance, disease resistance, enhanced BNF and shorter cooking time. Results of this project would contribute to improved yield, farm profitability and human resources in the host countries and indirect benefit to participating U.S. Institutions and bean producers.

Summary Checklist ***(select as many as appropriate)***	
x	Project does NOT involves the use of proprietary transgenes or the generation of genetically modified organisms (GMOs)
x	Project does NOT involves human subjects and requires approval
x	Project does NOT involves animal use and requires approval
x	Project does NOT involves the use of agricultural pesticides and requires a Pesticide Evaluation and Safe Use Action Plan
x	Project involves M.S. or Ph.D. degree training of HC personnel at a U.S. university (How many?) __4____

A. Technical Approach

1. Problem Statement and Justification

Uganda and Zambia are among the Least Developed Countries (LDLs) situated in Sub-Saharan Africa (United Nations, 2011). The LDL designation is reserved for countries with an annual income of under $1,000 US based on indicators of nutrition, health, education, adult literacy and economic vulnerability. In East Africa, yields of staple crops are the lowest in the world due to biotic and abiotic stresses which limit productivity. Improved varieties and/or affordable production inputs are needed to combat these stresses. This project addresses the most important diseases and stresses affecting common bean (*Phaseolus vulgaris* L.) and also seed quality characteristics including cooking time and mineral bioavailability. The common dry bean is the most important food legume crop grown worldwide and is the main component of the production systems and a major source of protein for the poor in Eastern and southern Africa (Beebe et al., 2008, 2012; Broughton et al., 2003). Beans are considered by many to be the perfect food as they complement cereals and are nutrient dense with high contents of protein, micronutrients, vitamins, dietary fiber, and also have a low glycemic index (Wortmann and Allen, 1994; Bennink, 2005; Widders, 2006). The crop plays an essential role in sustaining livelihoods of smallholder farmers and their families, providing both food security and income. Nutritionally, protein-rich beans play an especially significant role in human diets, and, although less important than cereals as a source of calories, beans often supply a significant proportion of carbohydrates (FAO, 2001). Like other legumes, beans are also a key source of minerals, especially iron and zinc (Graham et al., 2007). Economically, in the developing world, beans are traditionally a small farmer crop, often grown in complex farming systems in association or rotation with maize, sorghum, bananas or other crops (Broughton et al, 2003). Dry beans also provide these small farmers with marketing opportunities to urban centers. Similarly, green beans are an important source of income for small holder growers in East Africa who produce the crop primarily for export markets typically in Western Europe, but they are also increasingly important for domestic markets where they too provide an important source of nutrition and income (Wasonga et al., 2012). Agriculturally, beans bring to cropping systems the crucial capacity to decrease or eliminate the need for direct applications of some fertilizers, particularly nitrogen and phosphorus. In many regions of the world, farmers simply do not have access to fertilizer, and in regions of industrial agriculture where fertilizers are available, the skyrocketing costs of fertilizer are resulting in economic pressure on farmers (Sinclair and Vadez, 2012).

However, the full potential of the bean crop is not realized due to a number of constraints that include several fungal, bacterial and viral diseases and abiotic stresses which prevent the crop from achieving its yield potential. In East Africa, diseases are estimated to be the second biggest constraint to bean production, after low soil fertility (Wortmann and Allen, 1994). Keeping up with changes in pests and diseases of beans is a challenge as the climate changes. Drought, due to insufficient or unpredictable rainfall, is a worldwide production problem exceeded in magnitude only by bean diseases. Drought affects over 60% of dry bean production worldwide and is endemic in the major production areas of northeastern Brazil and north-central highlands of Mexico (Broughton et al., 2003). In developing countries which include East Africa, annually 62% of common bean suffers from water-limited stress at some stages of its growth (White and Singh 1991). In the semiarid Western US dry beans cannot be grown successfully without supplemental irrigation (Singh, 2007). Consequently, some drought estimates are even higher: Fairbairn (1993) indicated that 93% of the production areas are subject to physiological water-deficit stress at some time. Water shortage problems have been accentuated with demographic expansion and climatic changes. The importance and urgency of developing high yielding, drought resistant cultivars that use water efficiently, and reduce dependence on irrigation should reduce associated production costs and stabilize yield in drought-prone environments, while increasing producer profit margins. The drought situation may be particularly acute in East Africa (Wasonga et al., 2012) given climate change-associated temperature increases being experienced across sub-Saharan Africa (Hulme et al., 2001; King'uyu et al., 2000). Drought frequencies are clearly increasing in Uganda, particularly in the drylands area, referred to as the "Cattle Corridor," which stretches along a broad swath from the southwest to the northeast encompassing 84,000 square kilometers. This area experiences periodic and extreme drought, and encompasses some of the country's most fragile ecosystems. Although there are projection uncertainties (Lobell and Gourdji, 2012), temperature increases in sub-Saharan Africa are expected to exceed the projected global mean increase of 2.5 ºC by 0.7 to 1.1 ºC by the end of the 21st century (Christensen et al., 2007; Williams et al., 2007 cited by Beebe et al., 2012). This is aggravated by the fact that these areas are among the poorest and most food-insecure and currently lack the infrastructure and institutions needed to fully participate in and have access to global (and sometimes even regional) markets (Lobell and Gourdji, 2012). Accordingly, given both current and expected climatic problems the vulnerability of common bean to drought has been variously identified at morphological, physiological and biochemical levels (Beebe et al., 2010, 2012; Devi et al., 2013; Molina et al., 2001; Porch et al., 2009; Rao, 2001; Wentworth et al. 2006), and there are numerous physiological studies with a focus on shoot and/or root traits that potentially contribute to improved adaptation to drought in common beans (Beebe et al., 2012).

Diseases are the other major constraint to bean production worldwide. The most important foliar diseases of common bean in Ecuador and East Africa are angular leaf spot (*Phaeoisariopsis griseola*), bean common mosaic virus (BCMV), and anthracnose (*Colletotrichum lindemuthianum)*. Ascochyta blight (*Ascochyta phaseolorum*) and halo blight (*Pseudomonas syringae pv. phaseoli*) are important diseases at higher and cooler altitudes (over 1700 m above sea level), while common bacterial blight (*Xanthomonas syringae*) and bean rust (*Uromyces appendiculatus*) are more destructive in the warmer lower altitudes zones (1000 – 1400 masl). Angular leaf spot (ALS), anthracnose (ANT) and root-rots alone cause annual crop loss of 761,900 tons in the eastern Africa region. This accounts for 52% and 43% of the total grain yield loss attributed to all the biotic and abiotic stresses respectively (Wortmann et al., 1999). Due to population pressure in many countries and shrinking household land of less than 1 ha, cultural crop husbandry practices like fallow and crop rotations are not being followed in farmers' fields. This exacerbates the cumulative effects and pressure of the diseases and pests on the bean crop. Assessing the pathogenic variability of these organisms in each production zone is critical before breeding efforts to control these pathogens can be effectively implemented.

Climbing beans play a vital role in the farming system and livelihood of small producers in Ecuador and East Africa. Climbing beans offer the advantage of high yields (approaching 5 tons/ha), extended harvest period in those highland areas where precipitation is adequate and cropping options are limited. The traditional intercropping system with maize predominates in Ecuador where 90% of the beans are grown in the highlands on landholdings that vary in size from 0.2 to 5.0 ha. Climbing beans are cultivated along the length of the inter-Andean mountain chain mainly in association with maize and part under semi permanent trellis systems. The area cultivated with climbing beans has fluctuated annually, but is currently estimated at near 100,000 ha. It is estimated that 90% of the area is planted with landrace or local varieties such as Cargamanto which is sold largely as green shell. In other countries climbing beans are grown as a monocrop requiring farmers to stake the crop but they benefit from higher yields under this system. The majority of climbing beans are very susceptible to seed borne diseases, so losses are high as farmers save their own seed for subsequent plantings. Seed borne diseases such as ANT, bacterial blights (*Xanthomonas, Pseudomonas* spp), BCMV can be devastating and rust, and insect pests (*Diabrotica* spp) also contribute to yield and quality losses. Similar problems exist in East Africa and could be corrected relatively easily by backcrossing in major gene resistance(s) to these diseases once the pathogenic variability present is characterized and marker assisted backcrossing implemented with more robust marker systems.

A foundation of any successful agricultural system is that it must be sustainable i.e., the outputs of nutrients in both agricultural products and unwanted losses must be balanced by inputs. The challenge in Africa is to develop sustainable agricultural systems that meet the present and future needs of rapidly growing populations. Nitrogen fixing legumes provide the basis for developing sustainable farming systems that incorporate integrated nutrient management in developing countries. When compared with other legumes, common bean is generally considered to be poor in nitrogen fixation (Bliss, 1993). There is, however, adequate variation for biological nitrogen fixation (BNF) and its associated traits such as nodule number and nodule weight (Graham and Rosas, 1977; Graham, 1981; Attewell and Bliss, 1985). In common beans, rates of nitrogen fixation range from 20 to 115 kg/ha/cycle, depending on genotype, maturity class, growth habit and environmental conditions. When beans are clustered into maturity groups, early-to-flower cultivars tend to be weaker in nitrogen fixation than the later flowering genotypes. Most of the lines that are superior in nitrogen fixation tend to flower late or are late maturing. Graham and Rosas (1977) reported greater BNF in indeterminate and climbing cultivars with fixation correlated to carbohydrate supply to the nodule and the high nodule: root mass. Variability has also been observed in the traits associated with BNF such as nodule number, nodule mass and nodule activity. These traits have been found to be correlated positively with total plant N content (Rennie and Kemp, 1981), and therefore may be useful as selection criteria for improving N_2 fixation. Since common bean does not fix as much nitrogen as other legumes, some farmers tend to apply some nitrogen to maximize yields. However, most farmers in Africa are resource poor and cannot afford nitrogen fertilizers and many governments in Africa are cutting back on fertilizer subsidies (e.g. Zambia). Biological nitrogen fixation is certainly the cheapest and probably the most effective tool for maintaining sustainable yields in African agriculture where the price of nitrogenous fertilizers is unaffordable by many farmers (Mafongoya et al., 2009). Enhancing BNF in common bean grown in Africa would not only increase productivity but also help in developing a sustainable cropping systems for the resource poor farmers that guarantee food security for the growing population. To realize the potential benefits of BNF there is need for more research to identify superior nitrogen fixing genotypes and to develop breeding tools that can facilitate breeding for enhance nitrogen fixation. Past research on breeding for enhanced BNF has had limited success mainly because of the polygenic inheritance patterns of the trait.

For maximum utilization of beans by food-insecure consumers, it is essential to address the long cooking times required to make beans palatable. Food-insecure consumers often are also faced with limited cooking fuel and firewood. Often, when firewood supplies are limited, women change their food consumption patterns and this includes reducing food items that have long cooking times, including beans thereby compromising families' nutritional intakes (Browuer et al., 1989). Genetic variability exists for cooking time and landraces or varieties that cook quickly are especially valued by consumers and fetch a premium price in the marketplace (Correa et al., 2010). The faster-cooking varieties have major implications for the rural and urban poor, gender equity, and conservation of biodiversity. The direct effects are savings in cooking time and fuel costs, mainly for women, who are responsible for cooking (Biran et al., 2004). Adoption of fast-cooking varieties reduces the quantities of firewood used and the time spent gathering it (Elia et al., 1997; Brouwer et al., 1989). For urban consumers, money will be saved on expensive fuels (kerosene, charcoal, electricity, and natural gas). There is limited information on the genetic control of cooking time in beans. Such knowledge would aid breeders in the development of fast cooking varieties and ensure that women farmers and head of households benefit from this research.

Molecular marker technologies offer the possibility to dissect quantitative traits that condition resistance to many biotic and abiotic stress resistance and cooking time into their single genetic determinants. Quantitative trait loci (QTL) mapping of individual loci can be targeted for indirect selection using marker-assisted selection (MAS). In addition, co-localization of QTL provides evidence of functionality and is a powerful tool for map based cloning of specific genes contributing to complex traits. Despite the fact that drought, heat and limited soil N are important constraints to bean productivity, a limited number of QTL studies for these traits in common bean have been conducted (Asfaw and Blair, 2012; Blair et al., 2012; Asfaw et al., 2012; Mukeshimana, 2013). Of the few that have been conducted for drought for example, studies have focused on populations from Middle American gene pool. Additional QTL analyses of drought resistance in populations developed from crosses between gene pools or from crosses within the Andean gene pool are needed to explore additional diversity for drought resistance QTL alleles, and to analyze the effect of genetic backgrounds on the expression of QTL that have already been identified. QTL analysis with Andean germplasm is also needed to identify genomic regions involved in heat tolerance, BNF capacity, and cooking time.With new molecular resources such as the bean genome sequence and SNP markers it will be possible to better understand the genetic architecture of these traits and more effectively develop molecular tools based on QTL and to circumvent some of the breeding challenges that come with relying solely on phenotypic selection.

The *Phaseolus vulgaris* genome sequence v1.0 (released 07/13/12) and 6000 SNP marker beadchip resources developed through the BeanCAP program are now available to the bean community. The MSU breeding program has used to SNP beadchip to map QTL for tolerance to drought, white mold and Empoasca species and those studies are being published. The genomic coverage in all three studies approached near 100% of the estimated bean genome of 1200cM. Bean breeders now have access to tools that can be used to map quantitative traits with confidence. Even though QTL analysis may be restricted by the limited number of parents used to develop the mapping population, the products of this analysis can result in practical output such as new white mold tolerant pinto bean variety Eldorado (Kelly et al., 2012). Genotype by sequence (GBS) can also be readily applied to any genotype of interest. The most exciting application of the GBS technology is to be able characterize genomic regions where resistance gene clusters are known to reside. In beans resistance gene clusters for ANT, ALS, CBB, rust, virus are known to reside on bean chromosome Pv01, Pv04, Pv08 and Pv11 (Ferreira et al., 2013) and smaller clusters exist on other chromosomes. Genetic data has shown linkage between genes that condition resistance to ALS and ANT on Pv01, Pv04 and between rust and ANT on Pv11. These linkage suggest that detailed bean sequence information now available on Andean (US bean sequence) and Mesoamerica (Mexican European sequence) genotypes will allow geneticists to identify specific sequences associated with specific genes. Using this information, SNP markers that flank these sequences can be identified between contrasting genotypes that would allow for the development of both SSR and Indel markers that can be used by breeders. The bean breeding program has the genetic stocks and populations that can be used to assist in identifying genomic regions for individual resistance genes for ANT, ALS and rust. This work would be done in collaboration with NDSU with support from S01.A4 project and PSU project on climate resilient legumes. The molecular markers currently in use for disease resistance breeding rely on old, inefficient marker technologies and weak or suspect linkages between the marker and the gene. With the newly available genomic tools it will be possible to take a directed approach on defined regions of linkage groups of interest to identify durable, easy to use markers for MAS. Robust markers linked to major resistance genes will be particularly advantageous for introducing major gene resistance into long-season climbing beans grown in Ecuador and East Africa.

With the broad genomic coverage offered by the SNP chip and the availability of GBS information, association mapping is now possible in beans but the outcome of these studies will differ from that of more traditional biparental QTL studies. Association mapping requires the evaluation of large diverse populations and the bean community has assembled a 400 genotype panel of Andean genotypes known as the Andean Diversity Panel (ADP) from the Americas and African continents. One very positive aspect of the widespread phenotypic evaluation of the ADP by different groups in different countries will be the accumulation of data on the phenotypic diversity in Andean bean genotypes. Progress in breeding Andean beans has been limited by the lack of genetic diversity available to breeders. This is illustrated in many studies (most recent: Bitocchi et al., 2012), but breeders may not actually be aware of the potential diversity present in the cultivated gene pool. That situation may be reversed with data on the ADP and could provide breeders will better opportunities to select diverse parents for future breeding activities rather than the narrow parental selection used in the past. Cyclic breeding schemes will be needed to introgress this diversity and should be deployed as opposed to the single cross strategies most programs continue to use. Data generated by association mapping will be more basic genomic information on traits of interest, so breeders will still need to exploit these traits in marker assisted breeding programs. Association mapping does not create new or novel genetic combinations, but only identifies associations that currently exist in the bean germplasm under study. Association mapping will be useful in the identification of potential candidate genes and QTL for improved BNF, drought and cooking time in a collection of Andean bean germplasm. Phenotypic evaluation will take place in Ecuador, Uganda, Zambia and the U.S. and be focused on the major red mottled, solid red, yellow, white and purple genotypes grown in these countries.

2. Objectives

Objective 1: Integrate traditional and marker-assisted selection (MAS) approaches to combine resistances to economically important foliar diseases, drought and improved biological nitrogen fixation (BNF) and assess acceptability of fast cooking, high mineral content in a range of large-seeded, high-yielding red mottled, white and yellow Andean bean germplasm for the Eastern Africa highlands (Zambia and Uganda), Ecuador and the U.S.

Objective 2: Characterize pathogenic and genetic variability of isolates of foliar pathogens collected in Uganda, Zambia and Ecuador and identify sources of resistance to angular leaf spot (ALS), anthracnose (ANT), common bacterial blight (CBB), bean common mosaic virus (BCMV) and bean rust present in Andean germplasm.

Objective 3: Use single nucleotide polymorphism (SNP)-based genome-wide association mapping to uncover regions associated with drought tolerance, disease resistance, cooking time and BNF to identify QTLs for use in MAS to improve Andean germplasm.

Objective 4: Develop phenometric approaches to improving the efficiencies of breeding for abiotic stress tolerance, especially drought.

Objective 5: Institutional Capacity Building and Training.

3. Approaches and Methods

Objective 1:

Quantitative trait loci (QTL) mapping and development of molecular markers associated with drought resistance, disease resistance for use in breeding programs.
The ADP panel will be evaluated under drought stress at Scottsbluff, NE and Ecuador, and for multiple disease resistance reactions in Zambia and Uganda. Disease screening of the ADP panel to CBB will be conducted in an augmented replicated trial at North Platte and at the greenhouse facilities at Scottsbluff. XAN 159, USPT-CBB-6, and Neb#1 Sel. 27 will be used as resistant checks and Orion as susceptible check. At flowering, plants will be sprayed with a bacterial solution using strains SC-4A and LB-2 and will be evaluated at the pod filling stage using a 1-9 scale, where 1-4 will be considered resistant and from 5-9 susceptible. The ADP will be evaluated in replicated trials under irrigated and non-irrigated conditions in Scottsbluff, NE. Selected ADP lines from Nebraska trial grown under drought stress will be evaluated at both NE and Ecuador under drought and non-drought conditions. Plant phenology, plant architecture, yield, and yield components will be evaluated in each trial. In order to quantify drought severity, drought intensity index, drought susceptibility index, and geometric mean will be calculated in order to predict the performance of a line under stress and irrigated conditions. Water stress will be implemented at flowering stage. Each ADP lines will be genotyped using the 6K SNP chip. Linkage disequilibrium will be estimated of the squared allele frequency correlation (R^2) using TASSEL: v.2.1. Estimation of population structure and kinship relationship will be derived using only loci that have pairwise R^2 value <0.5 for all possible combinations. Different linear regression models will be used for marker-trait association using the MIXED procedure in SAS. Tightly linked SNP markers for major diseases and drought will be identified. Significant association between the drought tolerant and marker genotype will be determined by one-way ANOVA using PROC MIXED (SAS, 1994). Linkage relationships will be determined by MAPMAKER/EXP group command. Linkage groups and interval analysis for QTL identification will be constructed. The drought tolerant genes and linked markers identified in the QTL analysis could be pyramided into commercial cultivars through hybridization and MAS in order to enhance development of high-yielding, drought-tolerant cultivars. The molecular markers could be used in drought-specific marker assisted selection schemes, so that only genotypes carrying the drought tolerant alleles would be advanced. Use of agarose based markers (Indels) will be implemented in Ecuador, Uganda, and Zambia.

In addition the following RIL populations will also be employed:

- A large RIL population of 345 lines derived from the cross of Buster/SER22 combines races Durango and Mesoamerica, and that has expressed excellent drought tolerance in Nebraska and Puerto Rico. Trialing of the population will continue at these locations.
- Concepcion*/RAB651 is an inbred backcross of large purple mottled Andean bean with a drought tolerant small red bean RAB651 from CIAT. This intergene cross has produced excellent seed quality and the population lacks the incompatibility problems common in other intergene pool crosses. This population is already in Rwanda and will introduced into Uganda.

Assess acceptability of fast cooking, high mineral bean germplasm in Uganda, Zambia, and Ecuador.
This objective will utilize data generated through the USDA-ARS FtF project described under 'Collaborations'. Through the USDA-ARS FtF project, data on cooking time, seed nutrient composition, and mineral bioavailability is being collected on 250 genotypes from the ADP. This information will be used to identify lines with superior nutritional and cooking characteristics. The top lines (~5-10) will be evaluated for sensory quality in Uganda, Zambia, and Ecuador, largely by women participants. Sensory evaluations will consist of consumer acceptance tests performed on cooked beans. All sensory analyses will be performed by an untrained panel of approximately 75 consumers of beans. Panelists will be asked to rate the sensory characteristics

of the beans with respect to appearance, color, flavor, mouth-feel, and overall likeability using a hedonic scale of 1-7 with 1 being highly undesirable and 7 being highly desirable. The lines with the highest likeability will be grown in on farm trials in each of the countries and compared to a local check. Lines will be evaluated for farmer acceptability based on agronomic and cooking characteristics. These results will guide breeding efforts for fast cooking high mineral beans by providing information on which trait combinations are most important to consumers and farmers.

Objective 2:

Characterize pathogenic and genetic variability of isolates of foliar pathogens and identify sources of resistance to ALS, ANT, CBB, BCMV and bean rust present in Andean germplasm. The foliar diseases ANT, ALS, CBB and rust are constraints to bean yields in Ecuador, Uganda and Zambia as well as other countries in the Americas and Africa. Resistance is the preferred disease management strategy because it has no costs to the farmer. To successfully develop disease resistant bean lines the race(s)/strain(s) prevalent in different countries needs to be determined. In all three of our focus countries collections of each of the pathogens will be made in primary bean production regions. A data base of past collections of the same pathogens and race structure will be compiled and shared with the broader *Phaseolus* community. New isolates of all pathogens will be inoculated onto differential bean genotypes in a greenhouse. The determination of prevalent races/strains by their disease reaction on the differentials will guide the race(s)/strain(s) to be inoculated onto breeding lines to select for lines resistant to one or more of the pathogens. Another approach that will be used is to plant breeding, RIL or prospective parental lines in fields where the pathogen(s) over seasons and favor the pathogen by application of overhead irrigation, planting in the rainy season and /or application of pathogen spores/cells directly to plants or in spreader rows The NIFA ADP nurseries and UNL drought tolerant germplasm nurseries will be leveraged to collect information on foliar pathogens in Zambia and Uganda. We also will partner with S01.A4 project to characterize isolates of the web blight pathogen in different host countries to use in search for an improved screening method for resistance.

Objective 3:

Identify QTL for nitrogen fixation and its related traits in the Andean gene pool using genome-wide association analysis.
The Andean Diversity Panel (ADP) is a collection of over 400 Andean genotypes from Africa, Europe, South, Central, and North America. The panel includes landraces, elite lines and released varieties. Materials in the diversity panel are from numerous market classes and seed types, including red mottled, purple, yellow, cranberry, and kidney. The ADP will be evaluated for biological nitrogen fixation in the greenhouse and field. Greenhouse evaluations will be conducted at MSU while field evaluations will be conducted on low nitrogen fields in US and Zambia. A nitrogen difference method will be used to determine the nitrogen derived from the atmosphere (NDFA) by each genotype. Genotypes will also be evaluated for nitrogen fixation using the N^{15} natural abundance method. Genotypic data for the panel will come from the Illumina SNP Chip developed through the BeanCAP. Association mapping will be conducted with the phenotypic and genotypic data as follows: The population structure will be considered using the mixed linear model (MLM) analysis described by Pritchard et al. (2000) to reduce spurious associations between phenotypes and markers that are not linked to any causative loci. In this method, non-linked markers will be used to estimate the population Structure (Q) and the kinship matrix (K) or coefficient of parentage. The population structure will also be estimated using principal components analysis. The K matrix and the rest of the association analysis will be performed using TASSEL software (Bradbury et al., 2007). Additionally, alleles at low frequency (<5%) will also not be considered so as to reduce the chances of finding false associations.

Identify QTL for nitrogen fixation and its associated traits in a Bi-parental mapping population. Bi-parental mapping will also act as confirmation study for the Genome–wide association study. A bi-parental population of 200 $F_{6:7}$ of recombinant inbred lines (RILs) derived from 'Mecosta' and 'CELRK' will be used. Currently, this population is being developed using single seed descent method at Michigan State University. 'Mecosta' and 'CELRK' are highly contrasting for nitrogen fixation capacities and for the associated traits such as nodule number and nodule mass. These two parents were chosen for population development following the field and greenhouse evaluation of 200 ADP genotypes. In both evaluations, 'Mecosta' consistently had high nodule weight and superior nitrogen fixation. In contrast, 'CELRK' had low nodule weight and inferior nitrogen fixation.. Both 'Mecosta' and 'CELRK' are light red kidneys with determinate growth habit. Similarities in many phenotypic traits except nitrogen fixation render these two genotypes most suitable for developing a population for genetic studies on nitrogen fixation. The 200 $F_{6:7}$ RILs and their parents will be evaluated in a greenhouse screen at MSU in the spring of 2014. The population will also be field evaluated in MI for two seasons in 2014 and 2015. The nitrogen difference method and N^{15} natural abundance methods will be used to determine the nitrogen fixation abilities of the RILs. The population will be genotyped with the 6K BeanCAP SNP chip. The SNP data will be used to develop a linkage map and phenotypic data will be associated with SNP markers to identify QTL. Resulting QTL will be compared to association mapping results to identify candidate markers for MAS.

Develop bi-parental mapping populations and identify QTL for cooking time.
Through collaborative efforts with the USDA-ARS Bean FtF project, genotypic variability for cooking time has been evaluated on approximately 250 ADP lines. Preliminary results indicate that cooking time is independent of seed size and color. Within a single seed type, genotypic variability for cooking times from 15 min to 70+ minutes has been observed. As part of the USDA-ARS Bean FtF project, the ADP has also been evaluated across locations and environments for disease resistance, including ALS, CBB, and rust and aboiotic stress tolerance, including low soil fertility. Based on cooking time data, as well as the other phenotypic data collected, parental genotypes will be identified for RIL population development and genetic improvement. Two populations will be developed using seed types favored in Uganda, Zambia, and Ecuador. Field evaluation will begin in the F6 generation on approximately 200 lines per population and will be conducted in Zambia and Michigan.

Cooking time will be measured using a pin drop (Mattson cooker) method. With this technique cooking time is determined based on the texture of individual seeds. The Mattson cooker apparatus consists of a dish with 25 seed size openings and 25 weighted pins. One seed is held in each hole with its corresponding pin resting on top of the seed. The dish is filled with 25 seeds of a single genotype that have soaked in distilled water for 12 hrs. The apparatus is placed in boiling water. When a seed is fully cooked the pin penetrates the seed and drops through the hole in the dish (Baojun, 2008). Cooking time is recorded as the time in minutes, starting when the seeds are placed in boiling water until the pin drops through the seed. Optimum cooking time for each line will be recorded as the time for 80% of the plungers to pierce the seeds (Wang, 2005). The feasibility of applying visible/near-infrared spectroscopy and imaging techniques and image processing methods, such as those applied in multi- or hyperspectral imaging systems of whole uncooked seed as a rapid, non-destructive to predict cooking time will also be investigated.

The populations will be genotypes with the bean 6K SNP chip. To perform the QTL analysis the function 'Composite Interval Mapping' in R/QTL will be used. The LOD threshold for each QTL will be set with 1,000 permutations. The percentage of variation explained for each QTL will be determined. The nearest marker to the LOD peak of each QTL will be selected. Based on results of the QTL studies, candidate markers for MAS will be identified and tested.

Objective 4:

Phenometric approaches to improving the efficiencies of breeding for abiotic stress tolerance, especially drought and heat.

A major objective is targeting specific photosynthetic (Ps) traits, identifying and avoiding drought sensitive components of the Ps process, which should almost certainly lead to elite genotypes important to breeding improvement. A related focus is to use Ps traits as indicators of stress sensitivity among genotypes. The advantage here is that phenometrics can now probe photosynthesis and dissect the processes involved so as to identify which processes are susceptible to perturbation by environmental parameters and stress. As part of this, we propose to take advantage of next generation phenotyping technology developed at MSU to probe Ps parameters under controlled yet dynamically fluctuating conditions that are relevant to those that impose drought and heat stress in the field (Kramer and Evans, 2011; Kohzuma et al., 2009). We will use these new technologies towards a phenotype-assisted breeding approach to transfer key heat and drought resistance traits to productive lines. Ps is also closely interconnected to a variety of other physiological processes. It is a major system for controlling cellular redox state, thus playing an important role in regulating enzyme activity and many other cellular processes (Buchanan and Balmer, 2005; Hisabori et al., 2007). There is also significant natural variation in Ps rates per unit leaf area (including overall efficiency, resistance to stresses etc.), as well as tolerance or resistance to drought (Lawson et al., 2012; Wentworth et al., 2006; Mencuccini and Comstock, 2000). These can be monitored by existing Chl fluorescence and gas exchange measurements, developed especially for Photosynthesis research, and these parameters are now widely used in stress biology and ecology.

Objective 5: Graduate and Short term training

Degree Training:

- Kelvin Kamfwa, Zambia, Doctorate student at MSU in Program Area of Plant Breeding, Genetics and Biotechnology with Drs. Kelly and Cichy; Thesis Title/ Research Area: Genetic dissection of biological nitrogen fixation in common bean using genome-wide association analysis and linkage mapping; Projected Completion Date: September 2014.
- Grady Zuiderveen, US, Doctorate student at MSU in Program Area of Plant Breeding, Genetics and Biotechnology with Dr. Kelly; Thesis Title/ Research Area: Not yet determined, focus on marker development and use in breeding for resistance; Projected Completion Date: September 2017.
- Jesse Traub, US, Doctorate student at MSU in Program Area of Plant Physiology, Breeding, Genetics and Biotechnology with Dr. Loescher; Thesis Title/ Research Area: Physiological differences among *Phaseolus vulgaris* cultivars differing in drought tolerance; Start Date: August 2013 on Legume Innovation funding for one year. Current graduate student with University Distinguished Fellowship from MSU for his first and final years of study FY11 and FY15 at MSU; Projected Completion Date: September 2015.
- Isaac Dramadri, Uganda, Doctorate student at MSU in Program Area of Plant Physiology, Breeding, Genetics and Biotechnology with Drs. Kelly and Loescher; Thesis Title/ Research Area: Not yet determined, focus on physiological process related to breeding for drought resistance; Start Date: August 2013 with BHEARD funding; Projected Completion Date: September 2017.

Short-term Training:

- One week Drought and Disease Screening methods to orient staff that will be involved in the day to day data collection and monitoring of drought and disease nurseries so as to get reliable and common parameters at ZARI, Kasama, Zambia; Anticipated numbers of Beneficiaries: 10 (6 females and 4 males); Support from PABRA/SABRN will also be sought for this activity.

- One week Drought and Disease Screening methods to Take staff through drought screening protocol, isolation and inoculation techniques for ALS, Rust, CBB at NaCCRI, Namulonge, Uganda; Anticipated numbers of Beneficiaries: 12 (5 females and 7 males); Support from CIAT/AGRA will also be sought for this activity.
- The project is planning to send participants to the other workshops being planned by the S01.A4 project. The actual individuals and participants will be identified later based on needs. Two of the graduate students at MSU will participate in the workshop on molecular techniques and analysis being planned as part of the S01.A4 project at NDSU.

4. Collaboration with Host Country Institutions

The project will interface with scientists working for national programs in Ecuador, Uganda and Zambia. The scientists are heading up active bean breeding programs in each country and in the case of Uganda and Zambia are networked with CIAT in Colombia and Uganda. All breeding programs need sustained funding to meet the long term objectives of the program and many of the current collaborative projects are short term with very specific objectives dictated by donors. The outcomes of most breeding programs can be the source of future long term funding if managed correctly. The scientists in all three programs recognize the constraints to bean production and equally the opportunities that new varieties can provide farmers and generate revenue not only for the same farmer but for the institutions where they were developed. Broadening and strengthening these programs is vital to the long term sustainability of the agricultural sector in all countries. Having the network to exchange germplasm when dealing with similar biotic and abiotic constraints promotes more rapid advancement and increases the opportunity of finding valuable genetic stocks that may result in future varieties with significant impact in the future.

5. Coordination with other International Grain Legume Research Programs/Projects

- USDA-NIFA: “Developing Common Bean (*Phaseolus vulgaris*) Germplasm with Resistance to the Major Soil Borne Pathogens in East Africa” focused on Bean Root Health in Rwanda and Uganda – PI-Kelly, MSU with partners in USDA-ARS /OSU/SDSU/CIAT/PABRA
- USDA-NIFA: “Genetic Approaches to Reducing Fungal and Oomycetes Soilborne Problems of Common Bean in Eastern and Southern Africa” – PI-Steadman UNL with partners USDA-ARS/ ZARI/ Iiam, Mozambique
- USDA-ARS FtT project: Breeding locally-adapted pulse crops for enhanced yield and seed qualities: an integrated, outcome-based plan for ARS, involving Dr. Cichy with partners in USDA-ARS.
- Legume Innovation Lab Project S01.A04 “Developing, Testing and Dissemination of Genetically Improved Middle American Bean Cultivars for Central America, Caribbean and Eastern Africa” – PI Beaver UPR with partners in USDA-ARS, Zamorano, NDSU
- USAID program on Climate Resilient Legumes: “An Integrated Program to Accelerate Breeding of Resilient, More Productive Beans for Smallholder Farmers” PI-Lynch, PSU with partners NDSU/ZARI
- CIAT network [including Idupulapati Rao, Bodo Raatz] and CIAT-Uganda (Clare Mukankusi) PABRA network (Mathew Abang, Roland Chirwa).

A meeting of all the US partners involved in the USDA-ARS, USDA-NIFA, USAID program on Climate Resilient Legumes and LIL projects listed above was held in Lincoln Neb on June 25, 2013 to ensure coordination of activities between projects. The research being proposed in the current project is particularly complementary to the two USDA-NIFA projects in Rwanda, Uganda and Zambia as the focus is on root pathogens and the current proposal will deal with foliar pathogens and healthy root systems needed to sustain bean plants through periods of drought and contribute to enhance nitrogen fixation. CIAT Colombia and CIAT Uganda through the PABRA network are major partners in the USDA-NIFA project in Uganda and the same network will be used to foster collaboration and outreach of research findings from this project. The project has collaborating scientists from US institutions who are part of the other USDA-NIFA project and the USDA-ARS project but their activities have a distinct focus not being addressed in these other projects. Plans have been made with the other LIL bean breeding project to contribute to nurseries and share findings on certain pathogens that are not being addressed (vice versa) so as to complement and not duplicate activities. Both LIL projects will benefit from fine mapping of genomic regions where resistance gene clusters are located in the development of more robust markers for use in resistance breeding.

6. Outputs

- Established and evaluated (mobile) nurseries for ALS, ANT, CBB, rust and drought and identified source of resistance in Ecuador, Zambia and Uganda.
- Collected and characterized isolates of ANT, ALS, CBB, and Rust from different bean production regions of Zambia, Uganda and Ecuador.
- Initiated crossing of landraces with resistant sources of ALS, ANT, CBB, and Rust in Zambia, Uganda and Ecuador and conducted progeny screening for different for resistances.
- Identified Andean drought tolerant lines from a trial tested in Scottsbluff, NE.
- Assessed the acceptability of Andean lines with superior mineral bioavailability and short cooking times and initiated crossing for genetic improvement of Andean lines with superior mineral bioavailability, short cooking time and disease resistance and developed high throughput/non destructive methods for determining cooking time.
- Develop drought screening protocols (using both field and next generation phenometric based techniques) and assemble a drought nursery to be tested across locations in Africa and the US
- Seed multiplication and distribution to participant countries – work through PABRA.
- Characterized biophysiological (gas exchange and chl fluorescence) characteristics associated with drought.
- Developed improved bush and climbing Andean beans possessing drought and multiple disease resistance.
- Indentified more robust markers for ANT and ALS and identified QTL for enhanced BNF and drought tolerance for use in MAS.
- Compiled information for an Andean variety release(s) by MSU and INIAP.
- Enhanced country capacity building training: 2 PhD students for Africa and one MS for Ecuador and training of 16 staff (10 male and 6 female) in disease and pest identification in Uganda and Zambia and conduct FieldBook training in Ecuador.

7. Capacity Building of Partner Host Country Institutions

- Kelvin Kamfwa from Zambia is one of the PhD students to be trained at MSU in Plant Breeding, Genetics and Biotechnology under this project. Kelvin Kamfwa still has a position at University of Zambia. Upon successful completion of his PhD studies, he is expected to return to the University of Zambia where he will take up leadership role of the bean breeding program and help in strengthening graduate training.
- Isaac Dramadri will be trained under this project and is expected to return to Makerere University, Kampala upon the successful conclusion of his degree program.

- Other African students with funding through BHEARD and Mastercard programs will be actively recruited for degree programs as part of this project.
- A training workshop on the Integrated International Maize Information System (IMIS)-Fieldbook platform will be held in Ecuador. Hands-on training section will cover topics such as installing the software, general inventory, seed preparation for trials and nurseries, managing pedigrees, and analysis.
- Short term training programs in country will be conducted and additional training through LIL partner workshops, Borlaug LEAP program and WorldTAP short courses at MSU where the emphasis is to train the trainer in aspects of Molecular Breeding.
- Established linkages with BecA Hub in Nairobi and the African Biosafety Network of Expertise at MSU to expand capacity building and future training workshops.

B. Alignment with USAID Feed the Future Goals and Strategic Research Objectives

1. *Alignment*-

Genetic improvement of bean productivity and quality traits is the basis of the current project which is complementary with the Feed the Future goals of sustainable production. Project collaborators on this project serve as PIs or co-investigators on other Feed the Future programs which ensure an effective integration with feed the future goals to enhance bean production in a sustainable fashion.

2. *Gender Equity*-

Bean production in East Africa and in regions of Ecuador is an activity largely involving women farmers. Every attempt will be made through our HC partners to involve women farmers in field testing and evaluation of new bean lines, collection of pathogens for study and disease screening from local fields and bean types preferred and grown by women farmers. We will work through PABRA network to enhance gender equity in the area of extension education and seed distribution. Every effort will be given to recruit female students for graduate and short term training. We will work to leverage additional funding through BHEARD and Mastercard graduate training programs in order to ensure that women are well represented in overall training being provided through this project. All members of the team are acutely aware of this issue and will work to ensure that gender equity is addressed appropriately at all levels of the project. Identification and development of fast cooking bean lines will benefit women by reducing the fuel and labor involved in preparing beans for their families. A gender and poverty checklist for evaluating projects and activities will be distributed to and implemented by all project partners.

- ✓ Has a community of beneficiaries or end-users of the project activities been identified?
- ✓ Will both men and women be involved in pre- and post-harvest evaluation?
- ✓ Does the proposal promote a better understanding of women's and men's differential knowledge and contributions to dry bean production?
- ✓ Does the proposal address a significant constraint to dry bean productivity?
- ✓ Does the proposal consider women's practical and strategic needs?
- ✓ How does the proposal address the balance of access to and control of resources by women and men?
- ✓ Does the proposal encourage marketing opportunities in community based seed production for both women and men?
- ✓ Will there be a training component for women and men and or resource-poor farmers.
- ✓ Does the proposal consider analysis of disaggregated data by gender and socio-economic class?
- ✓ How will project outputs be disseminated in the community?
- ✓ Are technology delivery services in place so that the technology quickly reaches end-users?

- ✓ Does the proposal consider the functional participation of all stakeholders, including resource-poor farmers?
- ✓ Will there be positive environmental benefits?
- ✓ Has there been a change in economic growth rate before and after project implementation?

3. *USAID Mission Engagement*-

The PI plans to visit the regional missions in all three countries in company with HC PI to introduce the project to the Agricultural officers and emphasis the importance of the research to local bean production and consumption in each country. We would hope to obtain local information on NGOs operating in each country and link with other extension programs to help guide the program in outreach phase. Engagement of the local missions in the project will depend on mission staff and goals.

C. Impact Pathway Plan

The following seven outputs were listed in the impact pathway plan – complete details on the steps, program logic and potential impacts are shown on that plan:

- Release by MSU of a new Andean cranberry bean variety with superior overall performance by FY17; two superior quality Mesoamerican navy and black bean varieties will also be released by MSU.
- Release of two new Andean bean varieties by INIAP, Ecuador and two varieties by ZARI, Zambia. These varieties would differ in seed types so the specific seed type is not yet identified.
- Candidate genes for complex traits (drought, BNF) will be identified using Association Mapping techniques and robust markers for major resistance genes through GBS.
- Relevant pathogens such as rust will be characterized in Ecuador, Uganda and Zambia.
- Improved Andean germplasm in both bush and climbing types will be identified across participant locations in Ecuador, Zambia and Uganda for use in future breeding efforts
- Physiological components and characteristics associated with drought will be identified.
- Enhanced scientific capacity in Uganda and Zambia through graduate student training and short term workshop
- Enhanced scientific capacity in Ecuador through a short term workshop in Fieldbook.

D. **Project Budget - complete budget attached separately - Sept 4, 2013**

Legume Innovation Lab Project : BUDGET SUMMARY						
S01.A3 Improving Genetic Yield Potential of Andean Beans with Increased Resistances to Drought and Major Foliar Diseases and Enhanced Biological Nitrogen Fixation (BNF)						
	41/13 - 09/30/17					
	FY 13	**FY 14**	**FY 15**	**FY 16**	**FY 17**	**Total**
a. Personnel Cost						
Salaries	$9,851	$121,300	$108,240	$122,453	$123,403	$485,247
Fringe Benefit	$1,100	$15,568	$18,502	$24,587	$23,353	$83,110
b. Travel	$32,913	$55,000	$83,000	$52,500	$53,000	$276,413
c. Equipment ($5000 Plus)	$0	$0	$0	$0	$0	$0
d. Supplies	$20,000	$80,250	$65,550	$62,950	$64,634	$293,384
e. Training						
Degree	$0	$0	$0	$0	$0	$0
Non-Degree	$0	$32,000	$31,200	$20,000	$14,000	$97,200
f. Other	$10,000	$95,450	$85,350	$81,750	$79,950	$352,500
Other - Tuition	$6,440	$33,444	$23,188	$24,116	$25,080	$112,268
g. Total Direct Cost	**$80,304**	**$433,012**	**$415,030**	**$388,356**	**$383,420**	**$1,700,122**
h. Indirect Cost 51%	$32,571	$66,080	$57,039	$48,062	$45,053	$248,806
i. Indirect Cost on Subcontracts (First $25000) 51%	$5,100	$45,900	$0	$0	$0	$51,000
j. Total Indirect Cost	$37,671	$111,980	$57,039	$48,062	$45,053	$299,806
Total	**$117,975**	**$544,992**	**$472,069**	**$436,418**	**$428,473**	**$1,999,928**
Grand Total	**$1,999,928**					

Cost Share	**FY 13**	**FY 14**	**FY 15**	**FY 16**	**FY 17**	**Total**
In-kind	$11,307	$33,096	$35,807	$34,290	$35,152	$149,652
Cash	$0	$10,000	$10,000	$10,000	$10,000	$40,000
Total	$11,307	$43,096	$45,807	$44,290	$45,152	$189,652

Attribution to Capacity Building						
Percentage of effort	3.22%	10.36%	14.18%	15.61%	16.88%	13.62%
Amount corresponding to effort	$2,584.64	$44,850.27	$58,834.36	$60,614.86	$64,722.99	$231,607.11

1. Budget Narrative

- Salaries: All salaries and fringes costs at MSU are for graduate students, some listed under US for HC; Technical support and student labor at UNL; Technical support and labor in Ecuador; Technical support and top up salary for HC PI; Technical support and top up salary for HC PI.
- Travel; Largely international for MSU and UNL to visit participating countries; country wide travel in HCs.
- No equipment requests made
- Supplies: Include field, lab and greenhouse supplies at all locations
- Training: Non degree at MSU is exclusively for HC individuals to attend workshops, similar training at UNL and in country training at three country locations for staff, students, technicians.
- Other: Part of this category at MSU cover registration fees and tuition for graduate students; also includes contractual analysis for SNP analysis, growth chamber rental, phenomics studies, near infrared analysis for quality traits; Indirect administration institutional costs in Ecuador; Labor costs and institutional overhead costs-8%; in Uganda and; Administrative and institutional costs in Zambia.
- Overall split between the U.S and HC institutions is US/HC is 47/53%.
- Cost share for US at 20.1% for MSU and 14.7% for UNL for an average of 18.1%.
- Estimated 14% is dedicated to institutional capacity building- does not include international travel to conduct workshops overseas.

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