# Enhancing Biological Nitrogen Fixation (BNF) of Leguminous Crops Grown on Degraded Soils in Uganda, Rwanda, and Tanzania

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**Abstract of Research Achievements and Impacts**
This BNF-CRSP project is designed to identify production systems that enhance BNF, develop germplasm that benefits most from symbiotic inoculation, and aggressively share this new information with small landholder farmers in sub-Saharan Africa whose health and well-being depend heavily on legume production.

Achievements in FY11 that contribute to identifying production systems that enhance BNF include completion of initial field trials to evaluate genotype x inoculant interactions, establishment and application of common protocols for quantifying biomass, total plant N, petiole ureide content, nodule sampling/classes/occupancy, initiation of analyses of plant N, biomass, nodule classes, ureide levels, collection of soil samples from all U.S. and HC field sites for rhizobia analysis, characterization of soil and weather conditions at all U.S. and HC field sites, identification and distribution of non-nodulating bean lines essential for field BNF evaluations, completion of initial grafting studies to indicate potential for increasing nodule formation and effectiveness in beans, identification of potential rhizobia genetic markers for indigenous strains and those in trial inoculants, and completing a preliminary assessment of rhizobial content of HC-produced inoculants.

Achievements in FY11 that contribute to developing germplasm that benefit most from symbiotic inoculation include identification of most of the 330+ lines to be included in the BNF Diversity Panel and initiation of seed increase of these lines, documentation that there is level of genetic diversity among selected bean lines for BNF capability and response to inoculant that will be useful for association mapping and physiological studies, identification of BNF-responsive genes for supporting future studies to distinguish superior BNF lines.

Achievements in FY11 that contribute to sharing new information about inoculant technology with small landholder farmers in sub-Saharan Africa include completion of initial field trials to test local variety response to inoculants as planned, completion of a draft survey tool to assess farmer knowledge, practices, and attitudes (KPA) about bean seed inoculation, and hiring field staff to monitor and coordinate Extensionist and farmer trainings on inoculant technology.

**Project Problem Statement and Justification**

Common beans are the most important legume crop in Uganda, Rwanda and Tanzania occupying a very large proportion of land devoted to legumes. For example, over 45% of the protein intake by Ugandans comes from beans providing 25% of dietary calories. Likewise, over 75% of rural households in Tanzania depend on beans for daily subsistence. Common bean is an important source of protein for low-income families in rural and urban areas providing about 38% of utilizable protein and 12-16% of daily caloric requirements. Improved bean production in Uganda, Rwanda, and Tanzania offers unique opportunities to address the deteriorating food security situation there and elsewhere in sub-Saharan Africa.

Numerous studies have shown the potential of improving legume productivity by enhancing nodulation through proper use of a biological inoculant. Modern inoculant formulations designed to deliver a synergistic suite of biological and chemical enhancements for biological nitrogen fixation under stressful soil conditions have been made available to our collaborative research project by Becker Underwood, Inc. Becker Underwood's **BioStacked®** inoculant technologies for legume crops consist of well stabilized *Rhizobium* bacteria, a biological fungicide, plant growth promoting rhizobacteria, and other biologically derived proprietary biostimulant technologies which promote plant growth and overall plant health. We anticipate they will be particularly effective under degraded soil conditions encountered on small-landholder farms in Uganda, Rwanda, and Tanzania.

Although common bean has the potential for BNF, it is reported to have the lowest percent $N_2$ derived from N fixation among legumes. Genetic variation for BNF has been reported within the primary gene pool, and lines with superior BNF have been identified. Few breeding lines with improved BNF, however, have been developed. Marker-assisted selection (MAS) under such conditions is highly sought after as a means to facilitate breeding for traits like BNF with low to moderate heritability. Molecular mapping in combination with germplasm screening and MAS would be a powerful way to improve locally adapted germplasm for BNF in a host country. Recombinant inbred populations currently available are ideal for tagging and mapping genes that influence quantitative traits (QTLs). Few QTLs associated with BNF, however, have been identified to date, and those identified have not been validated. Identifying and validating QTL-conditioning enhanced BNF would be a major contribution to the scientific community, and represent a major step toward effective marker-assisted selection for BNF.

Our BNF-CRSP program objectives address the need to identify production systems that enhance BNF, develop germplasm that benefits most from symbiotic inoculation, and aggressively share this new information with small landholder farmers in sub-Saharan Africa whose health and well -being depend heavily on legume production. This Dry Grain Pulses CRSP Project also contributes directly to USAID Mission's "Feed the Future" strategic development objectives. Activities are focused in 3 of the 5 top tier FTF target countries in Africa (Uganda, Tanzania, and

Rwanda). The project explores the benefits of modern agricultural (microbiology) technology to increase pulse productivity and income to small-holder farms. The Uganda Mission in particular identified "Beans for Nutrition" as a priority value chain to reduce poverty, increase sustainability of livelihoods and economic empowerment of women. The outreach component of this project contributes to USAID's mission of strengthening producer's organizations by working with individual farmers and farmers groups.

**Results, Achievements, and Outputs of Research**

**<u>Strategic Aim 1:</u> Improve BNF and seed yields of common beans significantly using superior seed inoculants such as Becker Underwood's BioStacked® inoculant through farmer-based experimentation and adoption of innovative production techniques.**

***Objective1a. Evaluate effectiveness of biologically inoculants on local and improved germplasm.***

**Approach**
Identify common bean varieties to test at all Host Country trial locations, establish the first set of field trials at Host Country research stations, secure non-nodulating lines of common bean and increase seed for field trials, quantify yield responses to inoculation in field trials established at US collaborating institutions and Host Country sites, and establish a website for posting, storing, and sharing protocols and results.

***Objective 1b.*** *Quantify genotype by environment interactions and constraints to enhancing BNF of inoculated plants.*

**Approach**
Field studies - complete analysis of plant/soil/weather data, establish a weather database for all field sites, calculate seasonal and long-term (5-yr) soils temp and moisture profiles, finalize protocols for sampling/processing plant materials from field trails and controlled environment studies, identify BNF responses associated with inoculant x genotype x environment interactions. Greenhouse studies - determine indigenous rhizobia levels all US and HC sites and associate with environmental conditions, Characterize soil rhizobia soil populations and strain diversity at field sites, evaluate trial inoculants, Bait soil rhizobia populations and analyze nodule DNA for indigenous strains.

**Results: Host Countries**
Field experiments involving NaCRRI and Makerere University continued in the four sites located in different agroecologies, namely Namulonge (central Uganda), Mbarara and Kabale (south western Uganda). Students remained instrumental in data collection and analysis. Treatment remained as three rhizobia types sourced from USA (Becker Underwood), and from Universities of Nairobi and Makerere. A control without inoculation was also included to represent the indigenous strains. Other study factors included six bean varieties, namely 3 bush for Namulonge and Mbarara sites, and 3 climbing for Kabale highland site. Phosphorus being the most constraining nutrient in the soils of east Africa and yet very crucial to effective BNF, was considered as a treatment (0 and 40 kg P $ha^{-1}$) to evaluate to what extent the imported rhizobia

can withstand the limited P supply in the soil. The factorial combinations culminated into a total of 72 treatment plots.

Soil and weather data were collected from each research site. Typcial data are shown in Table 1 appended to this report. Additional data and long-term values are being evaluated to determine trends associated with observed variation in BNF and inoculant response. In addition, soil samples collected from trial fields in Uganda, Rwanda, and Tanzania were sterilized and analyzed at WSU for a wide range of chemical and mineral parameters. While most soils were low in N and P, a potentially more challenging concern in these soils evident from these analyses is Boron deficiency (Table 2). Boron is very low in all soils tested, and extremely low in several of them. Boron is needed at higher levels in N-fixing crops due to its involvement in nodulation signaling. Because it is a micronutrient, very small additions to soil or as a seed coating can make a big difference. Availability and use of B should be investigated within this project to enhance BNF.

In addition to the parameters described in the project workplan, data were collected and are being processed for leaf area index, 100 seed weight, and nodulation score. Nodulation score was computed based on Zaychuk (2006), which involves assessment of plant vigor, number of nodules with leghemoglobin pigment and nodule position.

Students under the supervision of Dr. Tenywa and Dr. Ugen, completed preparation and defense of their thesis proposals and are undertaking greenhouse experiments. Martha Abwate's topic is "Effect of drought spells and phosphorus application on common bean biological N fixation in a low P soil." This study is premised on the fact that drought a rising constraint to crop production due to climate change effects in the region, to the extent that there are pronounced drought spells even within a rainy season. This is anticipated to affect BNF particularly under the low P situation of most soils in the region. Peter Ssenyonga's topic is "Phosphorus and molybdenum management for BNF improvement in common bean in east African soils." This research is premised on the evidence of inconsistent responses of common bean in terms of BNF despite efforts to promote us of rhizobia inoculants. There are also scattered cases of Mo deficiency in Africa that needs to be validated in the case of east African soils. The student is preliminarily using to use soil from Uganda, Tanzania and Rwanda for the greenhouse experiment. He will then concentrate more on the soil that will present the most severe Mo deficiency scenario. A major constraint in his work is likely to be the procedure to use that will ensure sensitivity in detection of Mo at laboratory analysis level.

Field trials were established at Selian in Arusha and Sokoine University of Agriculture in Morogoro. This year each country tested different varieties. The varieties that have performed well in Tanzania will be selected to be evaluated in other countries. The treatments included P application (P+ and Po), Rhoizobia application (Biofix inoculum-from Makerere, Nitrosua inoculum-from SUA, **Bio-stacked**® inoculum from Becker Underwood and the control i.e. without inoculation). Five bean genotypes were used (Bilfa 4, Kablanketi, Rojo, Jesca, Maini or BAT 477). Phosphorous application was the main plot, rhizobia inoculation as sub-plots and genotypes as sub-sub plots.

Data were collected on the following variables: leaf N, seed N, roots fresh and dry weights, shoot fresh and dry weight, 100 seed weight, number of pod per plant, number of seeds per pod, seed yield and nodule weight. All data except for seed N and nodule weights (which is still being processed) were analysed. It appears that for the Tanzania HC trials conducted in 2011 at two locations, there was a slight yield advantage from using the local inoculant (NitroSUA) at one location and all three inoculants were better than the control at the other location, but all of these positive responses were under low P. With the addition of P fertilizer there was no yield advantage with any of the three inoculants and in fact the control plot had the highest yield at both locations. This interaction of P fertilizer on yield response to rhizobia inoculation warrants further study.

Although we are currently in the process of interpreting these field results, it is clear there was considerable variation in the response of the varieties to P+ and inoculation in terms of grain yield, yield components, and leaf N. Uneven stands and irrigation are possible causes for this variation, which cannot be corrected simply by adjusting the harvest population. Uniformity in plant establishment and irrigation will be priorities for the next planting season.

Soil samples from the two locations where trials were conducted have been analyzed and the results are available to the team. These data will be collated with those from other locations and correlated with observed variation in yield and estimates of BNF. Soil temperature data was not collected because equipment to do that is not available. The five-year weather data of the locations where trials were collected is available. Soil sample from the study areas were collected and sent to Washington State University to determine the presence of indigenous rhizobia levels. In addition, intensive study on soil nutrient/microbe interactions is being conducted by MS Student, Beata Khafa. And the potential of incorporating favorable genes from bean landraces to enhance BNF is being pursued by MS student, Charles Komba.

**Results: U.S.**
Field trials conducted at Prosser in 2010 and 2011 consisted of three treatments (low N, Biostacked inoculant, and high N), 24 genotypes, two replications and two locations. Across these trials there was no significant yield advantage observed for the inoculated plots. A similar experiment was conducted during the summer of 2011 except the number of genotypes tested was expanded to 36. The 2011 plots have been harvested and yield data are being analyzed.

Eleven non-nodulating lines representing Andean and Middle American origins and tropical, sub tropical, and temperate adaptation were obtained from CIAT in August (appendix: Table 1). Twenty seeds of each line were obtained. Five seeds of each were sent to Karen Cichy, six seeds were sent to Susan Nchimbi, five seeds were sent to Tim Porch, and four seeds were retained at Washington State University for seed increase in the greenhouse this fall.

For the BNF field trials conducted at Prosser during summer of 2011, we collected three representative plants from each plot (~400 plots total) at flowering-mid pod fill stage of plant growth. The plants were pulled from the ground by hand and the roots were cut off at the cotyledonary node. The dried whole plant samples were shipped to Iowa State University for processing and ureide analysis of leaf, petiole and stem tissues. In addition, whole plant samples (~400) will be sent off to UC-Davis for $N^{14}/N^{15}$ and total N analysis. For each plot, seed size,

yield, and other traits including flowering date and harvest maturity were measured. Seed samples from each plot will be ground and tested for total N.

Field experiments conducted at Michigan State University included evaluation of 49 lines from the Andean Bean CAP planted under normal and low N fertilizer levels. These materials were harvested on Oct 4, 2011 and yield data are currently being analyzed.

Yield data from the 2010 field season indicated seed protein and the percent N derived from fixation did not increase in response to inoculation with Becker Underwood stacked inoculant. On average, percent seed protein of 93 lines (including 72 RILs of Eagle x Puebla 152 plus 21 additional lines) was 24.6% in uninoculated treatments, with an average of 11% derived from N2 fixation. The value for the inoculated counterparts was 24.7%, with 11% derived from N2 fixation. There were significant differences among genotypes in percent seed protein and percent N from fixation, but there were no significant inoculation or genotype x inoculation effects.

As prescribed in the work plan, soils were collected from field sites tested for indigenous rhizobia strains, and sent to Washington State University for analysis.

Testing of soils at HC and US field sites for indigenous rhizobia strains is underway, but progress is delayed for several reasons. The soils brought back from Africa were under quarantine for 90 days. For the rhizobia trapping experiment, three local varieties were grown (Othello, A-55, G-122). Poor seed quality of Othello eliminated this testing line. DNA has been collected from nodules of A-55 and F-122, 5 plants (reps) per variety x site. A variable section of the 16SrDNA has been amplified in preparation for deep sequencing (454 analysis). Amplification of the marker DNA (nifD) has been problematic because it is a nested pcr product.

Preliminary analyses of indigenous levels of rhizobia associated with local environmental conditions indicate infection/nodulation levels are “lower than expected”. Three week old beans were inoculated with 1 mL soil extract diluted at 10-2, 10-4, and 10-6. Roots were inspected after two weeks and very few nodules had formed. There were not enough nodules to give a reliable population estimate, and not enough to collect a community for study. After two more rounds of soil inoculation using increasing amounts of soil (5 g per plant in the end), good nodule populations were obtained. These samples are now being analyzed. This study will be repeated using new seeds, and lower dilution levels.

Initial evaluation of trial inoculants for rhizobia titre generated some unexpected (and potentially problematic) results. Bacteria were isolated on Modified Arabinose Gluconate (MAG), which is recommended for rhizobia. Inoculants from Rwanda and Makerere do not appear to be rhizobia at all based on the first 3 16S sequences we have analyzed. All three sequences are characteristic of Enterobacteriaceae. Genera Serratia, Klebsiella, and Pantoea give the closest matches (98%+) in GenBank. These are not organisms or sequences we have seen before in our program, nor are they “normal” contaminants. While these organisms commonly survive in soil, they can be harmful to humans at high populations. These are NOT organisms that should be cultured and distributed. WSU will continue to sequence several more isolates from which we have already extracted DNA. Confirming this finding will affect whether the WSU lab works further with these inocula, as they are not equipped to manage potential human pathogens.

Greenhouse screening trials on selected lines for BNF response are completed. Fifty-one lines have been screened for BNF using 15N dilution. Plants were harvested in July. Nodules were counted, and above- and below-ground dry biomass was measured. Samples are in que for 15N measurement, from which we will determine the proportion of plant N fixed from atmospheric N2.

Preliminary studies were undertaken at ISU to test the potential to increase nodulation and capacity for N2 fixation by grafting shoots of aggressive N2 fixing lines onto roots of less efficient lines. Vigorous seedlings with the first set of healthy unfolded primary leaves were selected and grafted using the wedge technique. Self grafts, cross-variety grafts, and across species grafts were compared to non-grafted controls. Grafted seedlings were stabilized in under controlled conditions, then transplanted to 1L pots containing potting a N-deficient soil mixture in the greenhouse. After one week, the soil is inoculated with Becker Underwood **BioStacked®** inoculum according to manufacturers' instructions. Plants were harvested at flowering/podfill to assess nodule formation, total plant N, and ureide concentrations. A portion of the data on nodule formation are included in this report (Figure 2). Nodules were observed on the non-nodulating line when cross-graphed with a soybean scion. Surprisingly, nodules also were formed on several bean lines when R99 (non-nodulating bean line) was used as a scion on other bean root stocks. Both results are contrary to the current understanding of shoot regulation of nodule formation. Evidently, both root and shoot controlling factors need to be considered in attempts to enhance nodule formation and effectiveness.

**<u>Strategic Aim 1:</u> Achievements and Outputs**

- Initial field trials to evaluate genotype x inoculant interactions have been completed
- Protocols for quantifying biomass, total plant N, petiole ureide content, nodule sampling/classes/occupancy were established and distributed to all US and HC partners.
- Analyses of plant N, biomass, nodule classes, ureide levels prior to pod fill are underway.
- Soil samples from field sites were collected and sent to WSU for rhizobia analysis
- Soil and weather data were collected where available.
- Seed of non-nodulating lines were obtained and distributed.
- Grafting studies indicate potential for increasing nodule formation and effectiveness in beans.
- Potential rhizobia genetic markers for indigenous strains and those in trial inoculants are established.
- Preliminary assessment of rhizobial content (or lack thereof) of HC inoculants completed.

**<u>Strategic Aim 2:</u> Examine the inheritance of genetic and environmental variation in BNF in common bean, and to identify molecular markers associated with QTL conditioning for enhanced BNF.**

***Objective 2a: Identify parental materials for inheritance studies of BNF*.**

**Approach**

Identify and collect bean lines for a BNF diversity panel (BNF-DP) consisting of 300+ Andean bean lines for evaluation, identify and increase seed of non-nodulating tropically adapted bean

lines, characterize selected lines for BNF response as potential RIL parents, initiate phenotype screens in greenhouse on selected lines for subsequent SNP analysis, and advance RIL populations currently available.

Objective 2b: Phenotype existing mapping populations for BNF response, populate with molecular markers, and conduct QTL analysis.

**Approach**

Phenotype selected populations [Bean CAP and South American Core] for BNF response in US field sites, prepare samples for SNP analysis on bean CAP and SA Core collection for association mapping with phenotype data, initiate search for genes associated with BNF, establish mechanism to correlate BNF phenotype data from field and GH trials.

**Results**

Considerable progress has been made by WSU (Miklas) and MSU (Cichy) to identify and collect bean lines for a BNF diversity panel. Thus far 333 bean lines have been collected for the panel (appendix: Table 1). Most of these lines are Andean in origin and will constitute the Andean diversity panel for subsequent association mapping of BNF and other traits in conjunction with the Bean CAP project. About half of the 333 lines require seed increase prior to DNA and phenotyping analyses. This increase will be done in Puerto Rico this winter in collaboration with Tim Porch (Angola Project). In addition to the 333 lines in hand, there will be additional lines from CIAT, Mexico, and Ecuador increased in Puerto Rico to include in this diversity panel.

Phenotyping of selected lines for BNF response has been initiated at WSU in three BNF experiments. Exp I included 36 genotypes selected from mapping populations and based on BNF capability reported in the literature. These were tested at two locations, two reps, and against three treatments - low N, low N with Biostack inoculant, and high N via supplemental fertilizer. The plots have been harvested; seed yield and seed size are being processed. Plant and seed samples will be tested for ureide content and total N. Exp II included 49 genotypes, 47 Andean lines from the Bean CAP and 2 Middle American checks including a non-nod line. There were two reps and two treatments - low N and high N. Some lines have yet to be harvested due to late maturity. But preliminary assessment of plot yield suggests some of these lines could be very efficient for BNF (appendix: Table 2). Vast diversity for BNF response (% efficiency – Table 2) in this subset of the Andean Diversity Panel is a very positive indication of thepotential utility of these materials for association mapping BNF traits in common bean. Exp III was the same as Exp II except that it consisted of Andean lines primarily from the South American core subset from the NPGS repository in Pullman, WA. Detailed analysis of these materials was abandoned because of their poor stands. Nonetheless, seed was collected from about half of the lines for inclusion in the Andean Diversity Panel.

Plant samples for SNP analysis have been collected from plants in the CAP and SA Core collection. The SNP allele calls for the Andean Diversity Panel will be outsourced through the Bean CAP project. Arrangements have been made with the Bean CAP project director Phil McCean to analyze these Andean Diversity Panel lines with the available SNP chip(s), once the composition of Diversity Panel has been finalized. The subsequent SNP and association mapping

analysis of the phenotypic data will follow standard procedures established by the Bean CAP project.

Greenhouse screening trial for BNF response was initiated on selected lines MSU. The initial greenhouse screen of 22 genotypes of Andean and Mesoamerican origin was completed in June 2011. This screen involved growing plants in a perlite/vermiculite media without added nitrogen. Seeds were inoculated with rhizobia strain CIAT 899. Plants were harvested at flowering and shoot and root biomass and shoot N concentration were measured. As expected, the non-nodulating line R99 had the lowest shoot dry weight and N content. There was a wide range in tissue N contents (0.35 mg/g to 0.003 mg/g) indicating a large genotypic variation existed among these lines for ability to fix N, when no fertilizer N is supplied.

During the PI trip to Rwanda, a vine type Andean kidney bean genotype was identified as a potential RIL parental line for BNF response. This line is adapted and highly productive in temperate zone and appears to have more prolific root system than commercial bush types prone to root rots. This type of root system may provide for enhanced nodulation and this is being tested. This combination of phenotypic characteristics would be especially useful for developing an Andean x Andean RIL population.

Phenotyping of selected lines for BNF response at MSU is underway. Phenotyping of 2010 field materials is now completed. This included analysis of seed N and seed N14/N15. Significant genotypic differences in seed protein and percent N from fixation were detected. Of the 21 genotypes evaluated in 2010 (excluding the Eagle x Puebla RILs) Puebla 152 had the highest percent protein in the seed at 28.5% this corresponds to data on the percent N in the aboveground biomass at flowering, where it was also the highest. The line with the lowest percent protein was the navy bean Albion at 17.2%. Interestingly this line also had the highest N derived from fixation in the seed at 25%. Phenotyping of 2011 field materials included collection of aboveground biomass at R1. Ground plants were weighed and sent to ISU for total N and ureide analysis. Plots were just harvested in October, and are currently being processed to determine seed yield, yield components, and seed N.

Also, a number of selected RILs have been advanced to the F3 generation (Figure 3). F2 seed of Puebla 152 x G08263 (a MSU Great Northern breeding line) were planted in Frankenmuth MI and F3 seed were harvested from 120 plants. These materials will be advanced to F4 via single seed descent to develop a RIL population that can be used in future genotyping, mapping, and physiological research envisioned for this project.

The search for BNF responsive genes in silico has been initiated. This activity centers on gene expression studies involving *de novo* purine synthesis and ureide metabolism. Purine synthesis and ureide metabolism are key processes in biological nitrogen fixation. These genes were originally identified in *Arabidopsis* and then as *Phaseolus vulgaris* homologs by Dr. Carol Vance (University of Minnesota). Dr. Vance has kindly provided the *P. vulgaris* primer sequence information for these genes to use in RT PCR. The genes are listed below:

*De novo* Purine synthesis

1) At1g09830, 2)At4g34740 3)At1g31220 4)At1g74260 5)At3g55010

Ureide biosynthesis

**1)**At1g16350 **2)**At4g34890 **3)**At2g26230 **4)**At5g58220

We are investigating the expression patterns of these genes in common bean genotypes with varying biological nitrogen fixation abilities.

**Strategic Aim 2: Achievements and Outputs**

- Most of the lines to be included in the BNF Diversity Panel have been identified (50 lines from bean CAP, 50 lines from South American Core set, 200+ lines from Africa, Central America, US collections) and seed increase is underway.
- A level of genetic diversity useful for association mapping and physiological studies has been documented among selected bean lines for BNF capability and response to inoculant.
- BNF-responsive genes identified for support future studies to distinguish superior BNF lines.

**Strategic Aim 3: Improve the productivity, profitability, and sustainability of agricultural systems on degraded soils through effective dissemination of new information and technologies to small-landholder farmers.**

***Objective 3a: Improve farmer awareness of inoculation technologies***

**Approach**

Evaluate current farmer knowledge, practices, and attitudes about BNF and inoculation, initiate training materials on BNF and seed inoculation for Extensionists, community based trainers, farmers, create awareness among Extensionists at HC institutions on benefits of BNF and inoculant use as seen in soybeans.

***Objective 3b: Prepare fort on-farm demonstrations comparing inoculant strategies***

**Approach**

Prepare for on-farm trials by identifying potential farmer cooperators, and by training farmer cooperators on proper methods for conducting on-farm trials

***Objective 3c: Strengthen farmers' collective capabilities to purchase inoculants and incorporate them into a profitable and sustainable system for small landholders***

**Approach**

Initiate production of training materials to disseminate through the Participatory Ecological Land Use Management Association (PELUM )

**Results**

The program to evaluate current farmer knowledge, practices, and attitudes about BNF and

inoculation is underway. A survey tool was developed by VEDCO Uganda staff hired to support HC evaluation and training activities (attached to this report). This survey tool is being tested for suitability on a subset of the 1200 farmers in the Uganda ISU/VEDCO rural livelihoods program in Kamuli District. Based on these results, a common survey will be developed for Rwanda and Tanzania in consultation with HC Extentionists.

All field testing on inoculant response was conducted on research stations at Host Country sites selected to vary in agroecological conditions. Potential sites for on-farm trials are scheduled for the first planting season in 2012. To date, potential areas and farmer for the on farm trials have been identified in Siha and Hai districts in Tanzania. Comparable sites will be identified for on-farm trials in Rwanda and Uganda in 2012.

Based on input from the TMAC team in May 2011, activities on Objective 3b and 3c were delayed to Phase II of this project.

**Strategic Aim 3: Achievements and Outputs**

- Field trials to test local variety response to inoculants were completed as planned.
- A draft survey tool to assess farmer knowledge, practices, and attidutudes (KPA) about been seed inoculation completed.
- Field staff hired to monitor and coordinate Extensionist and farmer trainings on inoculant technology.

**Objective 4: Institutional Capacity Building "Increase the capacity, effectiveness and sustainability of agriculture research institutions which serve the bean and cowpea sectors in developing countries"**

Capacity building in terms of degree training includes formal education for seven (7) MS level graduate students and six (6) undergraduate students, five of which are from host countries. Two graduate students are being trained in the Soil Science Department at Makerere University under the direction of Dr. John S. Tenywa, Professor of Soil Science. Two graduate students are being trained at Sokoine University of Agriculture under the direction of Dr. Susan Nchimbi, Associate Professor of Plant Breeding and Genetics. One graduate student is being trained at Washington State University under the co-direction of Dr. Lynn Carpenter-Boggs, Assistant Professor of Soil Microbiology and Biochemistry, and Dr. Phillip Miklas, Legume Research Geneticist with USDA-ARS. One HC graduate student is being trained at Iowa State University under the direction of the program PI, Dr. Mark Westgate, Professor of Crop Production and Physiology. And one HC graduate student is being trained at Michigan State University under the co-direction of Dr. Jim Kelly, Professor of Crop Breeding and Genetics, and Dr. Karen Cichy, Research Geneticist with USDA-ARS.

Capacity building in terms of non-degree training include formal internships for five (5) undergraduate students and training of HC laboratory technicians, field agronomists and extension staff on use and agricultural benefits of seed inoculants. In FY11, three undergraduate students were assigned to the three field sites in Rwanda to assist in germplasm evaluation. These students were supervised by Dr. Augustine Musoni, and interact directly with US PIs

during their visits to the field sites. Two undergraduate interns were assigned to work with VEDCO staff on assessing farmer awareness of BNF technology and information dissemination. One undergraduate intern was assigned to work with HC graduate students to support their field projects on improving bean production and BNF at Iowa State University. This student was supervised by Dr. Mark Westgate, program PI.

**Degree Training: Seven host country M.Sc. graduate students**

| | |
|---|---|
| First and Other Given Names | **Mercy** |
| Last Name | **Kabahuma** |
| Citizenship | Uganda |
| Gender | Female |
| Training Institution | Iowa State University |
| Supervising CRSP PI | Mark Westgate |
| Degree Program for training | M.S. |
| Program Areas or Discipline | Plant Physiology |
| If enrolled at a US university, will Trainee be a "Participant Trainee" as defined by USAID? | YES |
| Host Country Institution to Benefit from Training | Uganda |
| Thesis Title/Research Area | Shoot and Root Control of BNF |
| Start Date | Fall 2010 |
| Projected Completion Date | Summer 2012 |
| Training status | Active |
| Type of CRSP Support | Full |

| | |
|---|---|
| First and Other Given Names | **Martha** |
| Last Name | **Abwate** |
| Citizenship | Uganda |
| Gender | Female |
| Training Institution | Makerere University |
| Supervising CRSP PI | Tenywa |
| Degree Program for training | M.S. |
| Program Areas or Discipline | Soil Science |
| If enrolled at a US university, will Trainee be a "Participant Trainee" as defined by USAID? | YES |
| Host Country Institution to Benefit from Training | Uganda Makerere University |
| Thesis Title/Research Area | TBD |
| Start Date | Summer 2010 |
| Projected Completion Date | Summer 2012 |
| Training status | Active |
| Type of CRSP Support | Full |

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|---|---|
| First and Other Given Names | **Peter** |
| Last Name | **Ssenyonga** |
| Citizenship | Uganda |

| | |
|---|---|
| Gender | Male |
| Training Institution | Makerere University |
| Supervising CRSP PI | Tenywa |
| Degree Program for training | M.S. |
| Program Areas or Discipline | Soil Science |
| If enrolled at a US university, will Trainee be a "Participant Trainee" as defined by USAID? | YES |
| Host Country Institution to Benefit from Training | Uganda Makerere University |
| Thesis Title/Research Area | Micronutrient limitations on BNF |
| Start Date | Summer 2010 |
| Projected Completion Date | Summer 2012 |
| Training status | Active |
| Type of CRSP Support | Full |

| | |
|---|---|
| First and Other Given Names | **Kelvin** |
| Last Name | **Kamfwa** |
| Citizenship | Rwanda |
| Gender | Male |
| Training Institution | Michigan State University |
| Supervising CRSP PI | Kelley, Cichy |
| Degree Program for training | M.S. |
| Program Areas or Discipline | Plant Breding/Genetics |
| If enrolled at a US university, will Trainee be a "Participant Trainee" as defined by USAID? | YES |
| Host Country Institution to Benefit from Training | Rwanda ISAR |
| Thesis Title/Research Area | Genetic control of BNF in beans |
| Start Date | Fall 2010 |
| Projected Completion Date | Summer 2012 |
| Training status | Active |
| Type of CRSP Support | Full |

| | |
|---|---|
| First and Other Given Names | **Michael** |
| Last Name | **Lege** |
| Citizenship | US |
| Gender | Male |
| Training Institution | Washington State University |
| Supervising CRSP PI | Carpenter-Boggs, Miklas |
| Degree Program for training | M.S. |
| Program Areas or Discipline | Soil Microbiology/Biochemistry |
| If enrolled at a US university, will Trainee be a "Participant Trainee" as defined by USAID? | YES |
| Host Country Institution to Benefit from Training | Tanzania Sokoine University |
| Thesis Title/Research Area | N2 fixation of rhizobial strains |
| Start Date | Fall 2010 |
| Projected Completion Date | Summer 2012 |

| | |
|---|---|
| Training status | Active |
| Type of CRSP Support | Full |

| | |
|---|---|
| First and Other Given Names | **Charles** |
| Last Name | **Komba** |
| Citizenship | Tanzania |
| Gender | Male |
| Training Institution | Sokoine University Agriculture |
| Supervising CRSP PI | Nchimbi |
| Degree Program for training | M.S. |
| Program Areas or Discipline | Breeding and Genetics |
| If enrolled at a US university, will Trainee be a "Participant Trainee" as defined by USAID? | YES |
| Host Country Institution to Benefit from Training | Tanzania Sokoine University |
| Thesis Title/Research Area | Breeding BNF with land races |
| Start Date | Summer 2010 |
| Projected Completion Date | Summer 2012 |
| Training status | Active |
| Type of CRSP Support | Full |

| | |
|---|---|
| First and Other Given Names | **Beata** |
| Last Name | **Khafa** |
| Citizenship | Tanzania |
| Gender | Female |
| Training Institution | Tanzania Sokoine University |
| Supervising CRSP PI | Mchimbi, Tindwa |
| Degree Program for training | M.S. |
| Program Areas or Discipline | Breeding and Genetics |
| If enrolled at a US university, will Trainee be a "Participant Trainee" as defined by USAID? | YES |
| Host Country Institution to Benefit from Training | Sokoine University Agriculture |
| Thesis Title/Research Area | Soil nutrient interactions/N2 fixation |
| Start Date | Summer 2010 |
| Projected Completion Date | Summer 2012 |
| Training status | Active |
| Type of CRSP Support | Full |

**Other Training: Internships**

**Two undergraduate internships at VEDCO in Uganda**

| | |
|---|---|
| Type of training | Internship |
| Description of training activity | Participation in field operations |
| Location | Varied, depending on staff and farmer group locations |
| Duration | 8 weeks |
| When did it occur? | Summer 2011 |

| | |
|---|---|
| Participants/Beneficiaries of Training Activity: | Undergraduate students |
| Numbers of Beneficiaries | 1 male, 1 female |
| PI/Collaborator responsible | VEDCO, Musoke\ |
| | |
| List other funding sources that will be sought | VEDCO |
| Training justification | Adaptation of technology requires user understanding of appropriate management, and pitfalls. |

**Three undergraduate internships at ISAR in Rwanda**

| | |
|---|---|
| Type of training | Internship |
| Description of training activity | Participation in field operations |
| Location | Varied, depending on staff and farmer group locations |
| Duration | 8 weeks |
| When did it occur? | Summer 2011 |
| Participants/Beneficiaries of Training Activity: | Undergraduate Students |
| Anticipated numbers of Beneficiaries | 2 female, 1 male student |
| PI/Collaborator responsible | ISAR, Musoni |
| Training justification | Adaptation of technology requires user understanding of appropriate use, management, and pitfalls. |

**One undergraduate internship at Iowa State University**

| | |
|---|---|
| Type of training | Internship |
| Description of training activity | Participation in field operations |
| Location | ISU |
| Duration | 6 weeks |
| When did it occur? | Summer 2011 |
| Participants/Beneficiaries of Training Activity: | Undergraduate student |
| Numbers of Beneficiaries | 1 female |
| PI/Collaborator responsible | ISU, Westgate |
| List other funding sources | ISU |
| Training justification | Identification of germplasm with improved BNF requires understanding of limiting factors under field conditions. |

**Explanation for Changes**

**Objective 1**

Securing long-term weather data for the research station locations in Rwanda and Tanzania has been delayed due to in country regulations. The process is on-going and data are yet to be received. These data are needed to complete the objective of modeling weather-related trends in rhizobia soil populations and BNF response to inoculant.

Ureide levels in leaf and stem tissues were not determined at all HC location due to lack of required facilities to conduct such tests. Local sources of laboratory equipment needed to conduct these analyses are being investigated.

**Objective 2**

Biological contents and titre of the 'stacked' inoculant from Becker Underwood has not been completed. This will be done during the first prior to the mid-year report for FY12.

**Objective 3**

Extensive assessment of farmer knowledge, practices, and attitudes regarding inoculant technology is delayed pending approval of a common survey tool for all three host countries. A draft tool has been developed and an initial survey will be completed prior to the mid-year report for FY12.

At the recommendation of the TMAC team during their review of this BNF-CRSP project in May 2011, on-farm trials in cooperation with local farmers is delayed until initial field trials at HC research stations are completed and results reviews.

**Objective 4**

Student training in BNF protocols was delayed to 2012. Michael Lege, MS student at WSU, was trained in the N15 isotope dilution technique to quantify BNF in 51 bean genotypes. Training of additional graduate students from all host countries was added as a new activity by the TMAC team. This activity was not budgeted in FY11. So net meeting methods need to be devised to train students by internet. Miklas, Cichy, and a few students will visit Dr. Phil McClean's laboratory for training for SNP analysis in Spring 2012.

Establishing plausible mechanisms to relate field and GH responses to inoculation was delayed pending data assembly from the 2011 field season, including yield and %N in aboveground biomass to determine the correlation between field and greenhouse trials. These data are expected prior to submitting the mid 2012 report. Variation in percent seed protein will be targeted as an indication of BNF capacity.

**Networking and Linkages with Stakeholders**

All project PIs and graduate students in Uganda participated in the CRSP team and TMAC meetings in May 2011. Two students in Tanzania are being funded by N2Africa program – "effectiveness of inoculants on bean yields using improved germplasm". Bean germplasm was obtained by Dr. Nchimbi from Dr. Roland Chirwa CIAT-Malawi and from Dr. Phil Miklas – USA for evaluation in projects at SUA. Bean RILs and unique parental lines were obtained by PI Westgate from Dr. Cichy for field evaluation and grafting studies.

**Leveraged Funds**

| | |
|---|---|
| Name of PI receiving leveraged funds: | Dr. Lukman N. Mulumba |
| Description of leveraged project and purpose: | Capacity building for integrated soil fertility management for Uganda and Rwanda |
| Dollar Amount: | >US$700,000 |
| Funding Source: | Alliance for Green Revolution Africa (AGRA) |

**Scholarly Activities and Accomplishments**
None

**Tables/Figures**
See attached.

**Literature Cited**
Zaychuk. 2006. Nodulation and nitrogen fixation guide for assessment of pulse crops. www.pulse.ab.ca/linkclick.aspx?fileticket=l2ect Accessed October 2010

**Contribution to USAID Gender Equity Goals**
In Tanzania, Rwanda, and Uganda, common bean is mainly cultivated by women who have low resources, which do not allow them to buy farm inputs including fertilizers. Use of rhizobia inoculants will be beneficial to these farmers who currently are not using fertilizers in bean fields. More than 50% of the people involved in the demonstration activities are female from the local communities. Four of the six undergraduate interns supported by the project in FY11 were female. Three of seven MS students being trained at HC and US institutions are female.

**Progress Report on Activities Funded Through Supplemental Funds**
Not applicable

Figure 1. Example of local weather data collecte from the field research sites. Rainfall and temperature data for Namulonge, Mbarara, and Kabale field sites in Uganda.

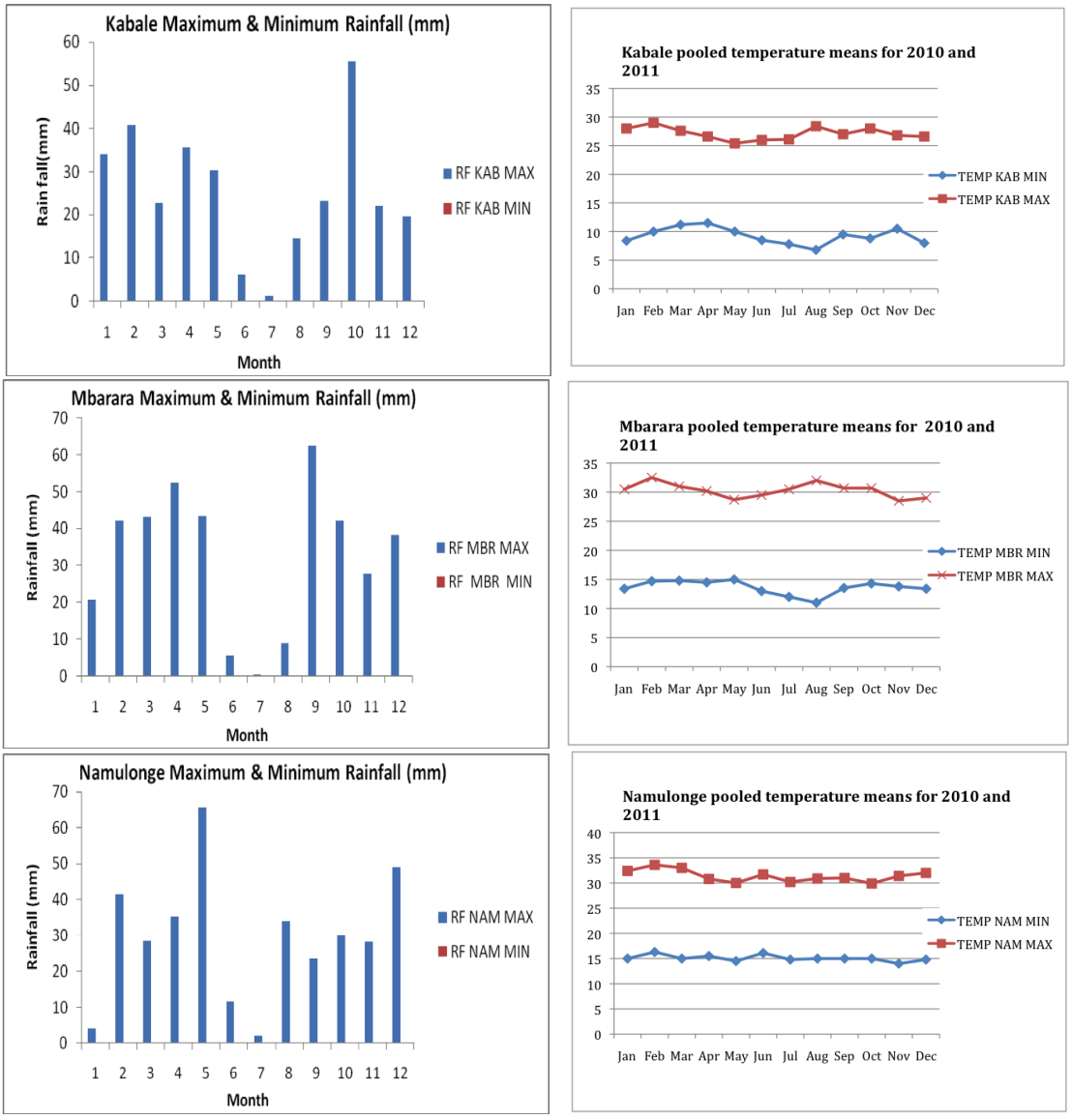


Table 1. Soil characteristics at field sites in Rwanda, Tanzania, and Uganda in 2011. Yellow boxes indicate out of optimum range. Red boxes indicate soils with extreme limitations.

| May-11 | soil samples | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | | | | | | | | |
| **town** | **country** | **code** | | | | | | | | | | | | | | | | | | | |
| Nyagatere | Rwanda | NYRW | | | | | | | | | | | | | | | | | | | |
| Rubona | Rwanda | RURW | | yellow | some concern | | | | | | | | | | | | | | | | |
| Musanza | Rwanda | MURW | | red | extreme | | | | | | | | | | | | | | | | |
| Selian | Tanzania | SETZ | | | | | | | | | | | | | | | | | | | |
| Morogoro | Tanzania | MOTZ | | | | | | | | | | | | | | | | | | | |
| Kamuli (Vedco wo | Uganda | KIUG | | | | | | | | | | | | | | | | | | | |
| Kampala | Uganda | KAUG | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | |
| Sample | LAB NO | pH | E.C. 1:1 | NO3-N | NH4-N | OM | P | K | Ca | Mg | Na | S | B | Zn | Mn | Cu | Fe | Eff. | A&E | REQ | Al |
| | | 1:1 | mmhos/cm | mg/kg | mg/kg | % | mg/kg | mg/kg | meq/100g | meq/100g | meq/100g | mg/kg | mg/kg | mg/kg | mg/kg | mg/kg | mg/kg | | pH | T/Ac | mg/Kg |
| NHRW | 9118 | 5.8 | 0.50 | 13.6 | 16.0 | 3.2 | 15 | 349 | 4.6 | 1.8 | 0.14 | 43 | 0.61 | 1.6 | 323.1 | 3.0 | 133 | 0 | 7.3 | 1.5 | 0.9 |
| RURW | 9119 | 4.3 | 0.56 | 33.0 | 19.6 | 2.3 | 31 | 187 | 0.9 | 0.4 | 0.12 | 66 | 0.02 | 0.3 | 301.1 | 2.4 | 205 | 0 | 7.1 | 3.6 | 55.5 |
| MURW | 9120 | 5.7 | 0.53 | 29.6 | 19.3 | 2.1 | 33 | 130 | 3.4 | 0.6 | 0.20 | 16 | 0.13 | 1.7 | 78.9 | 1.2 | 29 | 0 | 7.5 | 1.2 | 3.2 |
| SETZ | 9121 | 6.9 | 0.37 | 2.8 | 16.0 | 3.8 | 32 | 1700 | 13.1 | 3.4 | 0.44 | 32 | 0.55 | 6.5 | 320.1 | 8.3 | 32 | 0 | 7.2 | 0.0 | 0.8 |
| MOTZ | 9122 | 5.9 | 0.57 | 15.5 | 19.9 | 3.9 | 15 | 382 | 4.9 | 3.8 | 0.39 | 39 | 0.41 | 2.3 | 323.1 | 2.2 | 44 | 0 | 7.3 | 1.4 | 1.0 |
| KIUG | 9123 | 6.2 | 0.41 | 9.2 | 13.9 | 3.1 | 5 | 284 | 4.8 | 1.7 | 0.22 | 29 | 0.24 | 4.5 | 323.1 | 2.3 | 24 | 0 | 7.5 | 0.6 | 2.6 |
| KAUG | 9124 | 6.4 | 0.68 | 17.2 | 15.1 | 3.6 | 16 | 248 | 5.4 | 1.5 | 0.15 | 43 | 0.27 | 5.3 | 320.1 | 3.3 | 31 | 0 | 7.5 | 0.0 | 2.5 |
| | | | | | | | | | | | | | | | | | | | | | |
| | value too | low | | RURW, MURW high | | | low | MURW low | | | | low | low | low | | | high | | | | high |
| | | | | SETZ low | | | | SETZ high | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | |
| | | 6.5 | < 2 | 25 | | | 20+ | 200 | 1.2 | 0.4 | <0.35 | 30 | 1.3 | 1 | 4 | 0.8 | 10 | | | | |

Table 1. Effect of bean genotype, phosphorus rate and rhizobia inoculants type on common bean nodulation score at Namulonge, Uganda. 2011 BNF field trials.

| **Bean variety** | **Phosphorus Rate** (kg ha$^{-1}$) | **Rhizobia Strain** | | | |
|---|---|---|---|---|---|
| | | Makerere | Nairobi | Stacked | Indigenous |
| K132 | 0 | 10.8 | 5.3 | 11.5 | 7.0 |
| | 40 | 6.0 | 6.3 | 5.7 | 4.5 |
| Kanyebwa | 0 | 12.1 | 5.2 | 9.4 | 7.5 |
| | 40 | 5.9 | 6.7 | 11.5 | 8.8 |
| K131 | 0 | 12.3 | 12.9 | 9.5 | 10.6 |
| | 40 | 11.6 | 12.5 | 12.2 | 9.2 |
| $LSD_{0.05}$ | | | 2.3 | | |

Table 5: Effect of Rhizobia inoculants type on Nodulation score in climbing bean for bean varieties NABE 10c, NABE 12c and a local variety at Kabale, Uganda. 2011 BNF Field Trials. Only climbing bean genotypes were considered at the Kabale site.

| | Rhizobia Inoculants types | | | |
|---|---|---|---|---|
| | Makerere | Nairobi | Stacked | Indigenous |
| | 5.7 | 5.2 | 5.1 | 7.2 |
| $LSD_{0.05}$ | | 1.0 | | |

Table 6: Effect of bean variety, phosphorus rate and rhizobia inoculants type on nodule score in common bean at Mbarara, Uganda. 2011 BNF Field Trails. Bush bean genotypes evaluated at Namulonge were also used at this site.

| **Bean variety** | **Phosphorus Rate (kg ha$^{-1}$)** | **Rhizobia inoculant types** | | | |
|---|---|---|---|---|---|
| | | Makerere | Nairobi | Stacked | Indigenous |
| K132 | 0 | 12.8 | 10.2 | 9.7 | 7.3 |
| | 40 | 11.2 | 12.0 | 11.1 | 9.3 |
| Kanyebwa | 0 | 11.3 | 10.5 | 11.0 | 8.5 |
| | 40 | 12.9 | 12.2 | 12.8 | 6.2 |
| K131 | 0 | 12.0 | 6.7 | 9.1 | 6.2 |
| | 40 | 10.6 | 12.0 | 12.1 | 12.8 |
| $LSD_{0.05}$ | | | 1.6 | | |

Table 2. Example of field data collected on commonly grown bean varieties grown at research stations in Uganda in 2011. Stacked strain is Becker Underwood Biostacked inoculant. Nodulation score is a relative evaluation of the number of functional nodules. Note that this score varies with genotype, inoculant, and P level.

Figure 2a. Nodule number on roots of grafted plants using the soybean line PI132.217 as the scion grafted onto various rootstocks. PIR32 and R99 are bean lines. R99 is a non-nodulating line under normal condition. Evans and PI132.217 are soybean lines varying in seed N. Note the large number of nodules formed on R99 and PIR32 roots in the cross-species grafts.

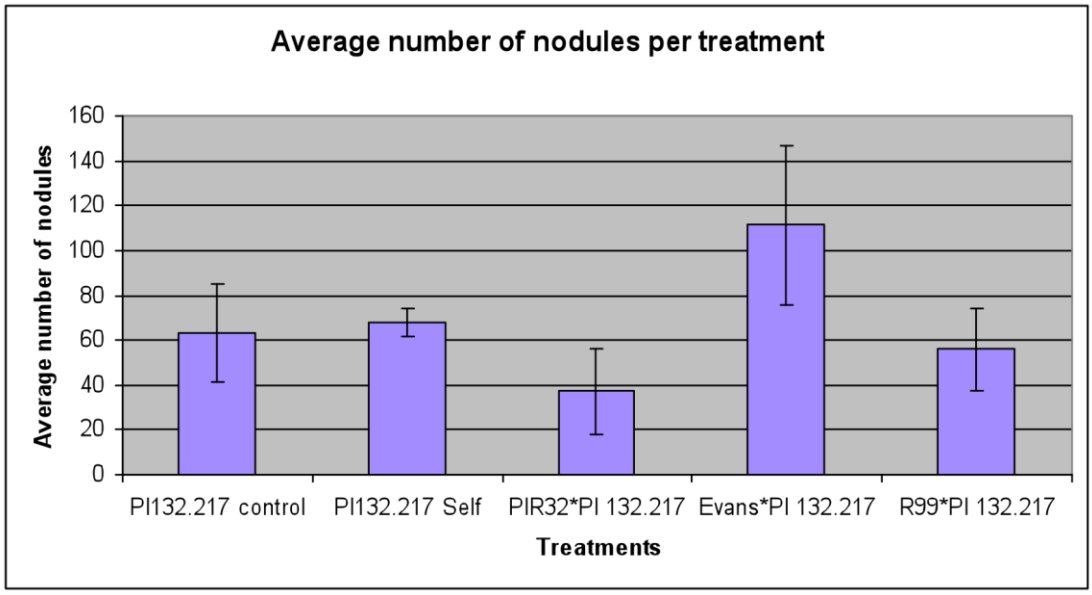
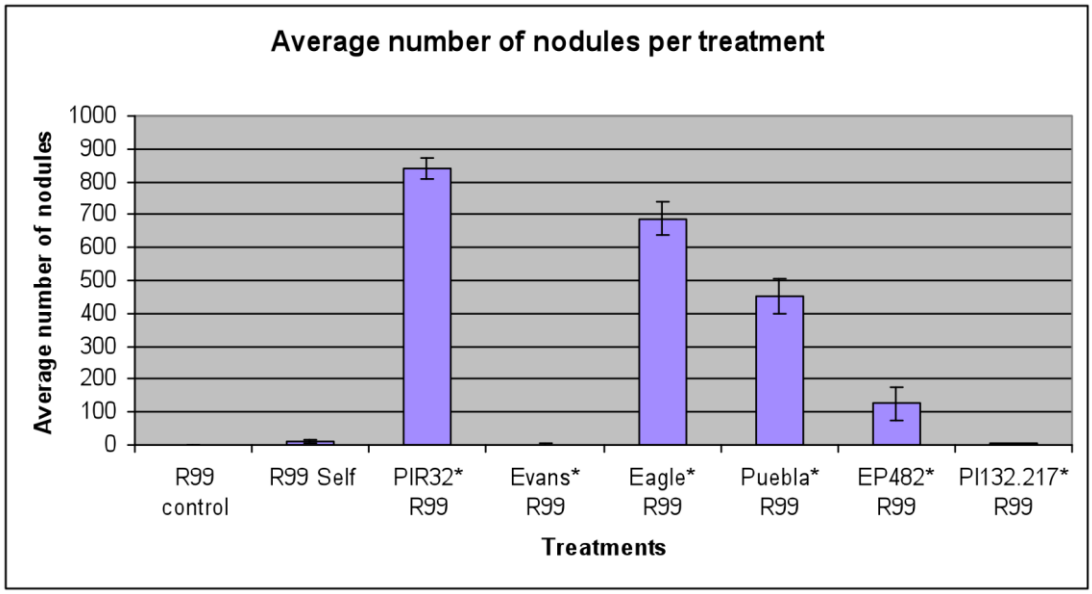



Figure 2b. Nodule number on roots of grafted plants using the non-nodulating line (R99) as the scion grafted onto various rootstocks. PIR32, Eagle, Puebla, and EP482 are bean lines varying in capacity for BNF. Evans and PI132.217 are soybean lines varying in seed N. Note the large number of nodules formed on the bean roots when R99 was the scion. No nodules were formed in the cross-species grafts.



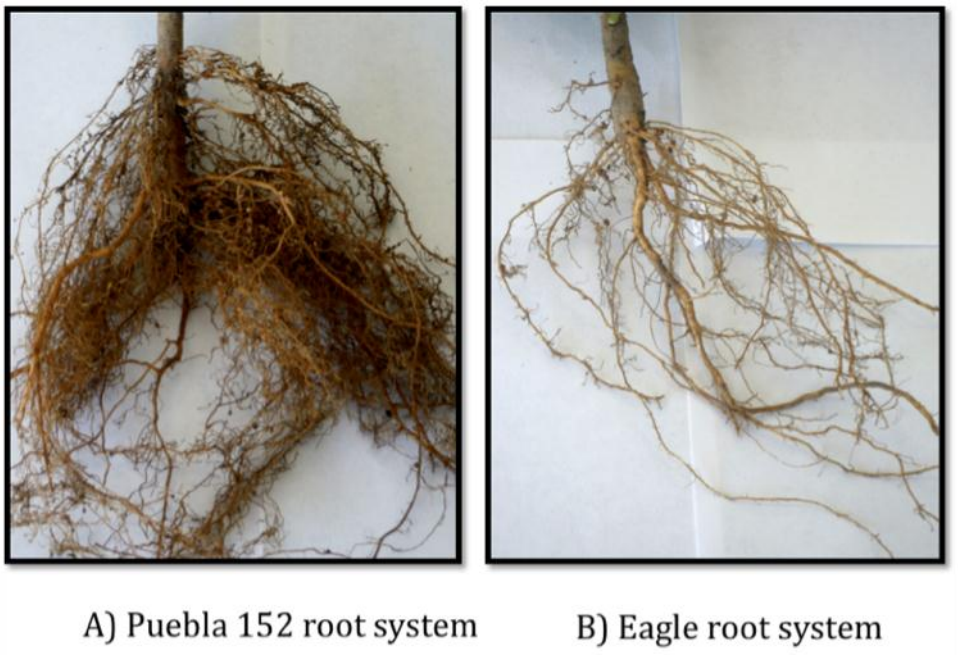
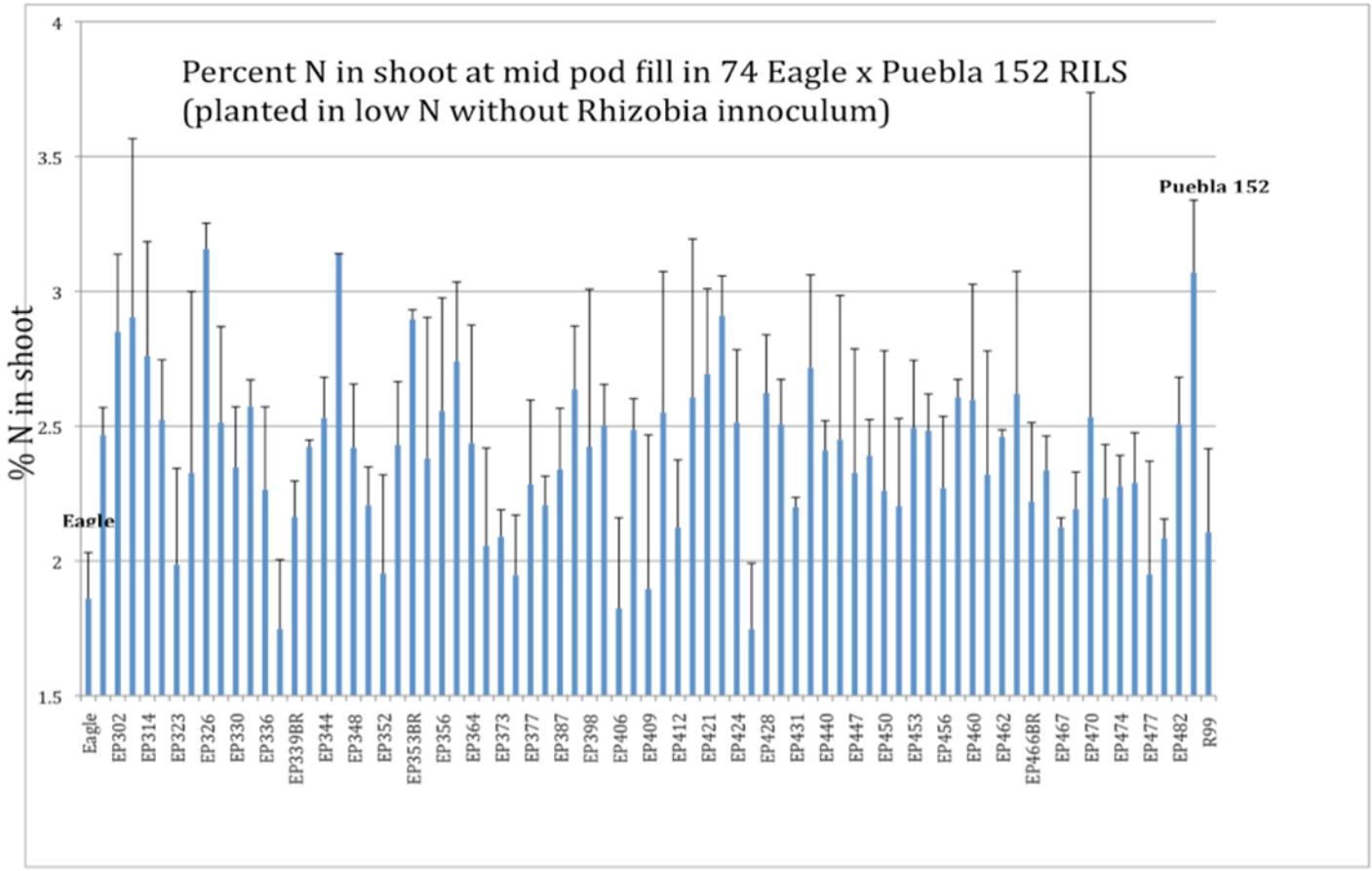



Figure 3. A) Image of root system of Puebla 152, a tropical black bean, considered to be efficient at biological nitrogen fixation. B) Image of root system of Eagle, a snap bean cultivar. Both plants were grown at the Saginaw Valley Research Farm.



**Dry Grain Pulses CRSP**
**Research, Training and Outreach Workplans**
**(October 1, 20010 -- September 30, 2011)**

**FY 2011 PERFORMANCE INDICATORS-SUA**
**for Foreign Assistance Framework and the Initiative to End Hunger in Africa (IEHA)**

**Project Title: Enhancing biological nitrogen fixation (BNF) of leguminous crops grown on degraded soils in Uganda, Rwanda, and Tanzania**

**Lead U.S. PI and University: Mark E. Westgate, Iowa State University**
**Host Country(s): Rwanda, Tanzania, Uganda**

| Output Indicators | 2011 Target (October 1, 2010-Sept 30, 2011) | 2011 Actual (October 1, 2010-Sept 30, 2011) |
|---|---|---|
| **Degree Training: Number of individuals enrolled in degree training** | | |
| Number of women | 4 | 4 |
| Number of men | 3 | 4 |
| **Short-term Training: Number of individuals who received short-term training** | | |
| Number of women | 50 | 4 |
| Number of men | 50 | 4 |
| **Technologies and Policies** | | |
| Number of technologies and management practices under research | 1 | 1 |
| Number of technologies and management practices under field testing | 1 | 1 |
| Number of technologies and management practices made available for transfer | 1 | 1 |
| Number of policy studies undertaken | | |
| **Beneficiaries:** | | |
| Number of rural households benefiting directly | 1000 | 0 |
| Number of agricultural firms/enterprises benefiting | 6 | 3 |
| Number of producer and/or community-based organizations receiving technical assistance | 50 | 0 |
| Number of women organizations receiving technical assistance | 3 | 0 |
| Number of HC partner organizations/institutions benefiting | 6 | 3 |
| **Developmental outcomes:** | | |
| Number of additional hectares under improved technologies or management practices | 1000 | 20 |

**Dry Grain Pulses CRSP**

**Report on the Achievement of "Semi-Annual Indicators of Progress"**

**(For the Period: October 1, 2010 – September 30, 2011)**

**This form should be completed by the U.S. Lead PI and submitted to the MO by October 1, 2011**

***Project Title:*** *Enhancing biological nitrogen fixation (BNF) of legumious crops grown on degraded soils in Uganda, Rwanda, and Tanzania*

***Abbreviated name of institutions***

| | ISU | | | MSU | | | WSU | | | VEDCO | | | NaCCRI | | | SUA | | | Makerere | | | ISAR | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Target | Achieved | | Target | Achieved | | Target | Achieved | | Target | Achieved | | Target | Achieved | | Target | Achieved | | Target | Achieved | | Target | Achieved | |
| ***Benchmarks by Objectives*** | ###### | Y | Nº | ###### | Y | Nº | ###### | Y | Nº | ###### | Y | Nº | ###### | Y | Nº | ###### | Y | Nº | ###### | Y | Nº | ###### | Y | Nº |
| ***(Tick mark the Yes or No column for identified benchmarks by institution)*** | | | | | | | | | | | | | | | | | | | | | | | | |
| **Objective 1** | | | | | | | | | | | | | | | | | | | | | | | | |
| Field Trial 1 established at HC sites | | | | | | | | | | | | | x | Y | | x | Y | | | | | x | Y | |
| Quantified yield advantage of inoculation in US and HC field trials | | | | | | | x | Y | | | | | x | Y | | x | Y | | | | | x | Y | |
| Established project website for data storage/sharing | x | | N | | | | | | | | | | | | | | | | | | | | | |
| Secured non-nodulating lines for field studies | | | | | | | x | Y | | | | | x | Y | | | | | | | | | | |
| Analysis of soil/weather data completed | x | | | | | | | | | | | | x | Y | | x | Y | | x | Y | | x | Y | |
| Common varieties to test at all HC sites identified | | | | | | | | | | | | | x | Y | | x | Y | | | | | x | Y | |
| Finalized protocols for field sampling and controlled environment work | x | Y | | | | | x | Y | | | | | | | | | | | | | | | | |
| Phenotypic responses to inoculant x genotype x environment identified | x | Y | | x | Y | | x | Y | | | | | x | Y | | x | Y | | x | Y | | x | Y | |
| Soil from field sites tested for indigenous rhizobia strains | x | | N | x | | N | x | | N | | | | x | Y | | x | | N | x | | N | x | | N |
| Established indigenous rhizobia levels associated with environment | | | | | | | x | | N | | | | | | | | | | | | | | | |
| Initial characterization of soil rhizobia populations conducted | | | | | | | x | | N | | | | | | | | | | | | | | | |
| Trial inoculants evaluated for rhizobia titre | x | | N | | | | x | | N | | | | | | | | | | | | | | | |
| **Objective 2** | | | | | | | | | | | | | | | | | | | | | | | | |
| Identified/collected bean lines for BNF diversity panel | | | | x | Y | | x | Y | | | | | | | | | | | | | | | | |
| Collected/increased seed of non-nodulating adapted lines | x | Y | | x | Y | | x | Y | | | | | | | | | | | | | | | | |
| Screened selected lines for BNF in low soil N +/- inoculants in HC field trials | | | | x | Y | | x | Y | | | | | | | | | | | | | | | | |
| Greenhouse screening trials initiated on selected lines for BNF response | | | | x | Y | | x | Y | | | | | | | | | | | | | | | | |
| Identify potenial RIL parental lines for BNF response | x | Y | | x | Y | | x | Y | | | | | | | | | | | | | | | | |
| Phenotyping of selected lines for BNF response at US site intiated | | | | x | Y | | x | Y | | | | | | | | | | | | | | | | |
| Plant samples for SNP analysis collected from CAP and SA Core collection | | | | x | Y | | x | Y | | | | | | | | | | | | | | | | |
| Mechanism to correlate field and GH reponses established | x | | N | x | | N | x | | N | | | | x | | N | x | | N | x | | N | x | | N |
| Iniated search for BNF responsive genes in silico | | | | x | Y | | x | Y | | | | | | | | | | | | | | | | |
| Selected RILs advanced to F2 | | | | x | Y | | x | | N | | | | | | | | | | | | | | | |
| **Objective 3** | | | | | | | | | | | | | | | | | | | | | | | | |
| Created awarenes among Extensionists at HC institutions in benefits of BNF | | | | | | | | | | x | Y | | x | Y | | x | | N | | | | x | | N |
| Evaluated farmer Knowledge, Practices, Attitudes on inoculation | | | | | | | | | | x | | N | | | | x | | N | | | | x | | N |
| Prepared for on-farm trails by identifying potential farmer cooperators | | | | | | | | | | x | | N | x | | N | x | | Y | | | | x | Y | |
| Prepared for on-farm trails by training cooperators on field methods | | | | | | | | | | x | | N | x | | N | x | | Y | | | | x | Y | |
| Initiated training materials on BNF and seed inoculation for exans/nists. | x | Y | | | | | | | | x | Y | | | | | | | | x | | N | | | |
| Initiated production of training materials to disseminate through PELUM | x | | N | | | | | | | x | | N | | | | | | | x | | N | | | |
| **Objective 4** | | | | | | | | | | | | | | | | x | Y | | | | | | | |
| Graduate students identified | | | | | | | | | | | | | | | | x | Y | | | | | | | |
| Graduate research programs initiated | | | | | | | | | | | | | | | | | | | | | | | | |
| Undergraduate student interns identified | | | | | | | | | | | | | | | | | | | | | | | | |
| Undergraduate student projects iniated | | | | | | | | | | | | | | | | | | | | | | | | |
| Team members trained in mehods of SNP analysis | | | | | | | x | | N | | | | | | | | | | | | | | | |
| Conducted short-term training of graduate students on BNF protocols | x | Y | | | | | x | | N | | | | | | | | | | | | | | | |

| | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Name of the PI reporting on benchmarks by institution** | Westgate | | | Cichy | | | Miklas | | | Mukoke | | | Ugen | | | Mchimbi | | | Tenywa | | | Musoni | | |
| | | | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | |
| **Name of the U.S. Lead PI submitting this Report to the MO** | | | | | | | | | | | | | | | | | | | | | | | | |
| **Signature** | | | | | | | | | ###### | | | | | | | | | | | | | | | |
| **Date** | | | | | | | | | | | | | | | | | | | | | | | | |