



Dry Grain Pulses  
Collaborative Research Support Program (CRSP)

# 2011

## Technical Highlights

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# Preface

## Dry Grain Pulses Collaborative Research Support Program (CRSP)

**FY 2011 Technical Highlights Report**  
(October 1, 2010 to September 30, 2011)

The Dry Grain Pulses Collaborative Research Support Program (Pulse CRSP) is a five-year research and capacity building program (2007–2012) funded by USAID’s Office of Agriculture Research and Technology, which focuses on pulses (edible grain legumes, including common bean, cowpea, pigeon pea, lima bean, etc.). This program seeks to build upon the scientific advances and technological achievements of the Bean/Cowpea CRSP while responding to the agriculture development priorities and objectives set forth in USAID’s Feed the Future (FTF) Global Food Security Research Strategy and in the Development Strategies by USAID Missions in FTF Focus countries and regions. These strategies focus on addressing the root causes of hunger and forging long-term solutions to these challenges. The strength of the Pulse CRSP is that it mobilizes the cutting-edge research capacities of U.S. universities in such strategic areas as genomics, marker-assisted selection, root biology, symbiotic plant-Rhizobia interactions, systems science, sustainable community livelihoods, clinical and community nutrition, gender, communication science, value-chain research, and market development to achieve its goals.

The global pulse industry is entering a new era characterized by the globalization of markets and fundamental changes in food value chains, presenting challenges and opportunities for smallholder farmers in developing countries and the U.S. to access these markets. At the same time, many developing countries continue to face food and nutritional insecurity and smallholder, grain-legume farmers continue to achieve unacceptably low levels of productivity due to poor soil, insect pests, plant diseases, climate change, etc.

The Pulse CRSP works to address these causes of food insecurity through science-based research projects that address the challenges of these smallholder farmers—through improved breeding programs, insect pest management, improved storage methods and technologies, and increasing soil nutrients and/or improving a plant’s ability to utilize soil nutrients, among others.

Pulse crops, including such edible grain legumes as common bean, cowpea, pigeon pea, chickpea, lima bean, lablab, etc., represent an important group of staple food crops that contribute to addressing household food security, generating income, enhancing soil quality—and thus the sustainability of agricultural systems—and, perhaps most importantly, providing nutrients (e.g., protein, vitamin B, essential micronutrients, and complex

carbohydrates) essential for nutritious and healthy diets for countless rural and urban poor around the world.

In September 2007, USAID awarded a five-year contract (Cooperative Agreement No. EDH-A-00-07-00005-00) to Michigan State University to serve as the Management Entity for the Dry Grain Pulses Collaborative Research Support Program.

The global vision of the Dry Grain Pulses CRSP, as outlined in the Technical Application, is to contribute to:

- Economic growth and food and nutritional security through knowledge and technology generation
- Sustainable growth and competitiveness of pulse value chains, utilizing socially and environmentally compatible approaches
- Empowerment and strengthened capacity of agriculture research institutions in USAID priority countries
- USAID’s development goals, as defined in the Feed the Future Research Strategy for Global Food Security, particularly “enhancing pulse productivity and the nutritional quality of diets”
- Achievement of Title XII legislation objectives, including the provision for dual benefits to developing country and U.S. agriculture

The Pulse CRSP seeks to achieve its technical vision through support for a portfolio of integrated, multidisciplinary, collaborative research, outreach, extension, institutional capacity building, and impact assessment activities on beans, cowpeas, and related pulses in accord with the following strategic Global Themes:

1. To reduce pulse production costs and risks for enhanced profitability and competitiveness
2. To increase the utilization of pulse grain food products and ingredients to expand market opportunities and to improve community health and nutrition
3. To improve the performance and sustainability of pulse value chains, especially for the benefit of women
4. To increase the capacity, effectiveness, and sustainability of agriculture research institutions that serve bean, cowpea, and related pulse sectors and developing country agricultural industries

For the initial five-year authorization of the Dry Grain Pulses CRSP, a two-phase technical program has been implemented with two project award cycles: Phase I (April 1, 2008–September 30, 2010) and Phase II (October 1, 2010–September 29, 2012). To this end, the Management Office (MO) issued a Request for Proposals (RFP) in November 2007. Among the proposals that were received and reviewed by an external advisory panel, eight projects were selected that best met the priority criteria established in the Technical Application of the Dry Grain Pulses CRSP and provided the highest likelihood of achieving developmental outcomes that benefited pulse value chains in developing countries

and the United States. The MO subsequently issued subcontracts to seven “Lead” U.S. universities for the implementation of these Phase I collaborative projects.

In 2009, following an increase in USAID’s authorization to the Pulse CRSP, a second RFP was announced and four new projects were selected following a competitive, peer-review process. These “Phase III” projects address strategic technical gaps in the Pulse CRSP research program, including biological nitrogen fixation, nutrition, and value chain research. These awards were subcontracted mid-fiscal-year 2010.

The seven Phase I and five Phase III projects presented in the FY 2011 Technical Highlights Report involve collaborative research, long- and short-term training, and technology dissemination activities in 13 sub-Saharan African countries (Benin, Burkina Faso, Mali, Niger, Senegal, Kenya, Rwanda, Uganda, Tanzania, Mozambique, South Africa, Zambia, and Angola) and three Latin American countries (Haiti, Honduras, and Ecuador). Of this group, ten are USAID Feed the Future focus countries. More than 25 host country institutions, including National Agriculture Research Institutions, agriculture universities, and NGOs collaborate with the lead U.S. universities in the Phase I and III projects.

This report highlights the technical progress and achievements made by the Phase I and III projects. We hope that readers will appreciate the importance and potential of the research and capacity-building investments that benefit smallholder pulse farmers and help improve the nutrition of the poor in developing countries. Readers should be aware that the *FY 2011 Technical Highlights Report* of the Phase I and III projects is only a one-year snapshot. Moreover, these highlights are condensed versions of more comprehensive technical reports that subcontracted U.S. universities are required to provide annually to the Management Entity and, ultimately, to USAID. These technical progress reports are valued and utilized for assessing Pulse CRSP program performance and reporting by USAID on Title XII and Feed the Future achievements and impacts to the U.S. Congress.

I want to encourage you to read the *2011 Technical Highlights Report* in its entirety. A comprehensive view of the scope of vital outputs generated by each project and the new knowledge, management practices, and technologies resulting from the research activities provide an excellent picture of how the Pulse CRSP uses collaborative science research to advance economic growth and food and nutrition security in developing countries. It is these outputs that will benefit stakeholders of pulse value chains— from producers in Africa and Latin America to the U.S. Some of the outputs include the following:

*Global Theme 1: To reduce pulse production costs and risks*

- Improved varieties of bean and cowpea with increased yield potential and resistance to drought and insect pests
- Improved delivery systems in West Africa to ensure that smallholder farmers receive quality seed of improved cowpea varieties for planting

*Global Theme 2: To increase the utilization of pulses*

- Systematic studies to determine the nutritional value of a bean-based diet for HIV-positive children
- Identification of 38 new compounds in cowpea grain with health benefits against chronic diseases

*Global Theme 3: To improve pulse value chain performance*

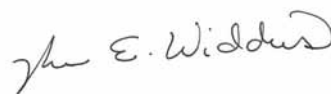
- Production and distribution in Rwanda of 5,000 booklets promoting the bean production chain
- Teaching and validating organic production methods in Honduras to help smallholder farmers supply U.S. specialty markets at fair-trade prices

*Global Theme 4: To increase the capacity, effectiveness, and sustainability of research institutions*

- More than 60 students from host countries enrolled in training associated with the Pulse CRSP
- Approximately 20 international students associated with the Pulse CRSP completed B.S., M.S., and Ph.D. degrees this year

For more detailed information on the Dry Grain Pulses CRSP, including the global program technical vision, project workplans, technical progress reports, project funding, brief bio-sketches of principal investigators, and links to websites with valuable information regarding pulse commodities, visit the program’s web page at [www.pulsecrsp.msu.edu](http://www.pulsecrsp.msu.edu).

As the director of the Dry Grain Pulses CRSP, I want to thank the Office of Agriculture Research and Technology, Bureau of Food Security, USAID–Washington, for its financial support for this worthy program. USAID’s investment in the Pulse CRSP reflects its recognition of the vital importance of pulse crops in contributing to the nutritional and food security of the rural and urban poor as well as to providing opportunities for resource-poor farmers and other value chain stakeholders to generate income and escape poverty. The host country and U.S. scientists and institutions partnering in this endeavor are also to be thanked and commended for their commitment to scientific excellence, to generating new knowledge and technologies that bring the hope of a better tomorrow, and to training a new generation of scientists and professionals who will provide leadership to the agricultural development of many African and Latin American countries.



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# Enhancing Nutritional Value and Marketability of Beans Through Research and Strengthening Key Value Chain Stakeholders in Uganda and Rwanda

ISU-1

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## Abstract of Research Achievements and Impacts

Activities during the past year have produced important achievements with regard to research and development goals. To improve bean quality and yield (Objective 1), adaptive research and trainings with farmers have focused on critical management practices and technologies:

- local community-based production and sale of quality seed
- improved postharvest storage through solarization and triple bagging
- training in group dynamics and gender equity
- strengthening group capacity and sustainability
- exchange visits with other farmer groups

Research continues on bean variety and fertilizer interactions that best increase yield. To effectively consolidate learning and to disseminate management practices to new farmers, extension materials using various media are being refined and translated into local languages.

To enhance nutritional value, appeal, and consumption of beans (Objective 2), we have developed appealing bean-based products and are teaching

farmers to prepare them. Our research has determined the starch digestibility and the sensory acceptability of a bean-based porridge as a weaning food that maximizes protein. Tests were also performed to evaluate the culinary and sensory characteristics of four local and five improved bean varieties. Farmers have been taught how to prepare bean flour, how to use it in making soup, and how to use it to augment or substitute for other ingredients in cooking. To increase knowledge retention and accessibility, extension materials were developed on such topics as the basics of feeding young children, methods of preparing beans to reduce cooking time and increase nutrient availability, and how to prepare and use bean-based flour.

In terms of increasing marketing and consumption of beans and bean products (Objective 3), the focus has primarily been on strengthening farmers' groups on how to analyze opportunities and constraints associated with collective marketing and then how to implement strategic plans. Training sessions increased farmers' understanding of group dynamics and business management, and facilitated initial success in marketing collectively. Promotion of new ways to process and consume beans is ongoing in rural communities, and urban sales and use of bean-based products are advancing via partnerships with private sector businesses and NGOs.

## Project Justification and Objectives

Agriculture in East Africa is characterized by women and men farming in small-scale, rain-fed fields with poor soil fertility, averaging two hectares per household. Erratic bimodal rainfall patterns in recent years have further challenged crop results. Farmers have limited access to training for improved agronomic

practices, quality seed, technologies to improve yields and reduce postharvest losses, and credit. Losses are very high throughout the bean value chain due to poor harvest and postharvest practices and poor on-farm storage facilities. Beans on the market are typically poor quality and infested. Producers are not well linked to profitable markets, especially emerging sectors of domestic and regional markets. Traders operate on a small scale with limited investment capability. The availability and use of processed products remains very modest. Hunger and poverty are widespread.

The lack of value-added bean products with reduced preparation times makes bean preparation laborious with high fuel requirements; consumers tire of monotonous flavor, reducing their bean consumption despite documented high nutrient content and health benefits. Optimized processing (hulling, soaking, milling, fermentation and germination, and cooking) can enhance digestibility and nutritional value by reducing phytates and polyphenols that limit iron uptake and create value-added, bean-based food products.

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*Agriculture in East Africa is characterized by women and men farming in small-scale, rain-fed fields with poor soil fertility, averaging two hectares per household.*

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Prospects of marketing increased quantities of beans and new agroprocessed bean products within the Ugandan and regional markets require carefully examining production

and marketing constraints (increased farm productivity, producer incentives, and access to better markets). Equally important is understanding prospects for increasing demand for beans and agro-processed products through collaboration with private sector businesses.

Our recent efforts to introduce new agronomic practices and technologies demonstrate encouraging progress. Ongoing collaboration since 2004 between Iowa State University (ISU), Makerere University (MAK), and Volunteer Efforts for Development Concerns (VEDCO) in Uganda's Kamuli District using a sustainable livelihoods approach increased food security and market readiness from nine to 77 percent among 800-plus farm households. The main crops are maize, beans, sweet potatoes, cassava, bananas, rice, and coffee. Most (90 percent) of participating households produce beans, but few (20 percent) sell some. The SL (Sustainable Livelihoods) approach focuses on understanding and supporting individual and community capabilities, assets (natural, physical, human, financial, social, cultural, and political capital), goals, strategies, and activities. In combination with SL approaches, scientific knowledge, improved technologies, financial assistance, and changes in government policies can have significant positive local impacts. Participatory research methods can generate knowledge that people can apply to improve their individual and collective well-being.

Beans provide a strategic opportunity to help meet Millennium Development Goal targets of reducing hunger and poverty. Improved beans production in Uganda and Rwanda offers unique opportunities to address the deteriorating food security situation there and elsewhere in sub-Saharan Africa. The short

growth period and two growing seasons offer great opportunities to contribute to rural poverty alleviation—playing an essential role in sustainable livelihoods of small-scale farmers and their families, providing food security and income to the most vulnerable group, the women and children.



## Objectives

1. Improve harvested bean yields and quality.
2. Enhance nutritional value and appeal of beans through appropriate handling and processing.
3. Identify solutions for constraints to increased marketing and consumption.

## Research and Outreach Approaches, Results, and Achievements

### Objective 1: Improve harvested bean yields and quality.

*Improve yields and quality through evaluation of better production practices.*

For seasons 2010B and 2011A, 12 trials were set up, two with each of the six farmers groups. For each season, trials were set up with different farmers in each group. Three varieties: K131, NABE 4, and Kanye bwa (farmers variety) were compared under four fertility treatments:

1. Control: no fertilizer added
2. FYM alone: (9 kg per plot, 10 T/ha)
3. Phosphorous alone: (36 g/plot, 40 kg/ha)
4. FYM (4.5 kg/plot, 0.5 T/ha) and Phosphorous (18 g/plot, 20 kg/ha)

The experiments were set in a randomized complete block design with two replicates. Each on-farm trial had 24 plots, each three m x three m. For season 2011A, an additional 30 seed health trial plots, each three x three m, were set up with each of the six farmer groups. Comparative analyses from seed health experiments are ongoing. Similar trials were established for the second season (2011B) but with FYM and Phosphorous amendments as 7.5 kg and 15 kg per plot, and 54 g and 108 g per plot, respectively, because laboratory soil analysis indicated lower levels of both P and N for the six trial sites.

At physiological maturity, beans were harvested from each plot and the following data were collected:

- number of plants per plot
- number of pods per plant determined from 20 randomly chosen plants per plot
- number of seeds per pod from 20 randomly chosen pods per plot
- total yield per plot
- clean yield per plot (shriveled, discolored, cracked seed removed)
- 100 seed weight
- moisture content for seeds from the various plots

The seed weight/yield was standardized to 13 percent moisture content and yield adjusted to kg/ha. Data from each of the seasons were analyzed separately.

Nitrogen and phosphorous are major nutrients required for good plant growth and development to achieve higher yields. Deficiency of both nutrients leads to stunting of bean plants, poor flowering, and flower abortion. Nitrogen and phosphorous were generally limited in the soils used for the trials and their application was aimed at increasing their availability in the soil for plant utilization. The trials demonstrated to farmers the need for soil fertility improvement to achieve better yields.

For Season 2010B, analysis of variance showed that there were significant effects of variety and location on total yields of the three bean varieties tested. Variety by location and variety by fertility treatment interactions were also significant.

Across locations (farmer fields), K131 showed more stability in yield, with average yields 365–668 kg/ha, and with 50 percent of total yields above the variety average of 499 kg/ha. This was followed by NABE4, with total average yields 201–562 kg/ha and 62.5 percent of the locations having average total yields above the variety average of 380 kg/ha. Variation in yields was greatest for Kanye bwa, with average total yields ranging from 36–570 kg/ha, and only 37.5 percent of the locations having average total yields above the variety average of 312 kg/ha.

Comparison of fertility treatments within varieties shows that application of fertilizers tended to increase the yields compared to the control. Within varieties, comparison of the highest fertility treatment yield to the control shows that NABE4 had the highest increase in total yield (43 percent), followed by Kanye bwa (38 percent) and K131 (13 percent), respectively.



For Season 2011A, analysis revealed significant location effects on plants per plot, pods per plant, seeds per pod, total yield, and clean yield. There were no significant variety effects on the total and clean yield. However, there were significant variety effects on the number of plants per plot, pods per plant, and seeds per pod. The main effects of fertility treatment on yields were, however, not significant. Interaction between variety and location was significant for pods per plant, seeds per pod, and total yield. Further, the variety by fertility treatment interaction was significant for total and clean yield.

Correlation analysis showed that the number of pods per plant and number of plants harvested per plot had significant positive effects on total yield.



Overall, K131 yields were higher than the other varieties. Application of farmyard manure together with phosphorous led to much higher yields for Kanyebwa, while application of farmyard manure alone led to much higher yields for K131 compared to the other treatments. The modest increases in yields with manure indicate that the nutrients still were not sufficient to meet the full requirements of the plant during establishment and seed filling.

Overall, analyses of data from all the on-farm trials show that:

- Improved varieties used in the study had higher averages and more stable yields across locations.
- K131 yields were consistently higher than other varieties; this could be attributed to its better resistance to diseases and drought.
- Improvement of soil fertility by application of manure and phosphorous has consistently led to moderately higher yields.

With agronomic controls, 180 plots were established for the seed health experiment among the six farmer groups. Data collection involved counting and classifying the number of pests observed on every plant, and data were entered using the recommended CIAT scale. The most common pests were bean beetles, aphids, thrips, whiteflies, and bean pod borers. Two experiments were set up. One assessed the effect of different seed sources on yield,

with treatments being farmers seed from the market and better storage by VEDCO and farmers. The other treatment involved NABE 4 from better storage by VEDCO and NACRRI.

The second experiment focused on the pathology and entomology of the most common bean variety in Kamuli (farmers seed). The treatments included five plots having seed with a seed dresser at planting; five plots sprayed with a pesticide; five plots with a fungicide; five plots with a combination of the pesticide, fungicide, and the seed dresser; plus five plots serving as the control. Both experiments had three replications, each with five plots. Analysis of data is pending.

Of the three bean varieties planted, K131 proved to be more tolerant to drought stress compared to farmers seed and NABE 4. In terms of yield per unit area, K131 performed best; NABE 4 had the lowest yields. Farmer seed was more susceptible to pests and diseases.

Of the six CRSP project farmer groups, five groups provided 10 kg each of beans that were used in the anaerobic storage technique. The materials used in this method were clean 10 liter jerricans with no holes and tightly fitting seals. There was significant improvement in the storage of beans, with minimal pest multiplication and bean damage.

*Support community-based seed production (CBSP) by farmers' groups/associations.*

CRSP farmer groups were able to plant 16 acres of beans in the 2011A season, well beyond the six acres that they cultivated the previous season. Despite the April hailstorm that devastated some fields, most groups were able to achieve a good harvest. CRSP farmers were trained in group dynamics and business management by NaCRRRI technicians and VEDCO staff to strengthen group cohesion for producing quality seed. Training covered major causes of group breakdowns as well as gender roles and gender equity. These trainings enhance mutual respect and rational assignment of duties within the group and maintain coherence in group activities. Refresher trainings on recommended management practices was also offered:

- use of approved seed at planting
- timely weeding and pest control
- drying of the beans on tarpaulins
- proper storage to avoid postharvest bruchid damage

All participating CRSP farmers were taken for a field exposure visit at the National Crops Resources Research Institute (NaCRRRI) at Namulonge to strengthen farmers' capacity in community-based seed production. Farmers were taken to on-station bean research plots to understand clearly the recommended site selection and field layout as well as criteria for selection of quality seed for planting.

All CRSP farmers were also taken to meet with members of the Gombe Seed Producers Farmers Association in Wakiso District (south of Kampala). During the visit, the farmers toured the bean fields of the hosts and shared their knowledge, skills, and success stories for growing beans. Gombe seed producers use a

hand-driven seed dressing machine and pack their own seed for sale using low-cost technology and a system of seeds packed in small affordable quantities (0.5 kg, 1 kg, 2 kg) for ease of sale to institutions and other farmers.

*Evaluate and promote adoption of improved postharvest handling and storage methods.*

At Makerere University, researchers evaluated the impact of solarization and triple bagging on beans in bulk storage. Of particular interest was reducing postharvest losses of beans due to bruchid infestation. Seed viability and the culinary properties of the beans are also of interest. For each 100 kg of beans, the entire lot was further divided by passing the grain through the Boerner divider multiple times, from which representative samples were then drawn

Characteristic	Units	Method
Moisture Content	%	Electronic handheld moisture meter
Foreign Material	Weight %	Hand screening and weighing
Damage, Insect Damage, Splits Beans	Weight %	Visual inspection and weighing
Number of Live Insects	# per 250 g	Visual inspection and weighing
Number of Dead Insects	# per 250 g	Visual inspection and weighing

Table 1. Methods of Determining Initial Characteristics of Beans and their Viability

The bean samples were divided into three lots: a control, triple bagged, and solarized and triple bagged. Solarization was done by placing the beans on a tarpaulin on the grass. The beans were then covered with a transparent plastic film and left in the sun. Triple bagging used woven polyethylene sacks free of holes and HDPE sacks 80 microns thick. Two HDPE sacks were placed inside the woven sack, filled with beans, and each tied with sisal string after eliminating all excess air. All three sample types were stored for up to six months in a vermin-proof store on pallets away from the wall. Characteristics of the beans are being assessed monthly and the results recorded.

Beans and maize purchased from farmers shortly after harvesting were dried to 12 percent moisture, solarized, and triple bagged. Control samples were stored in single polypropylene bags. Samples were taken from the controls every month. Preliminary results indicate that the combination of solarization and triple bagging is effective for killing weevils in both beans and maize stored in bulk. The control samples had increasing numbers of live weevils during a six-month storage period. The number of live insects in beans that were triple bagged remained low throughout the six-month storage period.

The moisture content of the triple bagged grains was maintained at about 12 percent. The beans were not sorted but stored as obtained. The maize had a higher initial viability (>90 percent). Viability of both beans and maize remained high following solarization and during storage relative to the original quality of the grain. Changes of about 10 percent were noted. The viability of poor quality, triple bagged beans is reduced by up to eight percent over six months. The reduction is less in good quality beans, consistent with earlier experiments. Triple bagging was also effective for protecting maize from insect damage and loss of viability (see figure 1). Experiments are ongoing.

Technicians from NACCRI with support from VEDCO staff trained all six groups regarding the roles of materials and procedures used during triple bagging and solarization.

Demonstrations used traditional interactive discussion, demonstrations, and animated videos.

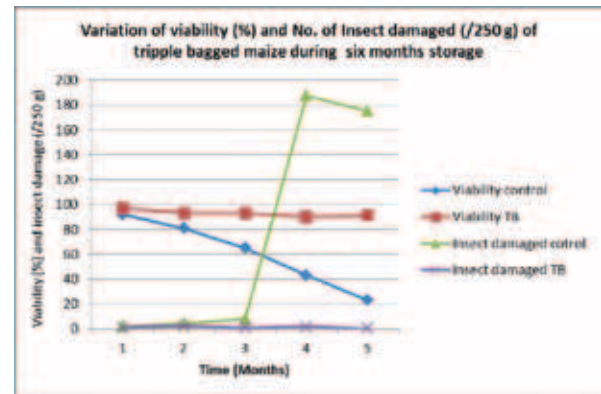


Figure 1. Seed Viability and Insect Damage of Triple Bagged Maize

At the beginning of the 2011A harvesting period, when CRSP farmers had beans with which to demonstrate triple bagging and solarization, all received bags and three meters of black and clear polythene materials. Triple-bagged containers of beans were opened after six months and assessed for bruchid survival and damage. Poststorage tests documented 96 percent germination. Beans stored under these conditions were planted in farmers multiplication gardens during the 2011B season.

A potential hindrance to sustainable use of these materials may be cost. Each bag costs approximately \$1.20 U.S. The real cost declines when bags remain in good condition and are reused; farmers are receiving follow-up training on how to handle the bags to avoid puncturing or tearing them. The process is initially labor intensive but ultimately labor saving, in contrast to biweekly resunning the entire bean harvest when using traditional storage methods. Future training will focus on ensuring that proper solarization conditions are achieved and maintained.

*Strengthen farmers' collective capabilities to learn and share innovative practices*

Project farmers' capabilities in seed production have been strengthened. CRSP farmers managed to cultivate 16 acres of beans in season 2011A compared to six acres in 2010B.

Existing extension training materials for bean production and postharvest management are continually being refined and translated into Luganda. Topics include proper site selection, plant and row spacing, weeding, pest management, harvesting, drying, threshing, moisture testing, sorting and seed selection, solarization, and storage (jerricans and triple bagging). PowerPoint slides, posters, and video clips on various agronomic and postharvest handling techniques have been developed to ensure quick and efficient knowledge transfer to farmers.

**Objective 2: Enhance nutritional value and appeal of beans through appropriate handling and processing.**

*Address nutritional and health problems among vulnerable individuals through increased consumption of beans, bean products, and complementary foods.*

Cold-extruded bean flour-based snack. The formulation of

composite flour of bean and maize was carried out after appropriate pretreatments. They were blended in different combinations and the cold-extruded snack was processed and subjected to organoleptic evaluation. The following combinations (see table 2) were found acceptable. Storage stability studies were conducted at 20–25°C for four months to monitor the snack foods. They showed no changes in color, flavor, or odor and were acceptable for consumption.

Sample	Bean Variety	Composition of Flour
1	Colta	100% Bean flour
2	Decelaya	70% Bean and 30% Maize
3	RWR 22-45	100% Bean Flour
4	White Beans	60% Bean and 40% Maize

Table 2. Organoleptically Accepted Combinations of Bean Composite Flour

A metallic hand-operated extruder was used to cold press the dough before deep frying in hot cooking oil. The dough made out of the blended flour was placed in the dough holder. The dough presser was placed on the dough and squeezed by pressing the handles of both the dough holder and the dough presser, resulting in the extrusion of the snack. The extrusion was made directly into the hot cooking oil and deep fried.

Acceptability of these snack foods varied. For RWR22-45, the accepted blend was 100 percent and for White variety it was 60 percent:40 percent beans to maize. Although the blend of RWR22-45 was not acceptable, it may be used as a single flour in processing snacks. This indicated that snacks from cereal-bean flour blends and bean flour alone can be processed.

In Uganda, a bean-based weaning food containing preprocessed (germinated and steamed) bean flour (40 percent), roasted grain amaranth (30 percent), and rice flour (30 percent) was developed. It was formulated to have protein and energy levels that contribute significantly to the Recommended Daily Allowances for children aged two to five years. A processing protocol aimed at reducing antinutrients and cooking time while improving protein and starch digestibility and the sensory acceptability for bean flour was developed.

The bean-based composite porridge gave an acceptable viscosity of 2500–3000 cP at a high solid content of 15 percent compared to eight percent for millet flour and seven percent for maize flour. The composite porridge could supply 97 percent of the Estimated Energy Requirements (EER) for females ages two to three years compared to 50 percent from millet porridge and 42 percent from maize porridge, with three servings daily. It could supply 84 percent of EER for males aged two to three compared to 43 percent from millet porridge and 37 percent from maize porridge, with three servings a day. For female children ages four to five, the composite porridge could supply 53 percent of the EER compared to 27 percent from millet porridge and 23 percent from maize porridge with three servings a day. For male children ages four to five, the composite porridge could supply 50 percent of the EER compared to 25 percent from millet porridge and 22 percent from maize porridge with three servings a day. Further, the composite could meet 115 percent of the Recommended Daily Allowance (RDA)

for protein of children ages two to three compared to 50 percent by millet and 38 percent by maize porridges with 1.5 servings per day. For children aged four to five years, the composite could meet 78 percent of the RDA for protein compared to 34 percent for millet and 26 percent for maize porridge.

In Rwanda, research was conducted to develop nutrient-dense, bean-based composite flour from bean and six other locally grown vegetables by analyzing its nutritive value and functional properties, processing a sample soup from the developed flour, and assessing consumer acceptability of the soup through sensory evaluation. The study is part of the effort to provide affordable adequate nutrition for low income families. Fresh beans were subjected to a combined treatment of soaking, germination, hulling, parboiling, drying, and milling into flour. For vegetables, the unit operations were washing, size reduction, blanching, drying, and milling. Flours obtained were mixed to different proportions. The mix was termed CRSP/KIST PANAMIX. Four samples of the composite flour were formulated using bean, moringa leaves, potato, tomato, carrots, leeks and garlic flours in the following ratios:

1. 70:10:8:4:4:2:2
2. 60:20:8:4:4:2:2
3. 50:30:8:4:4:2:2
4. 40:40:8:4:4:2:2



Four samples of soups were developed from these composite flours, and 15 trained panelists rated the prepared soups. Preliminary sensory evaluation showed that soups processed from samples 1 and 2 were the most acceptable. These two samples underwent laboratory analysis of selected functional properties and nutrients: bulk density, pH, oil absorption capacity, water absorption capacity, and wettability. Generally, both composite flours were nutrient dense, but the latter was found to be high in  $\beta$ -carotene, calcium, crude fat, crude protein, and total energy when compared to PANAMIX No.1.

Sample	Nutrients	Composition of Flour
1	Carbohydrates (g)	51.4
2	Crude fats (g)	7.1
3	Crude protein (g)	11.98
4	Energy (Kcal)	303.27
5	Fiber (g)	13.9
6	Moisture (g)	5.34
7	$\beta$ – carotene (mg)	79.12
8	Calcium (g)	0.87

Table 3. Nutritional Value of PANAMIX in 100g

Three topics were identified for which extension materials have been developed:

- Basics of feeding children aged six to 59 months
- Methods of preparing beans that reduce cooking time and enhance nutrient bioavailability

- Preparation of bean-based composite flour and utilizing the flour in porridge

The content is available in two forms: a summary training outline and a detailed training manual that will be translated into local languages, with illustrations added.

In Rwanda, two KIST lecturers and five students traveled to Rukomo sector, Nyagatare district, Eastern Rwanda, to demonstrate and conduct trainings. Villagers were encouraged to substitute 15 to 20 percent of the mix for wheat in cake, biscuit, and bread making. They also explained and demonstrated preparation of the bean-based soup and the cold extrusion method. In addition to 60 farmers who registered, several village leaders also participated. Moreover, many other farmers joined the event after being invited by the excited farmers who were registered.

Participants indicated that it could nourish vulnerable segments of the population (children, mothers, the sick, and the elderly). Training 60 health counselors in the district was proposed by the executive secretary of Rukomo sector. Demonstrating the techniques to farmers and village authorities in Mimuri sector is also on the agenda. Farmers in Kamuli District were also trained in preparation of the cold-extruded bean snack as well as methods (soaking and sprouting) to enhance the nutritional quality of bean dishes fed to children younger than five years.

Rapid appraisal of the basic knowledge of feeding infants and young children and the extent and use of beans were assessed; training materials for utilization of beans to improve the quality of meals served to infants and young children in Kamuli were piloted. In Rwanda, arrangements have been made with two NGOs (Africare and World Vision) to assist in this activity.

*Analyze culinary properties, sensory characteristics, and consumer acceptability of improved varieties of beans.*

A protocol for rapid screening of culinary properties of pulses was obtained to determine the following characteristics: water absorption capacity during soaking (g/kg), cooking water absorption (g/kg), seed coat splitting (%), cotyledon splitting (%), soluble solids content (° Brix), pigment leaching, and sensory characteristics. In Uganda, this protocol is being used at Makerere University to evaluate culinary and sensory characteristics of local and improved bean varieties.

The culinary properties of eight local and six improved bean varieties are being analyzed. Their moisture content was determined followed by drying the beans to approximately 12 percent moisture and storing them in the layers of ziplock bags. Samples are drawn and subjected to cooking experiments to determine the time required to cook. After cooking times are confirmed, the different bean varieties will be subjected to sensory acceptability testing using untrained consumer panels.

*Incorporate insights from the analysis of the private food processing industry regarding the development and commercialization of bean-based products.*

Four types of incubators were identified: local economic development incubators, academic and scientific incubators, corporate incubators, and private investor incubators. Engaged



in research and development, the Makerere University Food Technology and Business Incubation Centre (Mak-FTBIC) found the academic and scientific incubators most suitable for their activities.

Mak-FTBIC targets projects generated from internal research activities (technology development projects) and external projects, including satellite incubation activities in which private sector businesses are provided technical support in their premises outside of the Mak-FTBIC. Undergraduate students are inspired to undertake research projects with prospects for incubation and commercialization, while graduates are gainfully employed and have the opportunity to be entrepreneurs.

Bean processing into value-added products has so far been very limited in Uganda and the East African Community. This project is contributing to its establishment. The most common processing of beans is canning and is an important industry. Asian countries, especially India, sell precooked bean curries and sauces in sachets. Such products have potential for the urban market in Uganda.

Experience with Nutreal Limited to upscale production of the bean-based composite flour developed from this project indicates that the following are important:

- The technology developed should be compatible with the private company's mission and objectives.
- R&D-based technologies are valued because they increase the chances of products' market success; being associated with an R&D institution is also viewed very positively.
- Continued technical support and involvement of the R&D personnel from the institution is key; private sector businesses want to be assured of it.

- The availability of a University-based R&D and business incubation facility encourages private sector involvement.

The collaborative relationship between MAK, NaCRRI, and VEDCO provides an ideal framework for linking farmers' groups to industry. Both NaCRRI and VEDCO work with farmers and farmers' groups and are in position to link backwards to production and forward to R&D and processing. The partners already provide technical support to farmers' groups to enhance production and productivity and to improve postharvest handling and storage. Makerere University's FTBIC has provided opportunities for linking farmers' associations to private industries as suppliers of raw materials.

Nutreal Limited, a private company working in MAK-FTBIC, is currently collaborating with our research group to increase the bean-based product range and utilization of preprocessed bean flour. Composite flours, including preprocessed beans, are being developed for food use. Trials to incorporate preprocessed bean flour into baked products are also underway.



### **Objective 3: Identify solutions for constraints to increased marketing and consumption.**

*Assess capabilities and needs of farmer groups and associations.*

Farmers in Kamuli own and cultivate an average of two to two-and-a-half acres, and one-half borrow or rent land (averaging one acre) for their agricultural activities. Most were growing maize and beans, but only 15 percent were harvesting at least 50 kg of beans. Most households sell some agricultural produce almost exclusively, on an individual basis, to traders. One-half of all households engage in nonagricultural income earning activities. More than one-half borrowed money during the previous year.

Participating in the CRSP project has enhanced farmers' assets and capabilities—their social capital through strengthening connections with local groups and through exchange visits, their human capital through gaining technical knowledge and experience, and their political capital through leadership roles and awareness of their interests, rights, and capabilities with local government. A significant impact on cultural capital is that five of the six groups are currently headed by women. Their natural capital has been enhanced through increasing the amount of land cultivated in response to new opportunities to grow and sell beans. Their physical capital has been enhanced through acquiring improved bean varieties; some groups have acquired oxen and an ox plough.

*Strengthen farmers' successful engagement in value chain development.*

Farmer groups were trained and supported in the collective marketing of beans, which involved improving farmers' understanding of market price variation (among traders, markets, and seasons); enhancing their ability to manage harvested grain to obtain increased prices through loss-minimizing storage, negotiation skills, and coordination of collective marketing; and business planning, record keeping, and analysis. Results include:

- Project farmers responded successfully to an *Invitation for Bids* for purchase of beans.
- The project team created a two-page negotiated contract for all six groups.
- More than 1000 kg of two varieties were purchased from project farmers for scaling up plans to purchase two MT from CRSP project farmers when the current season is completed.

The CRSP project, in coordination with the CSRL program in Kamuli, has provided initial training and two varieties of improved bean seeds to 348 farmers to each planted 1/3rd acre of beans in 2011A, and an additional 200 farmers to each planted 1/6th acre of beans in 2011B.

Assessment of farmer groups' interests, capabilities, and needs has led to continued mentoring and support for the bean value chain stakeholders forum established in late 2010. This included sharing insights from experiences of collective marketing by farmers associations in Masaka, Rakai, and Kapchorwa.

Partner meetings were organized in two subcounties (Butansi and Bugulumbya) in the Kamuli district and facilitated by team members from Makerere University and VEDCO to analyze anticipated opportunities and constraints for participatory marketing from the farmers' viewpoint. They also discussed strategies to achieve successful participatory marketing, prioritizing activities, and drafting the activity road map. Farmers identified the establishment of storage centers as a key factor in obtaining better prices for their beans when bulking and selling collectively. They also identified limited access to microfinance and agro-inputs (pesticides and herbicides) as key constraints to boosting their production.



## Leveraged Funds

Drs. Mazur and Westgate have successfully leveraged more than \$90,000 in external funding for Ph.D. student support in Agronomy and Food Science and Human Nutrition, in part due to the assistance received from the Dry Grain Pulses CRSP.

## Publications

Musaazi, Aisha Nakitto. 2011. *Developing a quick-cooking bean flour*. Final thesis for M.S. degree. Department of Food Science and Technology. Kampala, Uganda: Makerere University.

Vasanthakalam, H. 2011. *Adding Value to Grains—Science, Nutrition and Technology*. (includes chapter “The Cold Extrusion Process” [pp. 109–111]). Germany: Lambert Academic Publishing, 188 pages.

## Networking and Linkages with Stakeholders

CRRI NaCRRRI has been multiplying more than 200 nutribean lines high in iron, zinc, and protein recently received from the University of Nairobi through CIAT. Through the Material Transfer Agreement signed between CIAT–Colombia and Iowa State University, ISU has been receiving germplasm from breeders that reflects variation in drought and seed nutritive composition. Recombinant Inbred Lines and their parents will be very useful in understanding the physiology of seed nutrient composition, the work of Ph.D. student Gerald Sebuwufu. NaCRRRI researchers Michael Otim (entomologist) and Pamella Paparu (pathologist) have been conducting research to quantify the incidence of insect pests (bean aphids, thrips, bean stem maggot, and flower beetles) and diseases (bean root rot, web blight, and bean rust). Findings will guide advanced training of farmers in pest and disease control to reduce crop losses.

VEDCO holds biannual community review meetings in its areas of operation; CRSP project partners and farmers participate in these review and planning meetings. VEDCO organized the first value chain stakeholder workshop in Kamuli in late 2010 that involved 25 participants from 15 organizations (farmer marketing groups and associations, government agencies, nongovernmental organizations, private sector traders, transporters, distributors, and processors). Ongoing research on value chain development for beans and maize corresponds to VEDCO’s commitment to continue playing a facilitative role.

Visits to ISU by the Co-PI from Makerere University resulted in further learning about parallel and complementary research interests and bases for long-term collaboration. ISU faculty members visited Uganda, bringing expertise in agricultural and biosystems engineering, agronomy, development communications, human nutrition, and sociology.



# Combining Conventional, Molecular and Farmer Participatory Breeding Approaches to Improve Andean Beans for Resistance to Biotic and Abiotic Stresses in Ecuador and Rwanda

MSU-1

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## Abstract of Research Achievements and Impacts

The bean breeding program at Michigan State University (MSU) released a new vine cranberry bean variety, *Bellagio*, that has improved plant structure, uniform maturity, resistance to anthracnose and bean common mosaic virus, and excellent seed quality for canning. In statewide trials in 2011, it outperformed the commercial variety Chianti; this new variety should help recover cranberry bean acreage in Michigan. The breeding program continues to evaluate black, navy, red, pink, pinto, great northern, and kidney lines for yield and resistance to common bacterial blight, rust, white mold, virus and anthracnose, and drought tolerance. In New York, root rot screening of new germplasm from MSU and Puerto Rico was conducted in the field and selections were made and returned to the research programs for use in breeding; greenhouse screening of lines from Ecuador against *Rhizoctonia* was also conducted. In Ecuador two new bean varieties were released to farmers in the northern valleys: *INIAP 483 Intag* is a large-seeded red mottled type and *INIAP 482 AfroAndino* is a small black-seeded variety released for the canning industry. The new red mottled variety *Intag* is the first to possess resistance to three important diseases (rust, anthracnose, and angular leaf spot) and is making impact into a broad area of the Intag Valley supported by the substantial outreach component of the program and the need for new bean varieties in the region. The varieties were released through evaluation and participatory selection with members of the CIALs in the provinces of Carchi, Imbabura, and Intag. Ten tons of basic seed of five varieties was produced for distribution to growers in the region, and the program continues to refine its nonconventional seed production in the Mira and Chota Valleys by working with specialized seed growers. In Rwanda, the breeding expanded the crossing program and successfully produced 10 tons of breeder and prebasis seed of bush and climbing beans that was distributed to NGO partners and seed companies for additional seed multiplication and distribution in small quantities to small farmers. Four climbing bean varieties for high altitude zones are under consideration for release in Rwanda in 2012. The lines are white, red, and red-mottled seeded types and have high yield potential (greater than 3 t/ha) and the red line has more than 90 ppm seed Fe. Two doctoral students conducted field research in Rwanda, one screening genetic populations for drought tolerance and the other evaluating participatory cropping systems in grower fields, comparing the interplanting of climbing bean varieties with and without maize. Bulletins, promotional materials, and booklets were produced in both countries to disseminate information on new bean varieties and bean production systems.

### Project Justification and Objectives

Common bean is the most important grain legume (pulse) consumed in Ecuador and the most important protein source in Rwandan diets. Around 120,000 hectares of beans are cultivated annually in Ecuador, and common bean is the most widely grown pulse in Rwanda. Both bush and climbing beans constitute an important economic income for farmers and a staple food for thousands of Ecuadorian families and the majority of small-scale farmers in Rwanda. Improvement of bean genotypes for



Ecuador's environment has a potentially significant spinoff for adaptation to Rwanda's upland farming systems. Smallholder farmers, many of them widows supporting families, are keenly interested in rebuilding their bean genetic stocks and expanding into new market opportunities. Building on international bean germplasm, but particularly on the Ecuador experience and germplasm, a valuable opportunity exists to develop and deploy improved bean varieties in Rwanda. An improved understanding of plant traits and genotypes with resistance to multiple stresses from abiotic (e.g. drought) and biotic (root rot and foliar pathogens) sources will provide unique materials for small-scale farmers in addition to insight into plant tolerance mechanisms for enhanced plant breeding methods. Results of this project should contribute to improved yield, farm profitability, and human resources in Rwanda and Ecuador and indirectly benefit participating U.S. institutions and bean producers.

### Objectives

1. Develop through traditional breeding and marker-assisted selection (mas) a range of large-seeded Andean Bean germplasm with differing combinations of resistance to major foliar diseases in contrasting bean growth habits for distribution and testing in the highlands of Ecuador, Rwanda, and the Midwest United States.
2. Develop inbred backcross lines in a range of commercial seed types for testing under drought and root rot pressure in Ecuador, Rwanda, and the United States.
3. Collect and characterize pathogenic and genetic variability of isolates of root and foliar pathogens in Ecuador and Rwanda.
4. Employ participatory plant breeding and agroecological methods to assist the breeding process in Ecuador and Rwanda to enhance the productivity and market quality of beans under development.

### Research and Outreach Approaches, Results, and Achievements

**Objective 1: Develop through traditional breeding and marker-assisted selection (mas) a range of large-seeded Andean Bean germplasm with differing combinations of**



**resistance to major foliar diseases in contrasting bean growth habits for distribution and testing in the highlands of Ecuador, Rwanda, and the Midwestern United States.**

1. Continue to select parental breeding materials for crossing in Ecuador, Rwanda, and United States
2. Expand group of lines from Rwandan breeding for crossing with newly introduced differential lines from Ecuador, MSU, UPR, and CIAT/PABRA-interchange.
3. Cross Rwandan sources of resistance for bean common mosaic virus (BCMV), angular leaf spot (ALS), rust, anthracnose, *Fusarium* wilt, and *Pythium* and major foliar pathogens into large-seeded lines with contrasting colors.
4. Confirm resistance of selected parental lines to target root pathogen(s), including *Macrophomina*.
5. Utilize markers in early-generation selection for major disease resistant traits in Ecuador and conduct inheritance studies in the greenhouse for anthracnose in Yunguilla and rust resistance in JE.MA.
6. Initiate marker-assisted selection at one central lab (Rubona) in Rwanda.
7. Initiate selection for diseases resistance under screenhouse inoculation conditions at Rubona.
8. Yield evaluation of advanced lines in range of seed types in Ecuador, Rwanda, and the United States and continue to exchange promising materials among the breeding programs.
9. Initiate characterization of biofortified lines for Fe and Zn for use as parents in Ecuador and Rwanda.
10. Evaluate lines and varieties for canning industry in both the field and lab in Ecuador.
11. Continue seed increase of most promising lines.
12. Expand on-farm trials with advanced lines.
13. Release elite climbing and bush bean varieties in different commercial types across agroecological zones in Rwanda and a bush bean variety with broad disease resistance for production in Ecuador



**Results**

- Foundation seed increases of the new vine cranberry bean variety Bellagio, released by the MSU breeding program,

were produced in the western United States in 2011. The plant type is less decumbent than the current vine varieties and produces a large (55g) round seed. The seed type would have commercial appeal in both Ecuador and Rwanda. A group of anthracnose resistant cranberry breeding lines from MSU was sent to Rwanda and Angola for testing in 2011.

- A total of 5,600 plots were harvested for yield and more than 2,600 single plant selections were made in the early generation nurseries as part of the MSU breeding program. Three new lines in three different market classes (pink, pinto, white kidney) are under consideration for release in 2012, based on continued high performance of upright full-season pinto line P07863 with white mold avoidance, early-season white kidney K08961, and an upright pink line S08418 with good seed color. Sources of common blight resistance were identified in advanced kidney and cranberry bean lines.
- Research continues to develop a stable transformation system for common bean. The bean breeding program in the Rwanda Agriculture Board (RAB, formerly ISAR) increased the number of crosses in 2011. More than 200 single and backcross crosses were made during this reporting period to incorporate multiple resistances to anthracnose, angular leaf spot, bean common mosaic virus, and/or micronutrients Fe and Zn.
- In other cross combinations, ten crosses with novel and unadapted Fe sources that were acquired through CIAT were developed. One hundred fifty F1/BC1 simple, double, and three-way crosses were initiated. An evaluation of new high iron bean populations was introduced from CIAT headquarters last year and further screening of rich micronutrients (Fe and Zn) advanced lines were undertaken at Rubona, Rwerere, and Karama stations.
- Different lines have been evaluated as high iron content at different sites both on RAB research stations and in farmer fields in two groups. The first included preliminary yield trials (PYT) with an evaluation of 104 biofortified bush bean lines at Rubona station and an evaluation of 182 biofortified climbing beans (under observation nursery) in Rubona and Rwerere station. The second included an evaluation of 13 advanced high iron content bush bean types; 28 high Fe content and drought-resistant varieties were evaluated in a PYT at Rubona station. Among these lines, 14 lines were selected in the advanced yield trial (Phase I).
- The phenotypic evaluation of 125 RIL population from the cross of SEA5 x CAL96 was conducted by Gerardine Mukeshimana in Rwanda in dryland research stations at Karama and Nyagatare. Both irrigated versus nonirrigated treatments were applied at both locations. Geometric yields ranged from 428 to 2342 kg/ha and phenological and harvest index data were collected for use in QTL analysis.
- The same RIL population is being genotyped at MSU with the goal of mapping QTL associated with drought resistance in beans. The genotyping of the entire mapping population is being conducted.
- Thirty-five bush bean lines for canning were introduced from CIAT Kawanda (Uganda) and evaluated at Rubona for adaptability and further seed increase. The characteristics of

the four new varieties planned for released in Rwanda in 2012 are shown in table 1. They are well adapted to the highlands of Rwanda, their yield potential is high (>3.4 tons per ha), and they meet farmer preferences in terms of seed size and color. Results from two different labs showed that some of the advanced lines are high iron content lines. The best line based on the iron contain was RWV3316.

Varieties	Days to Flowering	Days to maturity	Seed color	Seed size	Yield (Kg per ha)
RWV3006	58	110	White	Large	3,560
RWV2872	56	108	Red-mottled	Large	3,939
RWV2361	57	108	Red-mottled	Medium	3,485
RWV3316	58	110	Red	Large	4,000

Table 1: New Climbing Bean Varieties to be released 2012, their phenology, seed characteristics, and yield potential.

- Increasing seed of bush and climbing beans identified as micronutrient-rich (Fe and Zn) among the ISAR improved varieties was planned to facilitate dissemination.
- The program identified three bean varieties with moderate levels of drought resistance from the 15 recently released lines. About 150 kg of breeder seed have been produced for each variety this season in Nyagatare. At the three research stations, the program produced about 1,000 kg of foundation seed for each variety for farmer associations involved in seed multiplication.
- On May 31, 2011, the National Grain Legume team in INIAP, Ecuador, launched the first bush black bean variety *Afroandino* – *INIAP 482* in Tumbatú (Carchi) Chota Valley. The variety is resistant to anthracnose and root rots, and yield averaged 1.6 t/ha over seven locations compared to 1.3 t/ha for the local check.
- The new large-seeded, red-mottled variety *INIAP 483 INTAG* with resistance to three foliar diseases was released by INIAP in 2011. The line was resistant to the two pathogens in all locations, while the control variety Concepción was susceptible to angular leaf spot and intermediate resistance to rust. In addition, the Concepción \*/G916 -1 line produced an average yield of 1.6t/ha greater than the control, with 1.0t/ha. On September 9, 2011, *Tollo Intag* was officially handed over to bush bean producers in the area of Intag as the improved variety of bush bean variety named Intag. (Two leaflets describing the new varieties available for distribution and the pdf file can be viewed @[http:// www.iniap.gov.ec](http://www.iniap.gov.ec).)
- The breeding program in Ecuador continues to combine resistance to rust, anthracnose, angular leaf (ALS), and *Fusarium* wilt in all new breeding materials. Crosses were made using varieties and promising lines of commercial seed and parents of Mesoamerican origin for resistance to rust, anthracnose, and ALS. At harvest, individual F2 plants were selected and 66 F3 progenies derived from crosses for resistance to *Fusarium oxysporum* were combined with susceptible varieties Portilla, Concepción, and Paragachi Andino. At harvest, 23 progenies were selected that exceeded the controls in plant vigor, resistance to rust, pod load, and performance. In the next cycle, the 23 progenies were



re-evaluated and eight lines were selected for resistance to rust, *Fusarium oxysporum*, and *Empoasca*; good vigor of growth; overall yield; and grain quality. In addition to selecting bush types, selections with type II growth habit in red-mottled seed color were identified with high levels of resistance to rust and anthracnose and to *Empoasca*, good pod load, seed quality, and yield.

- Seed increases were initiated on several promising red seeded bush bean lines, black seeded Condor and AFROANDINO; red mottled line; and four top promising lines with multiple disease resistance. Seed of all new and old varieties continues to be multiplied for distribution to the CIALs.
- In the Department of Nutrition and Quality at INIAP, 12 bush bean genotypes were assessed for the canning process by applying a heat treatment and then evaluation based on physical parameter and nutritional and sensory properties. Based on analysis of crude seed:
  - Condor had the lowest hardness (3.6 mm) and the highest protein content (30.0 percent)
  - NSL had the highest starch content (74.5 percent)
  - G21212 the highest content of anthocyanins (534 mg /100g beans) and the higher iron content (89.2 ppm)
  - BRB 195 had the lowest tannin content (139 mg/100 g beans)
  - ICA Quimbaya had the highest zinc content (46.9 ppm)
- Soaking in the trial determined that the rate of hydration was related to seed size. The very small size took two to three hours to reach the proper moisture ratio (1.8) to start the thermal seed processing. Small seeds required three to five hours, the medium from five to seven hours, and large-seeded genotypes reached optimal hydration ratio at six to eight hours. At the end of soak period, the seed reached between 49 and 55 percent humidity. The thermally processed grain was characterized for physical, chemical, and sensory properties. Canning brine with CaCl<sub>2</sub> produced the best seed size characteristics, greater hardness, better color characteristics, lower viscosity, and lower drained weight, higher protein content, starch, anthocyanins, tannins, iron and zinc, lower moisture content. Brine without Ca exhibited opposite characteristics.

- Flour quality of six bean genotypes was assessed in three stages: first, the milling quality was assessed using three pretreatments (toasted, seed conditioning, and seed coat removal) to determine the best yielding bean flour; second, the functional and rehydration properties of the flours with higher extraction rate were assessed; third, the quality parameters of various formulations with different levels of substitution in flour-based bean biscuits were assessed. The pretreatment with the higher flour yield (67–75 percent) came from the preparation in which the bean seeds were soaked at 50° C for two hours, steam cooked for five minutes, dried at 60° C to 14 percent moisture, and subjected to milling. Flour was selected from Portilla after analyzing the functional and hydration properties of flour. Bean flour mixtures with different levels (5, 10, 15, 25, and 50 percent) of substitution with cereals were tested to produce biscuits. Levels of 25 and 50 percent did not significantly alter the color and aroma of the biscuit, while substitutions greater than 25 percent produced noticeable changes in flavor and texture.



**Objective 2: Develop inbred backcross lines in a range of commercial seed types for testing under drought and root rot pressure in Ecuador, Rwanda, and the United States.**

1. Evaluate specific populations developed at CIAT and MSU/ Ecuador at two sites for reaction to drought and nonstress in Rwanda.
2. Continue with the selection of lines with tolerance to drought and root rots in Ecuador.
3. Evaluate subset of best drought-tolerant lines from in Rwanda and other sources.
4. Continue characterization of new local traditional lines (bush, climbers) collected from growers in Ecuador to determine level of drought tolerance and root rot in Tumbaco.
5. Complete survey to identify field sites for root rot evaluation (*Pythium*, *Fusarium* wilt, and *Macrophomina*), and initiate screening of promising germplasm in Rwanda.
6. Characterize germplasm for reaction to individual root pathogens at Cornell using selected promising germplasm for Rwanda, Ecuador, MSU, and TARS (UPR).

**Results**

- In Rwanda, 100 Recombinant Inbred Lines (RILs) from an interspecific cross of common and runner bean for drought and aluminum toxicity resistance were introduced in 2010 and evaluated at Rubona and Nyamagabe research stations. Selected lines have now been evaluated in PYTs.
- From 15 lines (Mesoamerican bean genotypes, small red) in another trial evaluated in 2011B at Karama station, size varieties were selected and submitted to further evaluation using an on-farm Participatory Variety Selection (PVS) approach in multilocation trials. Phenotypic description of these six lines will be undertaken and their potential yields on farmer field conditions will be determined.
- Greenhouse experiments at MSU conducted by Gerardine Mukeshimana to identify bean lines with higher levels of drought tolerance in the shoot were concluded in 2011.
- The mapping population (CONCEPCIÓN \* 2/RAB651) was sent to Rwanda for an initial seed increase to be evaluated under moisture stress to identify QTL for drought tolerance
- Field evaluation of bean breeding lines and germplasm for root rot resistance in New York: A replicated field trial of 34 advanced materials was established in the bean root rot nursery at the Vegetable Research Farm, Cornell University. These bean lines differed significantly in their reaction to root rot pathogens when determined at the full flowering stage. Seed yield of a number of the promising lines is currently being completed and summarized.
- Cover crops for managing root diseases of beans and other agronomic crops: An ongoing collaborative project is assessing the efficacy of selected cover crops (rye grain + hairy vetch, oat, sudex, forage radish, red clover, rapeseed, buckwheat, wheat, and a fallow check) in suppressing root pathogens, including *Fusarium*, *Pythium*, *Rhizoctonia*, *Thielaviopsis*, *Pratylenchus*, and *Meloidogyne* and improving yield, soil health, and quality. In general, root rot severity was lowest and yield of bean was highest in the field with the highest soil quality and lowest disease pressure. In this field, bean yield was highest in the rye plus vetch cover crop and lowest in the buckwheat cover crop plots.
- Long-term tillage, rotation, and cover crop trail (soil health site): this collaborative site of the Cornell soil health team was planted to beans in 2011. The trial consists of 14 acres divided into 72 plots (18 treatments replicated four times). The treatments are represented by three tillage systems (no-till/ ridge-till, zone-till, and plow/conventional-till), three cover crops (no cover, rye grain, and vetch), and two rotations. One rotation includes primarily high value vegetable crops (R-1), whereas the second rotation also includes season long soil building crops (R-2). Interestingly, we were able to machine harvest beans in the zone-till and no-till plots without much difficulty, but not the plow-till plots because the harvester was getting stuck and rutting and compacting the soil. The data are still been analyzed, but the yield of beans was highest in the ridge-till system, in rotation R-2, and after vetch.
- Evaluation of selected pea varieties for resistance to root pathogens: Root rot diseases of peas are prevalent and

damaging. In recent years, disease symptoms commonly observed on roots of infected plants in New York were those of *Fusarium*-root rot and, less frequently, *Fusarium*-wilt. Symptoms of infections caused by *Thielaviopsis*, *Pythium*, and *Aschochyta* were also occasionally observed. Recently, large number of varieties and promising lines were evaluated in commercial fields with known histories of severe disease incidence and in greenhouse tests using naturally infested soil. In 2011, 47 varieties and lines were again evaluated in the greenhouse. Root rot severity ratings varied greatly among the peas tested, from 8.8 to 3.3 on the one (healthy) to nine (most severe, dead) evaluation scale. June, Marias, BSC 3048 generally exhibit the most susceptible reaction, whereas Boogie, Pendleton, and Lil'mo were among the most tolerant.



- Thirteen RILs previously selected under normal irrigation were evaluated for pod load growth and reaction to rust, *Empoasca*, and seed quality. The same selected RILs plus two checks were evaluated under two treatments with and without drought stress. Vigor data and pod load at harvest were taken. Under drought stress, obtained yields were 10 lines between 1.7 and 2.0 t/ha while the Portilla and Concepción checks yielded 1.6 t/ha. Under normal watering every week there was no difference in performance between the lines and the control.
- Twenty-eight black bean lines were subjected to terminal drought. Pod load data, plant health, and seed yield were collected. Under these parameters, 10 lines were selected for high performance and plant health. All 10 lines were superior in performance to the check Afroandino. They were also resistant to *F. oxysporum* and drought tolerant. Five lines were selected based on superior performance, rust resistance, and vigor.

**Objective 3: Collect and characterize pathogenic and genetic variability of isolates of root and foliar pathogens in Ecuador and Rwanda.**

1. Continue surveys to diagnose major root diseases in Rwanda and collect isolates of root pathogens for additional characterization.

2. Maintain the collection of root rot isolates previously collected in different production zones of Ecuador.
3. Further characterize root rot isolates collected previously in northern and southern production regions of Ecuador.
4. Phenotypic evaluation of Rwandan germplasm for resistance to local isolates of anthracnose, ALS, and BCMV under field conditions, screenhouse, and MAS.
5. Continue the collection of isolates of anthracnose and ALS in Rwanda and Ecuador from diverse agroecological zones for race typing.
6. Increase seed of the differentials for anthracnose, ALS, and rust in Rwanda; continue characterization of ALS in Ecuador. Continued race characterization of *Fusarium* wilt pathogen and the aggressiveness of isolates of *Macrophomina*, *Rhizoctonia*, and *F. solani* will be conducted on selected bean germplasm.
7. In Rwanda, document and summarize past studies on mapping and/or variability of *Fusarium* wilt, *Pythium*, ALS, and anthracnose by CIAT/ISAR and MS theses.
8. Continue to document and publish results of recent and ongoing breeding activities in Rwanda.

**Results**

- Anthracnose was a problem in Michigan in 2011. Isolates were collected from growers' fields and all typed as race 73. Adequate levels of resistance to this MA race are present in current cultivars, but farmers continue to plant bin-run seed of susceptible varieties without having it verified as disease free. New navy and black bean lines with resistance to anthracnose are currently being evaluated in yield trials.
- Rust was collected again from bean fields in Michigan, but it was more widespread and severe in 2010. The new strain characterized as race 22:2 defeats many of the current resistance genes deployed in Michigan. Resistance has been identified in elite MSU black and navy bean germplasm and crossing has been initiated to transfer resistance; an extensive screening of all MSU germplasm was being conducted in the greenhouse in winter 2012. The collection of new samples of leaves and roots infected with major pathogens (angular leaf spot, bean rust, and anthracnose) has been a continuous activity in Rwanda since 2009. To date, races 27 and 55 have been confirmed and race three is a tentative observation.
- Four new races of anthracnose collected near Urcuquí, Mira, and Pimampiro were characterized and the *Co-4<sup>2</sup>* and *Co-5* genes offer good levels of resistance to these races. A single race of angular leaf spot 62:0 was collected at in Urcuquí and Intag; it can be controlled with Mesoamerican germplasm.
- *Fusarium* wilt is becoming an increasingly serious disease in Ecuador. The program initiated the collection of isolates of *Fusarium oxysporum* in the localities de Urcuquí, Pablo Arenas, and Intag. The program continues to make good progress in selection for resistance to *Fusarium* wilt and has identified a hot screening site at Tumbaco where high levels of soil borne pathogen exist and allow for early generation screening to detect resistance.

- Efforts were made to develop and standardize inoculation methods for screening for ALS using detached trifoliolate leaves in a Petri dish. Additional work is needed to satisfactorily standardize the method that shows considerable promise for saving both time and resources.
- Since rust and ALS are highly variable pathogens, testing must be conducted at different locations in Ecuador to confirm resistance and identify new sources of resistance. Testing of 31 sources of resistance to ALS and rust using natural inoculation was conducted in the town of Peñaherrera in the Intag valley. Eleven lines generated by the breeding program and 15 from CIAT were highly resistant to ALS, and 18 lines were resistant to rust.
- The inheritance of resistance to rust in the JeMa cultivar was evaluated in a population of 165 F2 seeds derived from a cross with AND277 (susceptible to rust) inoculated with rust race 0:61. The results suggest the presence of a single gene based on 130 resistant and 35 susceptible individuals.
- Six promising lines were selected with combined resistance to rust, anthracnose, and angular leaf spot in Ecuador. Angular leaf spot was evaluated in the field (La Concepcion, Carchi) and confirmed in greenhouse tests along with reaction to specific races of anthracnose; rust was evaluated in the field at Tumbaco. The selected lines showed high genetic resistance to all three diseases in comparison with the check Portilla that was susceptible to angular leaf spot and had an intermediate resistance to rust.
- Another objective of the visit to Rwanda in May 2011 was to expand the survey to access the major soilborne pathogens and other pests impacting bean production in Rwanda. Again, the bean fly and BCMV were observed predominately during this trip, suggesting the need to devote more efforts for their management. Root diseases observed in the various research sites and growers' fields visited were primarily *Fusarium*-cortical rot, *Pythium*-root rot, root-galling by the root-knot nematode, and a low incidence of *Sclerotium*-rot.

**Objective 4: Employ participatory plant breeding and agroecological methods to assist the breeding process in Ecuador and Rwanda to enhance the productivity and market quality of beans under development.**



1. Compare and contrast advanced line selection practiced by breeders and farmers in mid-altitude and high agroecological regions in Rwanda.
  - Plan genotype by farmer participatory assessment of advanced lines within intercrops and sole crops.
  - On-farm assessment of promising lines conducted in sole crop and intercrop trials at eight sites in 2011/12.
2. Evaluation of 17 tests with 17 CIALs each growing cycle in Ecuador.
3. Expand nonconventional and conventional seed production.
4. Release two bush beans and one climbing bean in Ecuador using farmer participatory approach.
5. Continue to provide seed of elite and new varieties for postharvest quality evaluation at KIST.
6. Continue with farmer participatory approaches to identify appropriate and cost-effective innovations for staking climbing beans that would enhance the adoption in Rwanda.
7. Organize a visit of scientists from Ecuador to Rwanda to exchange experiences on population management, germplasm bank, evaluation of early generation materials at different stations, and farmer participatory and seed production.
8. Initiate exchange of experience in Rwanda on participatory methods and seed production for local community use with smallholder farmer members.

*Results*

- Experimental research on cropping systems and bean varieties were conducted in northern Rwanda using a mother–baby–grandbaby trial system. In season 2011B, two on-station genotypes by cropping system x environment mother trials were planted in northern Rwanda at ISAR Station Musanze and ISAR Station Rwerere to determine how genotypes perform differently under different cropping systems across different environments. Each on-station trial had four replications. Eight more single replications, or baby trials, were planted on farmer fields with farmer associations in Gakenke, Burera, and Muko. One replication consisted of 14 plots. Bean genotypes RWV 3006, RWV 2070, RWV 3316, Gasirida, and double controls of Ngwinurare and a local mixture were each planted as sole beans and intercropped with PL9A maize variety. A plot of sole maize and a plot combining the local mixture and maize in a traditional mixture were also included. Data on yield, agronomic traits, leaf area index, canopy cover, and soil samples were collected at each site, and plant biomass for total N analysis was collected from the research station trials.
- At each farmer field site participatory variety selection was used near the end of season B to evaluate cropping systems and bean varieties under monocropping and intercropping. Approximately 105 farmers (71 women, 34 men) participated.
- In season 2012A the trials were replicated on station and on farm. Data collection is ongoing. Approximately 70 farmers participated.

- Survey and short structured interviews are being carried out in Season 2012A to collect data on the grandbaby trials, farmer preferences for cropping systems and bean varieties, socioeconomic status, and the effect of land-use policies on farmers' ability to experiment.
- A participatory workshop will be carried out at the end of season A with practitioners in plant breeding and agronomy. Focus areas will include trial designs, focus groups, and practical tools that consider livelihoods and sustainable production, including conservation and nutrition aspects of client-oriented research.
- About 44 lines combining drought and heat tolerance resistance with high mineral density and high yield potential were subjected to participatory variety selection (PVS) in Rwanda. These lines were evaluated in two separate Genotype x Environment (GxE) trials.
- Two field screening trials under drought stress were conducted to evaluate advanced bean varieties for yield potential through a participatory plant breeding process at Karama research station. The total number of lines tested was 29, and from this set six lines were selected to be tested under multilocation trials on stations and on farm in Karama, Nyagatare, and Ngoma.
- To enhance the availability of seed and the adoption of improved varieties by farmers in the Nyagatare, Gatsibo, and Kabarore districts, more than six tons of new bush bean varieties adapted to the region for their early maturity, desirable seed types, and high yields (2.0–2.5 ton ha<sup>-1</sup>) were multiplied and distributed to 6,000 farmers by RAB.
- In all RAB stations, about 10 tons of breeder and prebasic seed of the prereleased and released bush and climbing beans mentioned above were produced on research stations. Seed was sold and distributed to farmers and farmers cooperatives; NGOs; and seed companies for secondary seed multiplication and distribution to more producers.
- Evaluation and participatory selection of bush bean germplasm with local CIALs continued in Ecuador. In the town of La Concepción, eight lines with resistance to rust, anthracnose, and angular leaf spot plus four checks (two resistant and two susceptible) were evaluated by CIAL members for plant and seed traits.
- In the two growing cycles, 10 t of basic seed of five varieties was produced. Six seed-producing CIALs in the Mira and Chota Valleys multiplied seed of four bush bean varieties, and the CIAL in the Intag Valley produced seed of the new Intag (1530 kg) variety.
- In Huigra (1300 m), Chimborazo, 10 bush bean varieties were planted. Participatory assessments were performed on the plants and most of the varieties were superior to the local controls. In Cañar, five quintals each of Portilla and Rocha varieties were planted and evaluated at pod filling. Varieties showed good adaptability and farmers were pleased with the reintroduction of beans after 16 years. Seed of bush varieties was planted, multiplied, and distributed to farmers in the province of Loja.

- The important health benefits of beans were promoted during the preparation of the “world’s largest stew” in San Miguel de Porotos (Cañar) following a 10 K road race where the prize was the bean stew.

## Extension Activities

- A booklet to promote the bean production chain has been developed and 5,000 copies printed and distributed to Rwandan farmers and other producers. Training on seed multiplication for 28 participants has been conducted to increase their capacity in seed multiplication, increase the qualities of seed produced, and build a common understanding of guidelines in the domain.
- A bean stakeholder meeting was organized in Rwanda to establish an innovation platform for bean producers and traders. Participants from different organizations attended. Discussions focused on the following themes:
  - updates on bean breeding in Rwanda
  - seed systems
  - the role of the ISAR seed program in promoting improved seed uptake and linkages with stakeholders in the seed industry
  - updates on Rwanda and regional markets
  - small packs in dissemination of improved bean varieties
  - update on Agriculture Extension and policies in Rwanda
- Three working groups were formed:
  - Group 1: Roles and responsibilities in technology development and dissemination
  - Group 2: Accessibility of information on improved bean production and commercialization technologies to potential users
  - Group 3: Seed increase of improved varieties and wider dissemination
- Drs. J. Kelly and G. Abawi traveled to Rwanda May 9–23, 2011, to meet with collaborators at ISAR, review research activities, visit field research sites around the country, and present a three-day workshop on bean pathology (major root and foliar diseases) and breeding (genetics of beans) to the ISAR technical staff. Dr. Snapp joined the trip in mid-May and we visited the research plots of Krista Isaacs and hosted the ISU CRSP team during their stay in Rwanda.

## Networking and Linkages with Stakeholders

The project forged closer collaboration with the Kigali Institute of Science and Technology (KIST). RAB provided KIST with 20 newly released varieties for postharvest and processing studies under CRSP MSU/ISU collaboration to build synergy between the KIST lead PULSES CRSP ISU and the current project in integrating agronomic and market traits with the nutritional and quality attributes of new bean varieties released and being developed by RAB.

RAB, farmers' cooperatives, and seed production agencies worked together to further the dissemination of bean technologies. NGOs, such as World Vision, AFRICARE, ADRA, CARITIUS, Catholic Relief Services, DERN; farmers organizations—COAMV in the north, RDO in the east, Iterambere ry'Abahinzi Borozi Muhanga (IABM) in the south and local Government Extension Agents (country-wide), Musasu Watershed (south), Sogwe Watershed farmers Cooperatives (south), Gakiragi Watershed Cooperative (east), Umutara Polytechnic University (east), IMBARAGA (Umbrella farmer organization in the country), DERN, DRD, TIN, CSC, Rwanda Seed Company (RWASCO), AGRA Climbing Bean, N2 Africa, CIALCA, and individual farmers were also involved in promoting bean technology. Government institutions such as KIST, and Higher Training Institute of Agriculture and Livestock (ISAE) were among partners with RAB in scaled-up programs.

The program interacts with the following in Ecuador: PRODECI, PRODER, CRUZ ROJA, Agricultural Organizations; COPCAVIC, 10 CIALs, Grupo de Evaluadores de Frijol de Bolivar, Assoc. de Productores de Frejol de INTAG; Government Organizations; MAGAP, INIAP, Univ. Tecnica del Norte, and Univ. Catolica de Ibarra.

### Leveraged Funds

In addition to the Dry Grain Pulses CRSP project, funding was secured through the Nitrogen fixation CRSP project with ISU for the bean breeding program in Rwanda, AGRA (Alliance for a Green Revolution in Africa), Harvest-Plus, Bio-Innovate, ASARECA, ACTESA/COMESA, and PABRA network. Support from the government was provided to the bean program for variety selection and further seed increase. Support was received from Borlaug LEAP fellowship for training a doctoral CRSP candidate.

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# Expanding Pulse Supply and Demand in Africa and Latin America: Identifying Constraints and New Strategies

MSU-2

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## Abstract of Research Achievements and Impacts

**Angola.** Semistructured interviews of small-scale traders were conducted in common beans markets in Huambo and Londimbuali. Preliminary research on bean markets indicates a preference for local over imported beans, yet smallholder farmers in the Planalto region lack marketing strategies to reach those markets, and trade organization appears weak, implying high transaction costs. Research identified the margins for traders in key common bean marketing channels and found that bean farmers are interested in improved production technologies but wait for projects to ensure the inputs. While high transport costs restrict marketing, some farmers are able to transport to regional markets. Interviews with farmer groups found that most farmers sell into local markets or to traders arriving in the village, even when farmers associations exist. For market information, farmers listen to the radio programs' broadcasting prices but indicated that these prices were not reliable. Phase II rapid appraisal market research on cowpeas in two provinces found that peak sales months vary among markets. Almost all cowpea traders are women; many both grow and trade cowpeas. Traders identified various transport problems. In the rainy season, some areas remain inaccessible. Price information for cowpeas is limited to word of mouth. Where phones function, traders use them to obtain price and other trading information. Traders identified quality as a key constraint to greater marketing; cowpeas are mixed with different varieties and sizes in the bags and infested with insects. While IDA distributed cowpea seeds in the region, IDA extension agents believed that many farmers ate the seeds. In the future, IIA and IDA need to do more to ensure adequate supplies of seeds and to develop delivery systems for inputs at the farm level. Estevao Chaves completed his MS thesis.

**Mozambique.** Semistructured interviews were conducted with 109 traders (54 cowpeas, 55 beans traders) in northern/central Mozambique and Maputo's wholesale market (15 traders). The market rapid appraisal by SIMA found that cowpea traders were more likely to sell to large warehouse agents (LWA), whereas bean traders were likely to sell to retailers—likely due to the developing value chain for cowpea processing and export. Since LWA establish longer-term links with local traders, cowpea traders are more local and less likely than common bean traders to work in more than one district. This value chain may lend itself more to developing targeted actions to improve quality/postharvest handling, since wholesalers can be more easily organized. For beans, traders are informal and sell directly to retailers since there are few processors and organized bean traders. Market prices follow each other across the country's markets. CRSP research to evaluate cowpea markets and producer activities in southern Mozambique will provide insight into how cowpea production/marketing might be improved. Interviews with market traders in Maputo found that they sourced beans depending on relative prices and availability, sometimes importing from Swaziland/South Africa. Supermarkets import

beans due to poor quality of Mozambique's beans but also buy beans in the main production zones. Research under PABREN focused on enhancing farmer access to appropriate information on markets, including varietal choices and prices. This research in the northern bean areas is complete and in 2012 researchers will meet with farmer focus groups in the southern production areas. The database on cowpeas/bean prices has been created and is revised weekly, based on weekly SIMA price collection. Farmer focus groups for beans were conducted in the north in collaboration with PABREN. Currently farmer focus groups are being conducted in the south for cowpeas. Research on bean trade between Mozambique and South Africa found that import parity prices from South Africa were important in determining bean prices in Mozambique. Reducing transport and other transaction costs were identified as actions needed to ensure competitiveness of local beans throughout the year, since South Africa beans appear in markets in the off-season while local

beans are more available during the production seasons.

Donovan worked with the USAID/Mozambique mission to develop the Feed the Future Strategy, which will include pulses. The project collaborated with the NSI to use cell phone

technology for price information dissemination. While 2007 population census data showed that only one percent of rural households had cell phones, there has been rapid growth in rural cellular networks, and cell phone-based information will be useful to traders and better-off farmers.

**Honduras.** Strategies for managing bean crops using organic fertilizers and household items were tested in on-farm trials and demonstrated in farmers' fields in collaboration with CIALs; Rhizobium inoculation trials were conducted. At least 100 CIALs farmers are using organic fertilizer and pest control practices produced locally; at least 50 percent are capable of producing their own organic fertilizer/pest control products. Results from Rhizobium inoculant tests on farmer fields indicate the usefulness and potential adoption of this practice. Technical assistance has been provided in stages:

1. Technical personnel and CIAL leaders were trained on the production of organic fertilizer and pest control products at Zamorano's Organic Agriculture Unit.
2. Technicians and farmer leaders train other farmers at the local level.
  - a. Facilities were established to produce organic fertilizer and pest control products at PRR and FIPAH.
  - b. Practical training courses and demonstrations were carried out.
3. Field trials and demonstration plots were used to promote adoption of these products.
4. Periodic visits from NGO technicians and extension farmers, were conducted to follow the production and use of organic products at the farm level.

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*CRSP research to evaluate cowpea markets and producer activities in southern Mozambique will provide insight into how cowpea production/marketing might be improved...*

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More than 50 farmers from CIAL received training locally and are producing/utilizing organic products on their commercial bean plots. Organic products are sold by PRR and FIPAH facilities and by farmers from CIALs locally. More than 15 NGO technicians/CIAL leaders have been trained on the production and use of organic fertilizers and organic pest control products. During FY2011, 15 young farmers were trained in five practical modules including one on organic agriculture. The use of plant extract as insect repellent and foliar organic fertilizers on bean crops by CIALs farmers is quite common. Currently, the Rhizobium inoculant technology is in the farmer field testing stage. During the Primera 2011, inoculated and noninoculated treatments were compared in farmers' commercial bean production plots and more than 12 farmers were trained on the use of inoculants on beans. IMO staff was contacted to obtain third-party fair-trade certification for the farmer association. The IMO agreed to provide fair trade certification (pending the outcome of an audit visit) for the association, and Whole Food Markets (WFM) guaranteed that it would purchase 20 MT of beans from the farmers at a farm gate price of \$ 0.60/lb, if the association obtained fair-trade certification from the IMO. In March 2011 the PIs met with association leaders to discuss IMO certification requirements and sales to WFM and other supply chain participants. While in early 2011 the farmers had agreed to accept WFM's price, they now wanted \$0.75/lb because the local bean price had increased. Upon returning to the United States, Bernsten visited WFM's office to provide background information about the farmers and request a higher price. While WFM agreed to pay \$0.75/lb, when Rosas later contacted the farmers to confirm that they would accept WFM's new price, the farmers wanted \$0.85/lb—the price that local traders were now paying farmers. Bernsten contacted WFM to discuss options. Because WFM could not pay that price, we agreed to postpone a purchase contract until early 2012 to see if the local price would decline. If the local price declined, WFM agreed to reconsider purchasing 20 MT of small red beans for delivery in mid or late 2012. Consequently, plans to get IMO certification and export to WFM were postponed.

## Project Justification and Objectives

*Angola.* Common beans and cowpeas are important crops for smallholder farmers in Angola, with approximately 36 percent of households in a recent survey indicating that common beans were the most important source of cash income from crops. About two-thirds of household production was sold.

However, marketing constraints are clearly found in household surveys in the Planalto region, such that farmers sell from farm gate or in local markets due to ease of trading. The sales period is strictly based on the harvesting period, rather than on strategic storage and pricing. In regard to common beans, many farmers sell into the local markets at harvest simply for convenience. Farmers who sell in more distant markets (27 percent in the region) indicate that they choose those markets to get higher

prices, but they have transport expenses as well as information constraints, making marketing more costly. Some 28 percent of farmers determine when to sell their beans based on price, yet the information available to help guide this choice is limited.

A key question is whether there are areas for greater efficiency in the marketing system to enable Angolan farmers to contribute greater quantities of beans, replacing imported beans and meeting unmet needs in urban areas. Phase II of the research and training identified the costs associated with the marketing channels. Txocaine's thesis provides evidence that intermediaries gain fairly high marketing margins. There are opportunities for farmers to increase their profits by selling into regional rather than local markets, but only a minority of farmers is organized to market in larger, more distant markets. Additional work with World Vision and other local organizations is needed to ameliorate the information gaps that are reducing profitability and overall volumes marketed for beans.

Cowpea cultivation in Angola is more dispersed and marketing seasons more varied than for common beans. There is basically no information on cowpea producers and their marketing channels; research will begin to fill in the gaps of information on cowpeas to assess potential.

*Mozambique.* In Mozambique, both cowpeas and common beans are marketed by smallholder producers, and the local market information system (SIMA) shows high seasonality in prices for the common bean, whereas cowpeas tend to have less dramatic variability, with more flexibility in planting seasons and locations. Cowpeas and common beans have different marketing channels. Wholesale common bean traders do not work with cowpeas or other legumes and prefer to specialize. Cowpea markets tend to be more localized, but recent developments suggest that new markets for processing may be arising, along with pigeon peas. IIAM researchers with SIMA have begun to identify some of the new initiatives in the processing of cowpeas and pigeon peas for use in dhal for export to Southeast Asia.

The costs for cowpeas and common beans through various channels were determined in the rapid appraisal survey, and traders have identified ways to minimize their costs using banking and cell phone technology. The current trading structure of common beans is primarily an informal sector and does not lend itself easily to the formation of a bean task force.

In July 2011, Pulse CRSP researchers helped TradeHub and AGRIFuturo to organize travel by South African traders and processors into the bean production zones of Mozambique, looking into possible linkages. Phase I research has identified the basic marketing channels for common beans, and work has begun on the marketing channels for cowpeas.

*Honduras.* Common beans, the second most important food crop (95,000 ha) after maize, are an important source of food and cash income for smallholder farmers; however, input costs are rising, which reduce farmers' profits, and most smallholder

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*Cowpea markets tend to be more localized, but recent developments suggest that new markets for processing may be arising, along with pigeon peas.*

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farmers sell their surpluses to traders at the farm gate and receive low prices. With the recent ratification of CAFTA, bean imports are expected to increase, thereby reducing bean prices and farmers' incomes. Smallholder farmers need new technologies (i.e., organic alternatives to fertilizer and pesticides) that will enable them to reduce their input costs and access to new markets to add value to their crop. This project focuses on testing organic crop management practices and developing new market opportunities for smallholder farmers by producing and exporting fair-trade beans (small reds) to the U.S. food retail market.

## Objectives

1. Angola: Identify efficiency in marketing channels and leverage points to increase farmer profits and trader volumes.
2. Mozambique
  - a. Identify efficiency in marketing channels and leverage points to increase farmer profits and trader volumes.
  - b. Develop a cell phone-based information system for beans to link farmers and traders to market prices and availability.
3. Honduras
  - a. Conduct on-farm trials to validate organic bean production practices, including organic fertilizers and pesticides based on extracts from local plants.
  - b. Provide technical assistance to farmer groups interested in establishing commercial organic bean plots.
  - c. Assist farmer groups to obtain fair trade certification.
  - d. Export fair-trade beans to a U.S. retailer.

## Research and Outreach Approaches, Results, and Achievements

In all three countries, this research focuses on identifying potential market enhancements for smallholder farmers in Angola, Honduras, and Mozambique.

### Angola and Mozambique

During Phase I, research in both Mozambique and Angola sought to describe and understand bean producers and their relationship to markets. The most recent efforts in Mozambique have involved evaluating the cowpea value chain. Existing analysis for farmers and trading systems in Mozambique and in Angola has highlighted some key features of bean production and marketing, and cowpea marketing:

- Beans are considered a cash crop by many farmers.
- Trade networks are responsive to change.
- Traders travel long distances and may specialize in beans.
- Traders are adapting to new technologies and services (for example, cell phones and automatic teller machines at banks).
- Farmers make investments in improved varieties.

In both Mozambique and Angola, training workshops and guidance on value chain research for common beans and

cowpeas included price analysis. One MS student has finished his study program and the second will finish in late 2011.



### Objective 1: Angola. Identify efficiency in marketing channels and leverage points to increase farmer profits and trader volumes.

Semistructured interviews were conducted in common beans markets in Huambo and Londimbuali. Preliminary market research on bean markets indicated a preference for local beans over imported beans, yet smallholder farmers in the Planalto region lack marketing strategies to reach those markets and trade organization appears weak. Txocaine's research identified the margins for traders in key common bean marketing channels for Londimbuali. On the supply side, bean farmers are interested in improved production technologies yet tend to wait for projects to ensure the inputs. The high cost of transport is a key factor restricting marketing, yet some farmers with higher levels of production are able to arrange transport to regional markets. Interviews with farmer groups of World Vision found that most farmers continue to sell into local markets or to traders arriving in the village, even when farmers associations exist. For market information, farmers listened to the new radio programs' broadcasting prices but indicated that the prices were not reliable. This reflects a challenge for the market information system to train farmers on competition and markets.

Dr. Kiala, Artur Paulino, and other UJES students focused Phase II rapid appraisal market research on cowpeas in markets of the Benguela and Huambo provinces. Cowpea traders are almost entirely women, and many of them both grow and trade

cowpeas. Traders identified various transport problems; in the rainy season, some production areas remain inaccessible. As with common beans, price information for cowpeas is mostly limited to word of mouth; cell phones function only in selected areas. Where phones do function, traders were interested in using them for price and other trading information. Overall the traders identified quality problems as a key constraint to greater marketing, since the cowpeas are mixed, with different varieties and sizes in the bags; there are also problems with insect infestations. IDA had distributed cowpea seeds in the region, but the IDA extension agent believed that farmers ate many of the seeds distributed, rather than planting them. In the future, IIA and IDA need to ensure adequate seed supplies and develop delivery systems for inputs at the farm level.

**Objective 2a: Mozambique. Identify efficiency in marketing channels and leverage points to increase farmer profits and trader volumes.**

Semistructured interviews were conducted with 109 traders (54 cowpeas and 55 common beans) in the northern and central parts of Mozambique. Additional interviews were conducted in the main Maputo wholesale market with 15 traders.

The most recent market rapid appraisal by SIMA found that cowpea traders were much more likely to sell to large warehouse agents, whereas the common bean traders were more likely to sell to retailers. This difference is believed to be related to the developing value chain for cowpea processing and export. Large warehousing agents establish longer-term links with local traders, and thus the cowpea traders tend to be more local and are less likely than common bean traders to work in more than one district. This value chain may lend itself more to developing targeted actions to improve quality and postharvest handling, since wholesalers can be a more easily organized interest group. For common beans, the traders are generally informal and tend to sell directly to retailers in other markets. As figure 1 indicates, market prices tend to follow one another across the markets of the south, center, and north.

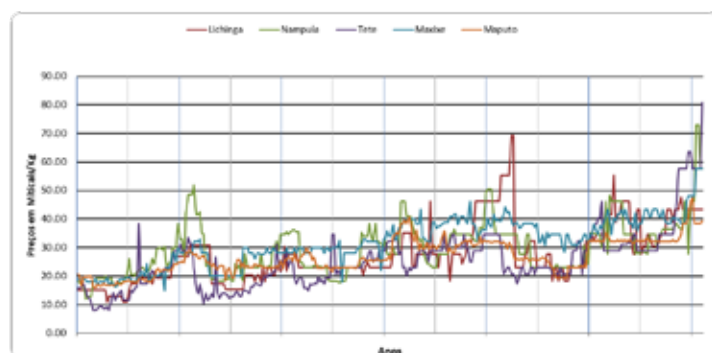


Figure 1: Common bean price tendencies in the markets of Lichinga, Maputo, Maxixe, Nampula e Tete, January 2005 – January 2011, in Meticais/Kg

There is ongoing IIAM research through the Pulse CRSP in association with PABREN to evaluate cowpea markets and producer activities in southern Mozambique to provide insight into how cowpea production and marketing might be improved in southern areas. IIAM/CESE researcher Isabel Cachomba

interviewed market traders in Maputo and found that they source beans depending on relative prices and availability. At times they purchase beans in Swaziland or South Africa, importing them informally to avoid import duties. Formal sector stores, such as supermarkets, also tend to source beans due to the poor quality of Mozambique’s beans and the drying processes used. Imported beans are also sorted.

Phase II Pulse CRSP activities are linked with PABREN network activities to enhance farmer access to appropriate information on markets, including varietal choices and prices in different key markets. Under the PABREN effort, IIAM researchers are evaluating farmers’ preferences for varietal choice.

The database on cowpeas and common prices is available and is continually being revised, based on weekly SIMA price collection. SIMA continues its efforts to disseminate the data via radio, television, newspaper, and e-mail. Estevao Chaves from Angola used the SIMA price series to look at the interdependence of prices among five key markets.

Ms. Gungulo, a CRSP student at University of Pretoria, researched trade in dry beans between Mozambique and South Africa. Preliminary results suggest that import parity prices from South Africa were important in determining bean prices for Mozambique farmers. Reducing transport and other costs were identified as actions needed to ensure the competitiveness of local beans throughout the year.

**2b: Develop a cell phone-based information system for beans to link farmers and traders to market prices and availability.**

Collaborative work will be implemented with the National Statistics Institute, involving farmer focus groups for pretesting messages and instructions and providing enumerator with short-term in-service training on SMS system use. The 2007 Population Census showed only one percent of rural households had cellphones; however, there has been rapid growth in rural cellular networks, so it is believed to be much higher. At least for now, cell phone information availability will be useful for a high percentage of the traders and better-off farmers when it gets out. A draft SMS system is in operation.

**Objective 3a: Honduras. Conduct on-farm trials to validate organic bean production practices, including organic fertilizers and pesticides based on extracts from local plants.**

Integrated approaches for management of bean crops using organic fertilizers (bokashi, compost, chicken manure, crop residues, and others) and household items (e.g., detergent, oil, ethanol) and plant extracts (Gliricidia, nim, hot pepper, onion, garlic, parsley) that have proven to be effective in reducing insect pests and disease incidence and damage continue to be tested and demonstrated in bean farmer plots in the Yojoa Lake, Yorito-Sulaco-Victoria, Vallecillo, and F. Morazán regions in collaboration with CIALs and NGOs. Rhizobium inoculation trials are conducted with farmer CIALs from the same regions and in the western bean production region in collaboration with the FAO-Seed for Development Project and in southern

Honduras with the CARE/CIAT Project. Farmers from participating CIALs of the Yojoa Lake, Yorito-Sulaco-Victoria, and Vallecillo regions have implemented the use of organic fertilizers and pest control practices produced locally. Rhizobium inoculant tests on bean farmer fields were conducted in several regions of Honduras. At least 100 farmers from participating CIALs are using organic fertilizer and pest control practices produced locally, and at least 50 percent of these farmers can produce their own organic fertilizer and pest control products.



**Objective 3b: Provide technical assistance to farmer groups interested in establishing commercial organic bean plots.**

Technical assistance has been provided in stages.

1. First, technical personnel and CIAL leaders were trained on the production of organic fertilizer and pest control products at Zamorano’s Organic Agriculture Unit.
2. Technicians and farmer leaders train other farmers at the local level.
  - a. Facilities were established to produce organic fertilizer and pest control products at PRR and FIPAH.
  - b. Practical training courses and demonstrations were carried out.
  - c. Organic products are sold at these facilities.
3. Field trials and demonstration plots were used to promote the utilization of these products.
4. Visits from NGO technicians and extension farmers followed the production and use of organic products.

*Results*

NGO technicians and farmer leaders have received training at Zamorano in the production and use of organic fertilizers and plant extracts for pest control. More than 50 CIAL farmers received local training and are producing and using organic products on their commercial bean plots. This year, 15 farmers were trained in five practical modules, including one on organic agriculture. The use of different plant extracts (onions, garlic, hot pepper, and others) as insect repellent and organic fertilizers based on earthworm humus, compost, and other organic nutrients is currently quite common by farmers from CIALs.

Rhizobium inoculant technology is at the farmer field testing stage. During the Primera season of 2011, inoculated and noninoculated treatments were compared in farmer bean commercial production plots and more than 12 farmers were trained on the use of inoculants in beans.

**Objective 3c: Assist farmer groups to obtain fair trade certification.**

The objective of this subcomponent is to work with a Honduran farmer association to produce and export fair-trade-certified beans to a U.S. food retailer (WFM); however, to sell beans and other commodities as fair-trade products, the producers must be fair-trade certified. Following WFM’s suggestion, we sought certification from the International Marketing Organization (IMO). Dr. Rosas met with the farmer association (ASOCIALAYO) to discuss the IMO’s requirements. The IMO agreed to provide fair trade certification (pending the outcome of the audit visit) for the farmer association and WFM guaranteed that it would purchase 20 MT of fair-trade beans from the farmers at \$0.60/lb, if the farmer association obtained fair-trade certification; however, after agreeing to this price, the farmers decided that they wanted a higher price—a price that WFM was unable to meet. Consequently, the IMO’s certification (audit) visit was postponed.

While we did not pursue securing fair-trade certification, cost of production data were collected, as required by the IMO for determining a fair price. Thus, having established contact with the IMO and having estimated the costs of bean production, if in the future the farmers agree to sell their beans at a price acceptable to WFM, the protocols are in place to schedule an IMO certification (audit) visit.

**Objective 3d: Export fair-trade beans to a U.S. retailer.**

Prior to contracting with farmers to produce beans for export to a U.S. food retailer as fair-trade beans, it is necessary to:

1. obtain fair-trade certification for the farmer association
2. document the links in the supply chain from the farm gate to the U.S. port of entry
3. estimate the costs associated with each stage of the supply chain (i.e., fair-trade certification, production, transporting a container to the village, cleaning/packing the beans, fumigation, transporting the container to the port, obtaining a Honduran export permit, Aphis inspection charges, U.S. customs clearance charges, shipping to the US/Houston TX, and the broker’s service charges)
4. identify a broker who can be contracted to transport the beans from the farm gate to the Honduran export port and complete the required export-related paperwork
5. identify a U.S. food retailer who will purchase the beans and provide a purchasing contract
6. identify a price acceptable to the U.S. retailer and the farmer association
7. obtain an export permit (due to supply shortages, the government frequently places an embargo on exports)

8. provide the farmers with a contract specifying the quantity and quality standards required by the buyer

Throughout the year, many phone and e-mail contacts were made with WFM's bulk commodity buyers to update them on the project and negotiate a purchase price. In early March 2011, WFM agreed to purchase 20 MT of small red beans for delivery in January 2012 at a farm gate price (US\$ 0.60/lb) that the farmers had agreed to in early 2011. After adding supply chain costs, this farm gate price translated to a price of US\$ 0.87/lb delivered to the U.S. port at Houston, TX. In late March, Rosas and Bernsten met with the leaders of the farmer association to discuss logistics and collected data from the farmers to estimate their bean production costs to document that the price offered by WFM was fair (required by the IMO). In addition, we met with a bean broker to discuss logistics and costs related to collecting the beans at the farm gate and transporting them to Honduras's export port and an Honduran government official to obtain information regarding the process for applying for an export permit.

Since early 2011, however, the local price of beans had increased substantially. Thus, at the March meeting with the leaders of the farmer association, they requested a higher price (US\$ 0.75) than WFM had originally agreed to pay. While WFM agreed to the new price that the farmers wanted (US\$ 0.75), the local bean price continued to increase. When Rosas met again with the farmers to offer them WFM's new price, the farmers noted that the retail price was now US\$ 1.00/lb and local traders were currently offering farmers US\$ 0.85/lb. Bernsten contacted WFM to discuss options. Because WFM could not offer the farmers the price that they now wanted, we agreed to postpone finalizing the purchase contract and wait until early 2012 to see if the local price declined to level that WFM was willing to pay. If the local price declined, WFM agreed to reconsider purchasing 20 MT of small red beans.

This situation highlights the challenge of getting farmers to agree to contract their bean production at a future price, when the local price of beans is highly volatile and the buyer is only willing to pay what U.S. consumers will be willing to pay. Farmers are used to selling their beans at market price, rather than contracting at a future price. Because farmers expected the price of beans to be close to \$ 0.85/lb in January 2012, they were unwilling to contract for a lower price in May 2011.

## Networking and Linkages With Stakeholders

*Angola:* The project PIs met in Huambo with World Vision. Donovan worked with their marketing officer to modify data collection and dissemination. Students will have access to the database of prices as it develops. Work with that project enables outreach directly to farmer associations in the Planalto Region of Angola. As suggested by the USAID mission in Angola, Donovan met with the Farmer to Farmer director for Angola and discussed potential linkages between the programs on extension messages and market information.

*Mozambique:* Discussions with TradeHub, Agrifuturo, and a private marketing firm (OLAM) focused on two aspects. For TradeHub, the effort was to link South Africa traders and processors to producers and markets in northern Mozambique. With OLAM, the discussions focused on using OLAM buying points for storage and technology dissemination.

*Honduras:* The PIs met with:

1. the leaders and members of the farmer association to discuss the IMO certification process, the certification timetable, the costs of bean production, and the price they required to supply WFM with 20 MT of beans
2. a bean broker in Tegucigalpa to identify steps that had to followed to export the beans and the associated costs
3. a government official to understand the process for obtaining an export permit
4. the bulk commodity purchasing staff at WFM to update them on the costs involved at each stage of the supply chain and to negotiate the price WFM would be willing to pay

## Leveraged Funds

The PIs and collaborators in this project have successfully leveraged external funding for market research, farmer focus groups, and research related in part to agrobiodiversity, seed production, and organic bean production, in part due to the assistance received from the Dry Grain Pulses CRSP.

## Publications

- Artur Paulino, *Potencial comercial de feijão macunde em Angola (Commercial potential for cowpeas in Angola)*. 2011. Thesis for UJES.
- Isabel Chachomba, *Report on Marketing of Common beans in Bazuka Market (Comercialização de Feijões no Mercado Basuka)*. 2011.
- Estevao Chaves. *Interdependência dos Preços de Feijão-Vulgar entre Cinco dos Principais Mercados em Moçambique. (Interdependence of common bean prices among markets in Mozambique)*. MS Thesis for University of Vicosa, Brazil.



# Improving Bean Production in Drought-Prone, Low Fertility Soils of Africa and Latin America— An Integrated Approach

PSU-1

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## Abstract of Research Achievements and Impacts

In the past year significant progress was achieved in the project. Numerous genotypes were screened for root traits conferring adaptation to drought and low soil fertility. These include inbred backcross lines developed at Zamorano and IIAM to introgress superior root traits into elite cultivars and a range of genotypes from Africa and the CIAT core collection. Further evidence was obtained for the value of two novel root traits, basal root whorl number (BRWN) and root etiolation for enhanced P acquisition. A field study in Mozambique showed that bean genotypes with superior root traits conserve soil fertility by reducing soil erosion. Socioeconomic survey data were analyzed, providing useful information for common bean seed preferences based on a broad representation of farmer households across the villages; these will be supplemented in Phase II with PVS activities at the sites. Survey data also include network data to conduct analyses of diffusion of bean seeds through trader networks, through seed sharing networks operative at the village level, and through the design and greater use of information networks that can be adapted to the needs of local farmers. Master's degree training continued for Malawian bean breeder Virginia Chesale, and IIAM researcher Samuel Camilo began his intensive English training at Penn State.

## Project Justification and Objectives

This project is premised on four well established facts:

1. Drought and low soil fertility are principal, pervasive constraints to bean production in Latin America and Africa.
2. The vast majority of bean producers in poor countries cannot afford irrigation and intensive fertilization.
3. Bean genotypes vary substantially for root traits that determine their tolerance to drought and low soil fertility, making it feasible to increase yields in low-input systems through genetic improvement.
4. To exploit the potential of this approach, we need intelligent deployment of root traits in bean breeding programs and better understanding of the socioeconomic and agroecological factors determining the adoption and impact of stress tolerant crops and cropping systems.

Drought and low soil fertility are primary constraints to crop production throughout the developing world, especially for common bean, which is typically a smallholder crop grown in marginal environments with few inputs. Phosphorus limitation is the most important nutrient constraint to bean production, followed by the acid soil complex of excess Al, excess Mn, and low base supply. The importance of nutritional stress in bean production systems of Latin America and Africa cannot be overstated. Fertilizer use is negligible in many developing countries, especially sub-Saharan Africa, which generally have the poorest soils. Integrated nutrient management, consisting of

judicious use of fertility inputs, management practices to conserve and enhance soil fertility, and adapted germplasm capable of superior growth and yield in low fertility soil is needed.

We have shown substantial variation in stable bean P efficiency across soil environments in Latin America. Analysis of the CIAT germplasm collection identified several sources with outstanding P efficiency—from 100 to 200 percent better than existent checks, such as Carioca. Studies with these genotypes identified distinct root traits that contribute to P acquisition through topsoil foraging, including root hair length and density,

adventitious rooting, basal root shallowness, and traits that reduce the metabolic costs of soil exploration, such as root etiolation and root cortical aerenchyma.

Genetic variation for these traits is associated with 30 to 250 percent variation in growth and P uptake among related genotypes in field studies. Several of these traits can be evaluated in rapid screens with young plants, greatly facilitating breeding and selection.

Drought is a primary yield constraint to bean production throughout Latin America and Africa. Beans vary substantially in drought tolerance, due to variation in root depth and seed filling capacity. Drought tolerance has been identified in several races of common bean, but is complex and associated with local adaptation. Utilization of specific traits in drought breeding through direct phenotypic evaluation or genetic markers (e.g., QTL) would be useful.

Genotypes that are more responsive to inputs may promote the use of locally available inputs in improved integrated crop management systems. Several African countries have reserves of sparingly soluble rock P whose effectiveness may be improved by the use of nutrient-efficient bean genotypes. Because beans are superior to maize in solubilizing P in their rhizosphere, the introduction of bean genotypes with superior root systems may enhance the utilization of rock P, thereby improving P and N availability (through symbiotic N fixation) in maize-bean systems. Similarly, bean genotypes with deeper root systems may be synergistic with soil management to conserve residual moisture. Our project will test these hypotheses.

A better understanding of socioeconomic factors determining adoption of stress tolerant bean germplasm and the effects such adoption may have on household income and nutrition is needed. Family structure may play a role in determining whether the introduction of more productive germplasm will have positive or even negative effects on household income and nutrition.

Recent developments in our understanding of root biology make it possible to breed crops with greater nutrient efficiency and drought tolerance. Such crops will improve productivity, enhance economic returns to fertility inputs, and may enhance overall soil fertility and system sustainability, without requiring additional inputs. The overall goal of this project is to realize the promise of this opportunity to substantially improve bean production in Africa and Latin America.

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*Drought is a primary yield constraint to bean production throughout Latin America and Africa.*

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## Objectives

1. Develop bean genotypes with improved tolerance to drought and low P.
2. Socioeconomic Studies.

## Research and Outreach Approaches, Results, and Achievements

### Objective 1: Develop bean genotypes with improved tolerance to drought and low P.

Several specific root traits that enhance bean productivity under drought and low fertility stress have been identified. Genetic improvement, of these traits, would improve bean production in Africa and Latin America.

The activities under this objective include collection of germplasm, phenotyping root traits, screening root traits for low P/drought tolerance, introgression of root traits into elite lines in Africa and Latin America, and evaluation and development of low P/drought tolerant varieties for farmers using PBV and PVS.

Bean germplasm from regional landraces, improved cultivars, and advanced lines will be collected from various breeding programs in Africa and Latin America: CIAT, SABRN, BILFA, and BIC and systematically screened for key root traits, including root hair length, root hair density, BRWN, basal root growth angle (BRGA), and adventitious rooting. Phenotypic screens will be conducted under controlled conditions and also as field root crown evaluations. The Latin America germplasm will also include landraces and improved lines from the Mesoamerican and Andean gene pools of *Phaseolus vulgaris* and interspecific lines from *P. vulgaris* x *P. coccineus* crosses.

Introgression of root traits conferring greater drought tolerance and P efficiency will be accomplished by developing inbred backcross (IB) populations from breeding lines that combine key root traits with multiple disease resistance and preferred seed types in the target regions. The initial cross will be made between the recurrent parent and the donor parents, followed by two backcrosses to the recurrent parent and three generations of selfing by single seed descent to develop IB populations.

Field selection will be based on the average performance of advanced IB lines in replicated drought and low P trials, complemented with field and greenhouse evaluations of root traits. Selected lines will be tested individually or in multiline combinations. Testing locations will be in Mozambique and Honduras. Selection for some disease resistance will be conducted in the field. Advanced lines will be evaluated in Malawi, Nicaragua, and Haiti.

Participatory plant breeding (PPB) and PVS approaches will be used in the field trials to evaluate the performance of the IB lines under drought/low P, agronomic adaptation, and commercial seed types. Participants in the value chain of common beans (production, processing, commercialization, and export) in the target countries will be invited to participate in these evaluations.



### *EAP/Honduras: Activity 1.1 Breeding Mesoamerican bean lines with greater tolerance to drought and low P availability*

A set of 50 inbred-backcross (IBC) lines were selected from three populations (of 310 lines) under drought and low P conditions from greenhouse and field trials in Honduras. Selection was based on plant architecture, disease resistance, maturity, vegetative and reproductive growth, yield and seed color, and shape and size of these lines. These IBC populations were developed by crossing the small red cultivar Amadeus 77 (recurrent parent) with lines selected at PSU having root traits associated with better adaptation to drought or low P stress conditions.

These 50 IBC lines were evaluated under greenhouse conditions and root traits were phenotyped by visual counts and observations of whorl number, basal roots number, and angle; root density, surface area, volume, and length were also determined. These 50 IBC lines were sent for root phenotyping at PSU; specific lines with contrasting root characteristics under low P greenhouse conditions were identified for further studies.

A selected set of 20 IBC lines was screened during the primera raining season under field conditions without fertilization in a low P soil at Zamorano. These lines were evaluated for plant growth, root traits (whorl number, basal root number, and angle) and nodulation at flowering, and seed yield and seed size at maturity. A small group of six IBC lines similar to the recurrent parent Amadeus 77 will be screened under farmer field conditions to determine their performance under different soil fertility and moisture conditions.

A set of 18 lines including drought and low fertility tolerant lines and two check cultivars (Amadeus 77 and the landrace Seda) were evaluated under field conditions at Zamorano in low P soil without fertilization. Plant dry weight, root whorl number, basal root number and angle, and nodulation were determined at flowering; seed yield and seed size were determined at maturity. Superior genotypes from this study will be used as donor parents for improving tolerance to drought and low fertility in small red and black commercial bean cultivars. The same set of 20 lines is being characterized for root hair density under greenhouse conditions to determine the importance of this trait under limited moisture and low fertility soil conditions. The importance of root hair density on rhizobia nodulation is being characterized.

The same low P soil plot at Zamorano was used to test a set of 20 small red and black bean improved cultivars without fertilization that are frequently grown under drought and low fertility soil conditions. Selected cultivars having superior performance under low fertility and drought will be used to identify elite parental genotypes for developing superior small red and black bean cultivars in crosses with the donor parents identified above.

Breeding lines developed under this project in previous years using the inbred backcross method are currently in advanced testing on farmer fields. At least one cultivar from this group of advanced lines will be released next year in Honduras and/or Nicaragua.

*IIAM/Mozambique and PSU: Activity 1.2. Evaluation and selection of bean genotypes with root traits adapted to drought and low P availability*

Low phosphorus (P) availability and drought are major constraints to common bean production in many developing countries. Root traits influence P and water acquisition from the soil. Genotypes with shallow roots, many basal root whorls, adventitious roots, and basal roots have advantages in acquiring P from low P soils, while genotypes with deeper basal roots and longer primary roots acquire water from deeper soil horizons. More than 160 bean genotypes from CIAT were screened in 2010 and 2011. The experiments were planted in a randomized complete block design with four replications in Mozambique and PSU. The number of basal root whorls and basal roots were evaluated eight days after planting in the laboratory and 45 days after planting in the field. Field evaluations included adventitious and basal root number; BRWN; length and branching of the adventitious, basal, and primary roots; number of nodules; and scores of the root rot infection. We found large variation in the number of adventitious and basal roots, and the whorl number among genotypes. Variation in root density (branching) and root length of adventitious, basal, and primary roots and the number of nodules was detected. Sources of tolerance to drought and low P adaptability were identified for potential use in breeding programs to develop bean genotypes adapted to specific stress. We identified 29 genotypes with a potential of up to 13 basal roots per plant, and 31 genotypes with long and deeper roots for drought tolerance. Figure 1 illustrates examples of root traits of genotypes adapted to drought and low P conditions. Root traits conferring adaptability to drought include deeper roots, while genotypes with shallower roots, numerous adventitious and basal roots, and long and dense root hair have advantage in acquiring P in the topsoil.



a) Genotype with two whorls and deeper basal roots



b) Genotypes with three whorls and deeper basal roots

**Figure 1.** Example of a genotype with few basal root whorls (a), and genotype with many basal roots (b) evaluated in the field.

### *Activity 1.3. Identification of common genotypes adapted to drought*

Twenty-one bean genotypes introduced from CIAT and local germplasm, including genotypes known to be drought tolerant, were used in this study. A field trial was conducted at the IIAM Agricultural Research Station in Chokwe, Mozambique, in 2011. Genotypes with two and three basal root whorls were also included. The experiment was planted in a RCBD with four replications. Seeds of each genotype were sown in three rows of five m. Weed and pest management and irrigation were applied as needed. Collected data included days to flowering and maturity, number of pods per plant, weight of 100 seeds, and yield. Preliminary results showed significant differences among genotypes in number of pods per plant, which varied from 17 to 44.8. Tio Canela and BAT 477 had 44.8 and 44.6 pods per plant, respectively. G 19833 had an average of 31.7 pods per plant. Tio Canela and BAT 477 are drought tolerant genotypes and previous studies showed that these genotypes have deeper basal roots. The control local varieties Bonus and Khaki had 28 and 26.2 pods per plant, respectively.

### *Activity 1.4. Development of P-efficient common bean genotypes and advance generations of selfing*

Crosses using parents contrasting in root hair traits were made to incorporate good root hair traits into adapted bean varieties. Our results showed genetic variation in root hair traits and basal root whorl number within populations. Selected lines will be tested in 2012 for adaptability and yield performance under drought and low P availability in Mozambique.

### *Activity 1.5. Phenotypic profiling of root traits of six genotypes tolerant to drought*

Bean genotypes with deep and steeper roots have advantages in acquiring water from deeper soil horizons. Six genotypes were planted in a RCBD with four replicates in Chokwe in 2011. Root architectural traits were evaluated 45 days after planting and soil cores were collected in three different horizons.



Variation in adventitious, basal, and primary root traits was found among genotypes. BAT 477, Tio Canela, and SEQ 1003 had 67.5°, 62°, and 57° basal root growth angle (table 1), respectively, corresponding to deep basal roots. These genotypes could acquire water from deeper soil strata under drought stress. The number of basal root whorls varied from two to four, while the number of basal roots varied from seven to 14. We also identified sources of tolerance to low P availability in local germplasm collected in Lichinga. Lines LIC-04-2-1 and LIC-04-3-1 had more whorls and basal roots, and shallower roots conferring adaptability to low P conditions. We collected other data to evaluate yield performance. We are drying the seed of these genotypes to measure yield performance and compare with root architecture. The roots from the soil core samples will be evaluated and compared with basal root growth angle.

Genotype	Basal root growth angle	Basal root number	Basal root whorl number	Adventitious root number
BAT 477	67.5a*	7.2c	4a	5.5b
Tio Canela	62a	7c	3b	6.25b
SEQ 1003	57a	8.25bc	3b	4.25b
Bonus	37b	10.5b	2.5c	8ab
LIC-04-3-1	27b	14a	2c	12.5a
LIC-04-2-1	26b	11b	2c	7.5ab

\*means followed by the same letter are not statistically different.

**Table 1.** Root traits of six common bean genotypes evaluated in Chokwe 2011. The data are an average of four replications measured 45 days after planting.



#### Activity 1.6. Seed increase

Seeds of different genotypes were increased in Chokwe and Sussundenga in 2011. The objective is to multiply seed for future research and promising lines and to maintain common bean germplasm.

#### Activity 1.7. Utility of BRWN for breeding P efficient lines

We conducted a field study on the functional utility of basal root whorl number for phosphorus acquisition efficiency in common bean. Six contrasting RILs derived from G2333 x G19839 were used in the experiment and were grouped in two root categories: genotypes with two whorls and genotypes with three whorls. A randomized design was used in the field experiments. Each treatment had four replications of each of three genotypes per BRWN category (two vs. three whorls).

The field study was carried out in Mozambique at the Sussundenga Research Station in Manica Province. The soil type is an Ustox with low pH and low organic matter. Three months before planting, the soil was limed to bring its pH to 6.2. Seeds were inoculated with rhizobia inoculum, on the day of planting. The experiment had phosphorus and low phosphorus treatments. All other nutrients were kept optimal through chemical fertilization. Simple superphosphate was the source of additional P for fertilized plots, applied at the rate of 100 kg per ha.

To evaluate root distribution with soil depth, we took cores in the field at 28 DAP, which consisted of extracting soil samples by hammering five-cm diameter metal cylinders vertically into the soil. Soil sections were then separated from the 0–15 cm depth and the 15–30 cm soil depth. Root fragments were recovered from each of these soil sections and scanned to determine root length from the cores.

Collected data included shoot dry weight, total root length, total leaf surface area, basal root whorl number, basal root number, and total phosphorus content. Plant samples were collected at 14, 21, and 28 days after planting (DAP). Shoot biomass was determined from samples dried at 60° for five days. Tissue phosphorus content was determined. During shoot sampling, leaf discs (6.6 cm<sup>2</sup>) were collected from five fully expanded leaves. The ratio of dry weight to area of these disks was used to estimate total shoot leaf area from total leaf dry weight. At plant sampling, roots crowns were excavated and placed in a 20-liter container with soap for washing. Detergent was added to the water used to wash the roots to help separate roots from soil particles without significantly damaging the root system or losing fine roots. Root samples were washed and rinsed in clean tap water and placed in vials with 25 percent ethanol solution for preservation. Roots were scanned and the images were analyzed for total root length and root length in each of the root classifications by root diameter. Although genotypes were selected based on their basal root whorl number, plants harvested at 14 DAP were re-assessed for basal root whorl number to confirm the root phenotypes. The evaluation consisted of selecting and excavating three representative plants from each replication, determining BRWN and BRN by counting root whorls and basal roots, and calculating the average value for each replicate.

Variation in BRWN and BRN was evaluated and was in accordance to whorl classes of the genotypes. Data analysis showed variation in shoot dry weight among genotypes with either medium or low phosphorus availability; however, the whorl class effect was greater in low phosphorus treatments than in high phosphorus. When genotypes were grouped by BRWN, statistically significant differences were detected between the two whorl classes under low phosphorus availability (fig. 2). Genotypes with three whorls showed statistically greater shoot dry weight compared to those with two whorls (fig. 3).

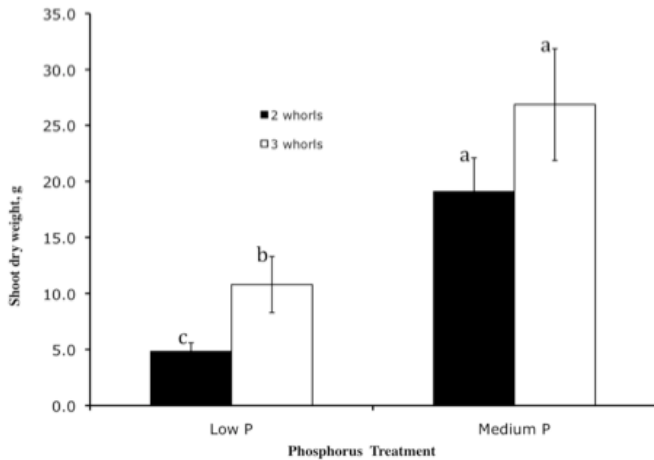


Figure 2. Shoot dry weight of genotypes with two and three basal root whorls (BRWN), grown under low P (5 ppm) and medium P (19 ppm) in the field. Plant samples were harvested at 28 DAP. Under low phosphorus, statistically significant differences were observed between the two root categories. Root categories did not differ in medium phosphorus treatments.

Under low P availability, two of three genotypes with two basal root whorls showed significantly less leaf area than genotypes with three basal root whorls. Statistical analysis showed significant differences in seed weights and interaction between whorl and weights, with F value =13.3 and 7.3, respectively.

Genotypes with two whorls had a 33.2 percent reduction in tissue phosphorus content when grown under high phosphorus availability while under low phosphorus; the two-whorled genotypes had a 46.5 percent reduction in tissue phosphorus content compared to genotypes with three whorls (fig. 3).

BRWN and BRN showed differences among genotypes in accordance with their respective whorl classes. Phosphorus treatment did not alter BRWN or BRN.

Genotypes were also evaluated for root hair length under both low and high phosphorus availability. All genotypes had greater root hair length under low phosphorus availability compared to high phosphorus availability, but there were no statistically significant differences among genotypes within low or medium phosphorus treatments.

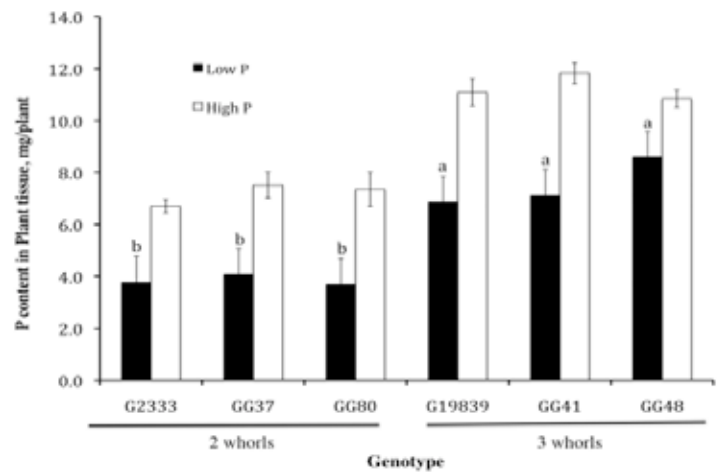


Figure 3. Phosphorus content among six genotypes contrasting for basal root whorl number (BRWN), grown under low phosphorus (5 ppm) and medium phosphorus (19 ppm) in the field. Statistically significant differences for phosphorus content were observed among genotypes grown under both medium and under low phosphorus availability. In low phosphorus treatments, statistical analysis showed that genotypes with three whorls showed significantly greater phosphorus content in plant tissue compared to genotypes with two whorls.

We collected soil cores at two depths in the field at 28 DAP to analyze root length distribution with depth. Root length in the cores collected at 0–15 cm depth showed statistically significant differences between whorl classes. Genotypes with three whorls showed more roots in the top 15 cm than genotypes with two whorls, while genotypes with two whorls had greater root length in the 15–30 cm segments. Total root length from soil cores (0–30 cm depth) was greater in genotypes with three whorls compared to genotypes with two whorls, which suggests that genotypes with an increased number of basal root whorls have a more extensive root system (in terms of total root length) compared to genotypes with fewer whorls. Results of data analysis for relative root length among genotypes showed that genotypes with three whorls showed greater relative root length compared to genotypes with two whorls under both low phosphorus and medium phosphorus availability. Differences in number of adventitious roots were observed among genotypes but were not influenced by phosphorus availability.

These results suggest that BRWN has the potential to improve plant performance under low phosphorus conditions not only by promoting an increase in BRN but also by enabling a more dispersed root system capable of maximizing the soil volume being exploited by the plant. The positive effect of BRWN on total leaf area, shoot dry weight, and shoot phosphorus content suggests that this trait helped the plants acquire phosphorus and other nutrients from the soil, enabling them to grow large and vigorous leaves for photosynthesis.

The variation in root hair length is less likely to be related to the basal root whorl number or total number of basal roots that a given genotype may have.

Adventitious rooting is important for topsoil foraging in common bean. Data analysis on adventitious roots among

genotypes showed no correlation with variation in BRWN; however, there was an increase in the number of adventitious roots in the low phosphorus treatment in all genotypes regardless of BRWN.

The differences observed in root length in the top 15 cm of the soil show that genotypes with three whorls had more shallow root distribution compared to genotypes with two, which confirms that the more whorls a genotype has, the more shallow its root system. Therefore, the main benefit of having an increased number of whorls is not the increased value of total root length but a more dispersed root system, with the roots from the upper whorls shallower. These plants then exploit a greater total volume of the soil and, in particular, more of the top soil, facilitating a top-foraging process, since shallower basal roots allow more intense topsoil foraging, which may be advantageous in P-limited environments since phosphorus availability in most soils is greatest in surface horizons.

There are several lines of evidence suggesting that shallower basal root growth enhances topsoil foraging and phosphorus acquisition efficiency. Deeper roots formed from the lower whorls can be important for drought tolerance, since one of the root characteristics for drought tolerance is the existence of deep roots in beans. This suggests that the number of basal roots is important not only for nutrient acquisition but also for drought tolerance.

The screening for basal root whorl number among Recombinant Inbred Lines (RILs) demonstrated the variability of this root trait among related genotypes. Our results show that genotypes with more root whorls have the ability to form more basal roots, and these basal roots are formed with a greater range of angles of insertion from the main primary root, which enhances the volume of soil exploited by the plant. The existence of variation in BRWN in common bean suggests that a breeding program can be devoted to developing genotypes with greater numbers of basal whorls that can perform well in conditions of low phosphorus availability.

#### *Activity 1.8. Utility of BRWN for drought tolerance in common bean*

In this activity we tested the value of increasing BRWN for drought tolerance in two field studies in 2010: one in Rock Springs, Pennsylvania, and one at the Ukulima Root Biology Center (URBC) in South Africa. This summary will focus on the Rock Springs' study.

Rain-out shelters at Rock Springs were used to impose a terminal drought treatment. Sensing precipitation, shelters were covered with clear greenhouse plastic film and moved over the drought treatment plots, reversing the direction to expose the plots at the end of the rainfall. Adjacent plots without shelters served as controls. Block 1 received 100.33 mm of precipitation/drip irrigation before imposing drought at 22 DAP, and Block 2 received 78.24 mm before imposing drought at 21 DAP. The control plots continued to receive precipitation/drip irrigation through the final harvest at mid-pod, Block 1 receiving 193.05 mm and Block 2 134.12 mm.

The trial design at Rock Springs was arranged as a split-plot for the water treatment. Each BRWN phenotype group (1, 2, 3) was

represented by four genotypes of the ALB RIL population, with five extra genotypes for comparison. The in-row spacing was 0.10m and the between row spacing 0.60m to mimic the planting practice in Latin America.

Twenty genotypes from two RIL populations ALB and DOR364 x G19833 were used for these studies. The ALB RILs were planted in the trial at Rock Springs. Both population sets were planted at the trial at URBC.

At both sites a TDR100 system was used to monitor soil volumetric water content (VWC) over time. At the trial in Rock Springs, soil VWC was collected continuously from Block 1. Soil cores for soil water content were taken at both sites to compare with the TDR-100 measurements.

At Rock Springs, variation in secondary growth of basal roots was measured over time. Five samples from four genotypes were harvested for root crown evaluation beginning at 13 DAP and continuing every seven days for four total harvests until R5 (42 DAP). Root crown evaluation included BRWN, BRN, the number of dominant basal roots, diameter of each basal root, diameter of a representative adventitious root, and the tap root diameter.

At the Rock Springs site, root crowns were evaluated at R5 (Block 1 42 DAP, Block 2 43 DAP) and R7. At both sites and harvest times the root crown evaluation included BRWN, BRN, the number of dominant basal roots, diameter of each basal root, the average basal whorl angle, and a compensation rating between the adventitious, basal, and tap root classes. The harvests at Rock Springs also included the diameter of a representative adventitious root and the tap root diameter.



Root distribution in the soil profile was measured from soil cores taken at the Rock Springs site in Block 1 at both R5 (48 DAP) and R7 (72 DAP) stages, and at the URBC site taken in all blocks at both R5 (only control) and R7 stages. At the Rock Springs site, two genotypes from each BRWN category, and at the URBC four genotypes from both populations were chosen for sampling. Three subsample cores were taken per plot.

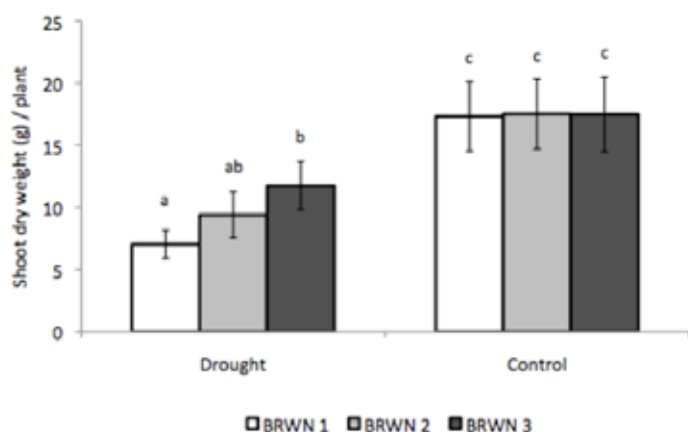
At the Rock Springs site several measurements were taken to determine the water status of the plants in the control and drought treatment plots. Before the R5 harvest, the predawn water

potential was measured. Four samples from four genotypes were measured from both control and treatment plots. The fourth or fifth trifoliolate was used for this measurement.

Leaf relative water content (RWC) was measured before imposing drought and again after the harvest at R5 and the harvest at R7. Two genotypes per phenotype were selected and sampled from both the well watered control and drought treatment areas.

Leaf area and shoot branching were measured before the harvest at R5 at Rock Springs to measure the effect of water deficit. At the URBC, after the harvest at R7, leaf area, branching, and number of pods indicating flower abortion were measured.

Greater BRWN was correlated with greater shoot growth of common bean under terminal drought. At R5, after 19 days of imposed drought, the plants under water stress had significantly less shoot dry weight than the well watered control. The well watered plots had no difference in shoot dry weight among the three BRWN phenotype groups, yet under terminal drought, genotypes with three whorls had 70 percent greater shoot dry weight than genotypes with one whorl (fig. 4). By R7 there were no differences in shoot growth among BRWN groups. Genotypes with two whorls did have a greater pod partitioning index than genotypes with one whorl in drought, and had pod weight in drought comparable to genotypes with three whorls under well watered control.



**Figure 4.** Genotypes with greater basal root whorl number (BRWN) had greater shoot dry weight at R5 under terminal drought conditions in the trial at Rock Springs, PA, in 2010. Genotypes with one whorl had significantly less shoot dry weight than genotypes with three whorls ( $p < 0.001$ ). Genotypes with two whorls were not different from genotypes with one ( $p = 0.0878$ ) or three whorls ( $p = 0.0949$ ). (Tukey multiple comparison analysis, columns with different letters are significantly different.) Columns represent the mean of four replicates  $\pm$ SE.

#### Other Research Activities

1. Evaluation of 43 bean genotypes for performance under low phosphorus availability. These evaluations identified a number of genotypes with yields greater than 1.5 MT/ha, which can preliminarily be considered phosphorus efficient genotypes.
2. Evaluation of 32 BILFA genotypes for performance under low phosphorus availability. Results of these evaluations have identified three genotypes with yields greater than 1.5 MT/

ha, which can preliminarily be considered phosphorus efficient genotypes.

3. Evaluation of 69 bean genotypes for performance under low phosphorus availability. Results of these evaluations have identified 39 genotypes with yields greater than 1.5 MT/ha, which can preliminarily be considered phosphorus efficient genotypes.
4. Seed increase of promising materials for P efficiency has been conducted in Sussundenga and Rotanda fields.
5. Biofortified bean lines have been evaluated for P acquisition efficiency in the fields of Sussundenga Research Station.

## Objective 2: Socioeconomic Studies Phase II

Phase II project activities build on Phase I survey research to understand constraints to adoption, income, and nutrition potential for households and intrahousehold impacts. Activities include:

1. engagement of farm households in PVS at our research sites,
2. on-farm testing followed by a farm household survey to determine critical constraints hindering adoption or reducing the diffusion of improved seed, including access to seed systems,
3. inclusion of survey questions specifically focused on disposition of newly-adopted beans (sales in alternative markets across supply chains, household consumption) by households, and
4. inclusion of both male and female perspectives in the survey to estimate intrahousehold impacts.

The economic network approach used in Phase I will be used to estimate the village-wide impacts of stress-tolerant germplasm. The use of this approach in Phase II allows for a short-run ex-ante/ex-post comparison, focusing on adoption constraints and impacts.

The farm household survey protocol for Phase II was developed and translated into Portuguese. The draft survey includes questions related to:

- constraints (agroecological, economic, social) to adoption and diffusion
- impacts on household income and nutrition attributable to beans
- intrahousehold impacts
- questions related to markets for beans
- seed sharing

In May, 2011 questions were pretested at two research sites near Sussundenga (Munhinga and Rotande). Both qualitative and quantitative survey approaches were used to assess villager understanding. The qualitative approach was used to understand seed sharing and constraints to adoption and diffusion.

All mapping of the relevant networks is now complete and includes male (i.e., male-to-male) and female networks (separately) as well as household-to-household networks without differentiation on the basis of gender. Simulations are being

developed to better understand the overall impact of the new technology on the bean-growing regions of Mozambique.

Collaboration with Michigan State University in the past year included sharing of GPS data on the eight study sites and meeting with the socioeconomic PIs in Maputo. In May 2011, the socioeconomic team met with USAID personnel in Maputo to describe progress on our work and seek their input.

## Networking and Linkages with Stakeholders

### *Central America*

During the current fiscal year, several advanced breeding lines tolerant to drought and low fertility developed by the project were distributed for testing in various Central American and Caribbean countries through the Bean Research Network. A similar group of lines was tested by farmer groups involved in the Participatory Plant Breeding Program for the Mesoamerican Region funded by the Norwegian Development Fund. From these groups of project lines, two cultivars have already been released in Honduras and are used by farmers and at least one cultivar will be released during FY12 in Honduras, El Salvador, and Nicaragua. These locally released cultivars are being validated and disseminated through the Bean Technology Dissemination Project from the DGPC/USAID in Honduras and Nicaragua. In addition, two promising abiotic tolerant lines have been included in the drought and heat tolerant bean lines trial distributed to national programs of CA/C for testing under the Bean Adaptation to Climatic Change in Central America and the Caribbean Project funded by the International Development Bank through the Red SICTA/IICA. All these trials are organized and distributed by Zamorano.

## Leveraged Funds

Dr. Lynch and his collaborators have successfully leveraged more than \$4,500,000 in external funding for research related to root biology and technology diffusion, in part due to the assistance received from the Dry Grain Pulses CRSP and the Bean/Cowpea CRSP.

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Nord, E.A., C.C. Zhang, J.P. Lynch. 2011. Root responses to neighboring plants in common bean are mediated by local nutrient depletion rather than self/non-self recognition. *Functional Plant Biology*, <http://dx.doi.org/10.1071/FP11130>.

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# Modern Cowpea Breeding to Overcome Critical Production Constraints in Africa and the U.S.

UCR-1

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## Abstract of Research Achievements and Impacts

Progress was made in three areas under the stated goal, “Develop improved, pest resistant, and drought tolerant cowpea varieties for target regions in sub-Saharan Africa and the United States.” The final testing and release of cowpea varieties follow:

- In California, the newly released blackeye cowpea CB50, with a larger, brighter white seed and which is sold by several warehouses as a premium export class, had production acreage increased in both 2010 and 2011. Four on-station evaluation trials for grain quality, yield, disease, and insect resistance were again positive in 2011 for the elite, novel, dry grain, all-white cowpea line.
- In Senegal, the line ISRA-2065, with thrips and aphid resistance, was released in 2011 under the name *Pakau*. Four hundred kg of foundation seed was produced in 2010 and an estimated 500 kg is expected in 2011. Following the advanced multilocation yield trials and on-farm tests, up to three new lines will be proposed for release as varieties in Senegal based on grain quality, yield, and disease and insect resistance.

Progenies of 28 to 40 crosses for developing new breeding lines were tested in Burkina Faso, Senegal, and California to combine high yield, grain quality, and abiotic and biotic stress resistance traits. Most crosses were advanced to F5 stage in 2011. Under the seed production and delivery systems objective, the following was achieved:

- In Burkina Faso, breeder seed of seven improved varieties was grown at Saria. Seven varieties of foundation seed were produced at Saria, Pobe-Mengao, and by individual farmers and farmers’ organizations. More than 50 MT of seven varieties of foundation seed will be sold to certified seed producers in 2012. Another group of 85 lead farmers were trained as certified seed producers.
- In Senegal, three ha each of Melakh and Yacine and one ha of Pakau foundation seed was produced at Bambey to supply the EWA NGO seed producer network. Sixty ha each of Melakh and Yacine Certified Seed was produced by farmers in 2010, much in the Touba Toul, Mekhe, and Merina areas where women and men farmer groups were trained in seed production. Certified Seed production and training focused on farmer organizations. Some seed was also produced at Dahra by a private group.
- A student from Angola completed the MS degree training in cowpea breeding and returned to Angola to aid in seed production and distribution system assessment. Multilocation trials of diverse cowpea lines were completed in Angola.

## Project Justification and Objectives

The primary project focus is to:

1. increase the productivity of African and U.S. cowpea producers through improved varieties with resistance or tolerance to the major abiotic and biotic stresses impacting production in these areas

2. expand grower marketing opportunities by breeding cowpea varieties with desirable grain characteristics
3. help ensure adequate seed of improved cowpea varieties

Genomics and modern breeding methods will be used to improve cowpea for yield-limiting constraints, with leveraging of genomic resources developed under a complementary cowpea project.



*Increasing Cowpea Productivity.* Low agricultural productivity is central to rural and urban poverty in Africa. On-farm cowpea yields in West Africa average 240 kg/ha, although potential yields are five to 10 times greater. Drought, poor soil fertility, insect pests, and diseases are major constraints. Cowpea varieties that yield more without purchased inputs especially benefit poor farmers, many being women who lack access to the most productive lands. Productivity is central to increasing rural incomes because less land, labor, and capital are then needed. The extra resources can then be invested in other activities to help boost total family income. Sustainable increases in cowpea productivity in Africa and the U.S. can be achieved by developing varieties with resistance to insects, nematodes and pathogens, drought tolerance, and the ability to thrive under low soil fertility.

*Increasing Marketing with Improved Varieties.* New cowpea varieties must have features desired by consumers and farmers, including grain appearance and desirable cooking and processing qualities for specific products. Landrace grain types are often preferred locally; if over-produced, prices offered to farmers can be low due to limited demand. Large, white grains with rough seed-coat are preferred throughout West Africa and can be marketed over a wide area, buffering supply (and prices) in the region. Large, white grains are also amenable to direct dry milling for use in value-added foods (e.g., akara, moin-moin) and prototype, value-added products. Development of adapted cowpea varieties with large, white grain and resistance to pests would increase the marketing opportunities of cowpea farmers and traders. There is also considerable demand for large, rough-brown seed type, especially in urban centers in Nigeria, but the standard rough-brown Ife Brown variety is susceptible to pests and diseases. Other new cowpea products are based on a sweeter and milder taste that could broaden cowpea consumption.

*Increasing Seed Supply of Improved Varieties.* Cowpea breeding by the Pulse CRSP, African NARS, and IITA (Senegal, Burkina Faso, Nigeria, and other countries) has led to improved varieties that are near release. However, only about five percent of the cowpea area in Africa is planted to improved varieties and their potential goes largely unrealized. Experience with improved common bean indicates rural African farmers will buy seed when available, indicating a probable market for cowpea seed.

Recently, effective models for production and dissemination of improved cowpea seed have evolved in Burkina Faso and Senegal, based on collectives and for-profit seed cooperatives, but a limited scope reflects insufficient quantities of breeder and foundation seed. We propose to help support increased production of breeder seed and work with producers of foundation seed to strengthen their production and marketing.



## Objectives

1. Develop improved, pest resistant, and drought tolerant cowpea varieties for target regions in sub-Saharan Africa and the United States using modern plant breeding tools.
2. Strengthen cowpea seed production and delivery systems in Angola, Burkina Faso, and Senegal to ensure delivery of improved varieties.
3. Technology dissemination (seed of improved cowpea varieties in West Africa).

## Research and Outreach Approaches, Results, and Achievements

### **Objective 1: Develop improved, pest resistant, and drought tolerant cowpea varieties for target regions in sub-Saharan Africa and the United States using modern plant breeding tools.**

Three main paths of work are being followed to achieve our research objective. We are completing final testing and release protocols of lines developed under the Bean/Cowpea CRSP, and other germplasm are in development. We are also initiating new short- and long-term breeding strategies to develop high-yielding, improved varieties.

### *Final Testing and Release of Varieties*

Several advanced breeding lines developed under the previous Bean/Cowpea CRSP at UCR and in Burkina Faso and Senegal have been released or are nearing release pending final evaluation.

*In Burkina Faso*, the six varietal candidate lines developed at INERA were planned for on-farm trials by five farmer groups at 10 sites in central (Saria, Nandiala, Donsin, Laongo, Manega) and northern (Pobe, Pissila, Titao, Pathiri, Gourcy) zones. The six new varieties were compared to a local variety in each site.

*In Angola*, cowpea field evaluations were conducted at three locations targeting the main ecological zones—Alto Capaca-Benguela, Cela-Kwanza Sul, and Mazozo-Luanda—to identify candidate varieties among local landraces and Bean/Cowpea CRSP and IITA varieties. The trials included 26 CRSP entries and about 26 local types. One or more of these candidates will become the first varieties for each of the production zones formally produced under the project. The Angolan materials were SNP-genotyped to enable association mapping comparisons for major mapped cowpea traits.

*In California*. One advanced all-green pinkeye line and one all-white line were tested in on-station trials in 2010 and advanced to large-scale strip trials in 2011. Twelve new advanced dry green breeding lines and five all-white lines were planned for replicated trials at two locations in 2011. Twenty-six new crosses involving these 12 lines were made in 2009 and F4 selections from these lines were evaluated in nurseries in 2011.

*In California*, for continued development and testing of new elite blackeye lines, a breeding nursery with several hundred F4 generation blackeye breeding lines was conducted.

*In California*, for development of lygus, nematode, and aphid resistant varieties, lygus resistant breeding lines have been developed and are in different stages of testing. In FY11, a subset of these lines was selected based on their performance in lygus screening trials and evaluated for grain yield and grain damage under lygus protected and unprotected conditions at Kearney. We also initiated a new round of crosses in 2010 for breeding high quality grain varieties with increased resistance to lygus. F4 lines developed from these crosses were screened in an unprotected nursery at Kearney under strong selection for resistance to lygus and for desirable grain quality. We are also breeding an improved version of CB46 with greater resistance to root-knot nematodes derived from IITA breeding line IT84S-2049. Line CB46-57Rk<sup>2</sup> is an advanced (BC6) backcross derived breeding line closely resembling CB46 with greater resistance to root-knot nematodes but with smaller grain size than CB46. This line was crossed with CB46 in 2010 to create the BC7F1. In FY2011 inbred BC7F2 lines were developed, evaluated for resistance to nematodes in laboratory growth pouch assays, and resistant lines increased in the greenhouse. For aphid resistance, breeding lines including 07KA-34 and 07KA-173 were developed (from resistance source IT97K-556-6) that show strong resistance to this pest in resistance screening trials. Following additional aphid resistance phenotyping in 2010, the most resistant lines were crossed with CB46 and CB50 as part of the process of transferring aphid resistance to adapted

varieties. In FY11, the F1s of these crosses were grown in the greenhouse to obtain F2 seed, and the F2 generation was scheduled for aphid screening nurseries at Kearney.

#### *Short-Term Breeding Strategy*

A two-tiered breeding strategy is being followed to meet the immediate and longer term needs of farmers. The short-term strategy uses improved and local varieties having both grain quality and agronomic features, such as appearance, taste, cooking qualities, yield stability, appropriate plant type, and maturity. Defects in local and improved varieties are being improved by breeding in resistance to diseases and pests—and other traits—using a rapid recurrent backcrossing approach.

*In Senegal*, progeny selection and advancement were made to develop varieties with medium to late maturity to cope with the changing crop season length in the northern zones and with the growing interest in cowpea in the south and eastern areas. These materials included thrips resistance, good grain size, and color qualities. For introgressing Striga resistance, Yacine was crossed with a more recent line (IT90K-76) for Striga resistance and Suvita 2 for Macrophomina. In FY11, these crosses were advanced to the BC2F2 and F4-F5 generations. These evaluations were combined with SSR markers for tracking Striga resistance.

*In Burkina Faso*, progeny selection and advancement is being made to develop varieties with increased seed size and important insect, disease, Striga, and nematode resistance traits. The national cowpea plan has stressed exporting surplus cowpea production to neighboring countries.

During FY11, Senegal and Burkina Faso recurrent backcross populations were advanced and inbred to the BC2F3 stage, then greenhouse or field selected based on the target traits for each round of backcrossing. Leaf tissue sampling for DNA extraction, SNP genotyping, and selection based on SNP marker complements were used to aid in the selection for multiple traits.

The California advanced and inbred BC populations were either backcrossed or tested for yield performance during the FY11 main growing season.

#### *Longer-Term Breeding Strategy*

The long-term breeding strategy is to pyramid resistance and grain quality in varieties desired by farmers using crosses between elite parents with complementary parental lines. To develop high performing, drought tolerant varieties, we are using a two-stream recurrent selection approach.

*Stream One* includes biparental crosses between highly drought tolerant lines. Selected drought tolerant families were planted in replicated field trials in Senegal and Burkina Faso in the main 2011 season for initial performance evaluation. *Stream Two* includes a set of popular local cowpea varieties chosen by breeders in Senegal and Burkina Faso for genetic improvement through MAS or MARS and hybridized to sources of thrips resistance and heat/drought tolerance. In 2009, 352 F3 families were screened for performance under post-flowering drought conditions and the seed bulked. The top 100 were re-evaluated

in California in 2010 and shipped to Burkina Faso and Senegal, where they were grown and phenotyped for thrips tolerance and grain production under drought/heat conditions in 2011. Some additional crosses with Yacine and Melakh made in Senegal were advanced by single seed selection to the F6 generation.

*In California*, Pigeonpea GA-1 was tested in 2011 in a large replicated strip trial with three irrigation regimes at Shafter. A four-fold replicated yield trial was conducted at Kearney in 2011 from 10 selections identified in 2010 evaluations.

#### *Final Testing and Release of Varieties*

*In Burkina Faso (INERA)*. Results of two years of testing indicate that among the five tested white-grain lines, KVx 442-3-25 is the most preferred because of its high yield (average of 1250 kg/ha) and large seeds. We will produce breeder seeds and have planned to produce at least 25 tons of foundation seed in 2011.

*In Senegal (ISRA)*. The breeding line ISRA-2065 was developed under the Bean/Cowpea CRSP from a cross between the high-yielding CRSP cultivar Mouride and aphid and thrips resistant local landrace accession 58-77 to develop a cultivar with the yield and stability of Mouride and with resistance to aphids and thrips. ISRA-2065 matures 60 days from planting and has desirable grain quality. ISRA-2065 with thrips and aphid resistance was released in 2011 as *Pakau*. The PADER project multiplied three ha of *Pakau* seeds in 2011, and 500 kg of foundation seed were produced in 2011. Up to three new lines will be proposed for release as varieties in Senegal.

*In Angola*, cowpea field evaluations were conducted at three locations targeting the main ecological zones to identify candidate varieties among local landraces and Bean/Cowpea



CRSP and IITA varieties. This included 26 lines. Results of the 2010 plantings enabled a performance ranking. All the tested Angola lines were SNP-genotyped using the Illumina GoldenGate Assay.

*In California.* Replicated small plot tests of new dry-green blackeye, pinkeye, and all-white varieties were conducted to determine varietal candidates. One advanced all-green pinkeye line and one all-white line were tested in on-station trials in 2010. Yields were disappointing, so a larger set of 20 new dry-green blackeye and pinkeye breeding lines were put into replicated tests at Kearney and Riverside in 2011 (table 1). Yields of all the dry green breeding lines were significantly less than elite blackeye variety CB46 in the Kearney trial. F<sub>2</sub> and F<sub>3</sub> progenies of 26 new crosses involving many of the all-green pinkeye and blackeye lines being tested were evaluated in breeding nurseries at Kearney and Riverside in 2010 and 2011 (table 2).

The all-white breeding line 07-11-557 had grain yields even higher than elite blackeye cultivars in the 2010 tests, potentially indicating that transgressive segregation for yield has been achieved. If 07-11-557 again outperforms the blackeye cultivars, we will initiate release. Line 07-11-557 and three additional all-white lines are being tested in trials conducted in 2011.

	Line	Type	Grain yield-Kearney (kg/ha)	100 grain weight (gms)
1	CB46	Blackeye	2970	21.33
2	10-11-735	Dry-green blackeye	2424	16.57
3	10-11-673	Dry-green blackeye	2248	17.49
4	10-11-664	Dry-green blackeye	2234	17.32
5	10-11-701	Dry-green blackeye	2168	14.70
6	10-11-718	Dry-green pinkeye	2088	17.88
7	10-11-757	Dry-green blackeye	2083	16.24
8	10-11-721	Dry-green pinkeye	2061	10.87
9	10-11-706	Dry-green blackeye	2041	19.46
10	10-11-682	Dry-green blackeye	2006	17.92
11	10-11-725	Dry-green blackeye	1917	18.58
12	10-11-698	Dry-green blackeye	1897	16.97
13	10-11-779	Dry-green blackeye	1861	17.14
14	10-11-662	Dry-green blackeye	1840	15.60
15	10-11-727	Dry-green blackeye	1826	14.23
16	10-11-685	Dry-green blackeye	1786	14.01
17	10-11-719	Dry-green pinkeye	1738	17.94
18	10-11-751	Dry-green blackeye	1690	16.03
19	10-11-656	Dry-green pinkeye	1648	16.64
20	10-11-741	Dry-green pinkeye	1571	18.39
21	10-11-693	Dry-green blackeye	1553	16.97
	LSD(0.5)		423	1.38
	CV(%)		13	4.99

Table 1. Grain yield and size of standard blackeye cultivar CB46 and 21 promising new all dry green blackeye or pinkeye lines tested in replicated trials at Kearney and UC Riverside trials in 2011 and that were used in crosses to generate new lines (data for 2011 UC Riverside Trial not yet available).

	Cross Number	Pedigree	Type	2011
1	2009-013	08-11-70-1 x 08-11-154	Green x Green	F <sub>4</sub>
2	2009-014	08-11-111 x 08-11-154	Green x Green	F <sub>4</sub>
3	2009-015	08-11-153 x 08-11-110	Green x Green	F <sub>4</sub>
4	2009-016	08-11-187-3 x 08-11-65	Green x Green	F <sub>4</sub>
5	2009-017	CB46 x 07-11-350	Blackeye x Green	F <sub>4</sub>
6	2009-018	CB46 x 08-11-70-1	Blackeye x Green	F <sub>4</sub>
7	2009-019	CB46 x 08-11-91	Blackeye x Green	F <sub>4</sub>
8	2009-020	CB46 x 08-11-187-2	Blackeye x Green	F <sub>4</sub>
9	2009-021	07-11-350 x CB46	Blackeye x Green	F <sub>4</sub>
10	2009-023	CB50 x 08-11-49	Blackeye x Green	F <sub>4</sub>
11	2009-024	CB50 x 08-11-60-2	Blackeye x Green	F <sub>4</sub>
12	2009-025	CB50 x 08-11-70-1	Blackeye x Green	F <sub>4</sub>
13	2009-026	CB50 x 08-11-132	Blackeye x Green	F <sub>4</sub>
14	2009-027	CB50 x 08-11-140	Blackeye x Green	F <sub>4</sub>
15	2009-028	CB50 x 08-11-186	Blackeye x Green	F <sub>4</sub>
16	2009-029	08-11-70-1 x CB50	Blackeye x Green	F <sub>4</sub>
17	2009-030	08-11-78 x CB50	Blackeye x Green	F <sub>4</sub>
18	2009-031	08-11-103 x CB50	Blackeye x Green	F <sub>4</sub>
19	2009-032	08-11-106 x CB50	Blackeye x Green	F <sub>4</sub>
20	2009-033	08-11-187-3 x CB50	Blackeye x Green	F <sub>4</sub>
21	2009-034	CB46 x 02053F1	Blackeye x Green	BC <sub>1</sub> F <sub>4</sub>
22	2009-035	CB50 x 02053F1	Blackeye x Green	BC <sub>1</sub> F <sub>4</sub>
23	2009-036	02053F1 x 07-11-350	Blackeye x Green	BC <sub>1</sub> F <sub>4</sub>
24	2009-037	02053F1 x 02082F1	Blackeye x Green	BC <sub>1</sub> F <sub>4</sub>
25	2009-038	CB46 x G747-1	Blackeye x Green	F <sub>4</sub>
26	2009-040	CB50 x G749-1	Blackeye x Green	F <sub>4</sub>

Table 2. Crosses made and advanced for selection of improved dry green varieties

Cross No.	Blackeye Crosses	Current Generation	Generation in 2011
2010-066	CB46 x 09Sh-3-2	F <sub>2</sub>	F <sub>3</sub>
2010-067	CB46 x 09Sh-3-4- sps	F <sub>2</sub>	F <sub>3</sub>
2010-068	CB46 x 09Sh-3-6sps	F <sub>2</sub>	F <sub>3</sub>
2010-069	CB46 x 09Sh-13-6	F <sub>2</sub>	F <sub>3</sub>
2010-070	CB46 x 09Sh-36-2	F <sub>2</sub>	F <sub>3</sub>
2010-071	CB46 x 09Sh-93-3	F <sub>2</sub>	F <sub>3</sub>
2010-072	CB46 x 09Sh-105-2	F <sub>2</sub>	F <sub>3</sub>
2010-073	CB46 x 09Sh-112-6	F <sub>2</sub>	F <sub>3</sub>
2010-074	CB27 x 09Sh-13-6	F <sub>2</sub>	F <sub>3</sub>
2010-075	09Sh-93-3 x CB27	F <sub>2</sub>	F <sub>3</sub>
2010-076	09Sh-113-6 x CB27	F <sub>2</sub>	F <sub>3</sub>
2010-077	524B x 09Sh-13-1	F <sub>2</sub>	F <sub>3</sub>
2010-078	524B x 09Sh-13-6	F <sub>2</sub>	F <sub>3</sub>
2010-079	524B x 09Sh-31-1	F <sub>2</sub>	F <sub>3</sub>
2010-080	524B x 09Sh-36-8	F <sub>2</sub>	F <sub>3</sub>
2010-081	524B x 09Sh-113-10	F <sub>2</sub>	F <sub>3</sub>
2010-082	09Sh-95-8 x 09Sh-13-7	F <sub>2</sub>	F <sub>3</sub>
2010-083	09Sh-36-6 x 09Sh-109-2	F <sub>2</sub>	F <sub>3</sub>
2010-084	09Sh-95-8 x 09Sh-113-12	F <sub>2</sub>	F <sub>3</sub>
2010-085	09Sh-113-4 x 09Sh-95-8	F <sub>2</sub>	F <sub>3</sub>
2010-086	09Sh-113-4 x 09Sh-3-6 sps	F <sub>2</sub>	F <sub>3</sub>
2010-087	09Sh-113-5 x 09Sh-13-6	F <sub>2</sub>	F <sub>3</sub>
2010-088	09Sh-113-5 x 09Sh-31-10	F <sub>2</sub>	F <sub>3</sub>
2010-089	09Sh-113-5 x 09Sh-36-6	F <sub>2</sub>	F <sub>3</sub>
2010-090	09Sh-113-4 x 09Sh-93-1	F <sub>2</sub>	F <sub>3</sub>
2010-091	09Sh-113-1 x 09Sh-93-3	F <sub>2</sub>	F <sub>3</sub>

Table 3. Crosses for development of high yielding, pest resistant blackeye cowpea cultivars. Many of the advanced lines used as parents in these crosses were subsequently included in replicated yield tests in 2011.

*In California*, for continued development and testing of new elite blackeye lines, a breeding nursery with several hundred F<sub>7</sub> generation blackeye breeding lines was conducted in 2010, and the seed of 15 new lines bulked for replicated tests conducted in 2011. Breeding lines, at F<sub>3</sub> generation are under development from 26 crosses between elite breeding lines and existing varieties and breeding lines (table 3).

*In California*, for development of lygus, nematode, and aphid resistant varieties, a range of very promising lygus resistant breeding lines have been developed. Based on results of two trials conducted in 2010, a subset of these lines was selected and evaluated for grain yield and grain damage under lygus protected and unprotected conditions at Kearney and under unprotected conditions at Riverside in 2011 (table 4). We also initiated a new round of crosses in 2010 for breeding varieties with increased resistance to lygus that possess high quality grain. The F<sub>1</sub>s were advanced to the F<sub>2</sub> in 2011. The second trial (unprotected only) had 10 new experimental lines from the 2009 lygus nursery screening program conducted at Kearney. Two lines had yields five to six cwt greater than CB46.

We have developed a breeding line with greater resistance to root-knot nematodes derived from IITA breeding line IT84S-2049: line CB46-57Rk<sup>2</sup>, which is an advanced backcross derived breeding line closely resembling CB46 with equivalent yield potential that has greater resistance to root-knot nematodes but a smaller grain size than CB46. This line was crossed with CB46 in 2010 to create the BC<sub>7</sub>F<sub>1</sub>. In FY2011, inbred BC<sub>7</sub>F<sub>2</sub> lines were developed and will be evaluated for resistance to nematodes in laboratory growth pouch assays and the resistance lines will be increased in the greenhouse to obtain sufficient seed for further tests in FY12.

For aphid resistance, breeding lines were developed that show strong resistance in screening trials. The most resistant lines are now being crossed with CB46 and CB50 as part of the process of transferring aphid resistance to adapted varieties. The F<sub>1</sub>s of these crosses will be grown in the greenhouse this winter to obtain F<sub>2</sub> seed.

#### *Short-term Breeding Strategy*

We initiated a new two-tiered breeding strategy to meet the immediate and longer-term needs of farmers. The short-term strategy is using improved and local varieties with both grain quality and agronomic features appreciated by farmers. Obvious defects in local and improved varieties will be improved by breeding in resistance to diseases and pests (and other traits), using a rapid recurrent backcrossing approach.

#### *Advanced Yield Trials*

The California blackeye lines being improved by recurrent backcrossing are summarized in table 4. The SNP-marker genotyping will be applied to check for resistance to root-knot nematode, Fusarium wilt, and aphid.

*In Burkina Faso*, one advanced yield trial of 192 lines and eight checks was conducted at Saria in 2011 to select high yielding lines with large seeds. The best lines will be reevaluated next year at Saria, Pobé, and Kamboinse.

Recurrent Parent Line	Trait donor parent	Trait being introgressed	Status at start of FY11 Workplan	FY10 and 11 activities
CB5	CB27	Fusarium wilt	BC2F7	Four advanced lines in replicated yield tests at UCR and Kearney in 2011.
CB46	UCR 03-11-747	Green grain	BC4F10	Test results for 2010 given in Tables 2 and 3.
CB46	IT84S-2049	Root-knot nematodes	BC6F9	Test results for 2010 given in Table 2, 2011, results pending harvest
CB46	Bambey 21(Senegal)	All-white grain	BC4F10	Test results for 2010 given in Table 2, 2011 results pending harvest.
CB46	IT97K-556-6 & UCR 779	Aphid resistance	BC1F6	Lines phenotyped lines for aphid resistance in 2010 and 2011 and crosses to be made in late 2011.
CB46	IT93K-2046	Lygus resistance	BC3F6	BC4 lines tested in replicated yield trials and new crosses made between promising lygus resistant lines.

**Table 4.** California blackeye lines being improved by introgression of specific traits using backcrossing at UCR.

From previous trials, on-farm test using the best three varieties was conducted 10 sites in 2011. K VX442-3-25 with an average yield of 1125 kg/ha was superior to the two others. Farmers preferred that variety and it will be released in 2012.

*In Senegal*, two advanced yield trials were conducted at the Bambey ISRA field station in 2010 and 2011. In 2011, 20 on-farm trials were conducted in the Louga and Mekhe areas with 10 lines selected from previous years.

#### *Crosses for Developing New Breeding Lines*

In Burkina Faso, progenies of new crosses were advanced to the F<sub>5</sub> stage during 2010. The F<sub>6</sub> bulk of these crosses was harvested in 2011 for planting in 2012 to select for desired traits. The goal is to increase seed size of the improved varieties for Burkina Faso. The range of crosses should allow selection of new larger seeded varieties carrying important insect, disease, Striga, and nematode resistance traits.

*In Senegal*, for introgressing Striga resistance, Yacine was crossed with a more recent line instead of Suvita 2. The Mouride x Monteiro lines will introduce large grain quality into a drought and striga resistant background. Additional crosses were also made and included ISRA-2065, Yacine, and Melakh, each crossed with Striga resistant lines for Macrophomina resistance.

Under the planned longer-term strategy to pyramid resistance and grain quality in varieties desired by farmers, several activities were conducted. To develop high performing, drought tolerant varieties, we are using a two-stream recurrent selection approach. *Stream One* includes a set of breeding lines developed from crosses between drought tolerant and elite African breeding lines. In 2008 the F<sub>1</sub>s were made at UCR, then advanced to the F<sub>2</sub> generation and subjected to screening for drought tolerance. Drought-tolerant F<sub>2</sub> individuals were advanced to the F<sub>3</sub> for each population. In 2009, 352 F<sub>3</sub> families from these crosses were screened for performance under postflowering drought conditions. Seed of the top performing 19 F<sub>4</sub> lines were re-evaluated in California in late 2010. The top-yielding lines will be shared with our African partners at the end of 2011 and intercrossed in an attempt to pyramid drought tolerance factors.



*In Burkina Faso*, 95 families of the crosses using the set of parents IT84S-2246, IT93K-503-1, and Mouride have been evaluated in a replicated trial at Pobé during the main 2011 season. In Senegal the  $F_3$  lines from these families were evaluated for drought tolerance and the best performing 100 selected and the family bulked for further evaluation. Individuals of the most drought tolerant lines that have large seeds will be used for crossing to the improved lines developed under the backcrossing program.

*Stream Two* includes two four-way cross populations developed from diverse yet elite African and California cultivars with a host of desirable traits. The resulting materials represent a very broad-based assemblage of elite genetics and will be shared with the new breeding program in Angola.

*In Burkina Faso*, 20 elite lines from the GCP-Tropical Legumes II project were tested for grain yield and agronomic characteristics in 2010. From these, the best performing lines were evaluated in advanced trials in 2011.

*In Burkina Faso*, two phenotyping trials were conducted. The data is being processed and the lines will be SNP-genotyped.

*Marker-assisted backcrossing (MABC)* is a breeding strategy that can markedly increase the rate of progress and the precision of backcross breeding outcomes. The new high-throughput SNP genotyping platform is ideally suited to the current task of introgressing key traits into locally adapted varieties via MABC. The trait-marker associations have been identified through QTL mapping efforts that combined AFLP and SNP marker data with extensive phenotyping data for drought tolerance, insect resistance, and continuing efforts for root-knot nematode, *Macrophomina*, *Fusarium*, and other disease resistance traits. Genotyping through the KBioscience SNP platform was conducted in 2010 and 2011 to aid in progeny selection.

*In California*. Pigeonpea GA-1 is being tested in a large-scale (3.7 acres) strip plot with three irrigation treatments at Shafter in 2011, to be harvested in 2011 and yields determined. A small-plot, four-fold replicated trial is near harvest at Kearney with entries that include 10 selections from the 2010 Kearney

pigeonpea nursery and single-plant selections derived from a plot of GA-1 grown at Shafter in 2010. If significant yield differences are observed among selections, seed of the most promising lines will be bulked for larger-scale trials and a fast-track release path followed.

## **Objective 2. Strengthen cowpea seed production and delivery systems in Angola, Burkina Faso, and Senegal to ensure delivery of improved varieties.**

Cowpea seed production and delivery systems in Burkina Faso and Senegal will be strengthened to ensure delivery of improved varieties. Adoption of improved varieties is constrained by inadequate supply of breeder and foundation seed, which limits the seed that can be produced.

The approach is to increase amounts of breeder and foundation seed available to certified seed producers, identify new certified seed producers, and strengthen and expand proven activities in Senegal and Burkina Faso through leveraged funding from NGOs and USAID Mission funding. Working with the national extension services in Senegal (ANCAR), Burkina Faso, and Angola (SENSE) to reach farmers' organizations in different communities will be coupled with strengthening the small private seed producers.

A strategy adopted by the newly created GCP/ICRISAT Legumes for Livelihoods project in Niger, Nigeria, Mali, Tanzania, and Mozambique is to improve farmers' access to cowpea seed and enhance widespread adoption of improved varieties through the development and promotion of community seed production available at local markets. In Senegal, Burkina Faso, and Angola, schools can act as a seed supply center in each village, with teachers trained on quality seed production. Several progressive farmers will be guided in seed production and supplied with quality foundation seed for multiplication.

*In Burkina Faso*, the primary effort is to produce foundation seed and certified seed of six newly released varieties and 10 existing varieties—up to 60 tons in seven provinces by trained farmers. Breeder seed will be produced in the off-season for five varieties. Foundation seed production will be made to ensure an adequate capacity on each of the three INERA stations, generating about seven tons of foundation seed at Tougan (Sourou province),



Saria (Bulkiemde province), Donsin (Oubritenga Province), Pobe (Soum province), and Pissila (Sanmatenga province). A target of 70 seed producers—women and men—will be trained in seed production, harvest, and postharvest handling.

*In Senegal*, the availability of foundation seed has been identified as a bottleneck for adequate supply of seed to farmers. To address this, one ha of Melakh, one ha of Yacine, and ½ ha of ISRA-2065 will be produced to complement the foundation seed production by the ISRA seed unit at Bambey. At each location, foundation seed will be provided and farmers will be trained in seed production, harvest, and post-harvest handling.

*In Angola*, we will continue to link with government and NGO institutions, including World Vision, Africare, CRS, and ADRA-Angolana to determine opportunities for advancing the cowpea seed system. Opportunities for a coordinated bean and cowpea seed system based on the breeder–foundation–certified seed system chain will be pursued.

*In Burkina Faso*. At the INERA station of Saria more than 70 tons of foundation seed was produced in 2010. A portion of breeder seed produced in 2010 has been used to produce foundation seed during the 2011 season. We anticipate a production of at least 55 tons of foundation seed in 2011.

*In Senegal*, three ha each of Melakh and Yacine and one ha of ISRA-2065 foundation seed was produced at the ISRA Bambey station. It is expected that at least 100 kg of each variety will be made available to the network of farmers' cooperatives (RESOPP) now set up by the NGO EWA. In the Thilmakha area, foundation seeds were distributed to two farmers for production of one ha of Melakh and one ha of Yacine certified seeds during the 2010 season. In 2011 these acreages were doubled to two ha for each variety and farmer. Certified Seed production was also conducted in collaboration with a farmers' union (UGPM) in Mekhe with 10 ha of Melakh and Yacine each. These acreages were raised to 25 ha in 2011. With the closing of the EWA activities in the Louga area, we supplied eight ha of certified seed production to the farmers' cooperative RESOPP.

### **Objective 3. Technology dissemination (seed of improved cowpea varieties in West Africa).**

*INERA, Burkina Faso*. In Burkina Faso during FY11, Breeder seed of seven improved cowpea varieties will be produced, which should yield 800 kg of breeder seed per variety. Theory and practical training of 120 farmers, of which at least 30 will be women, will be conducted at five locations from April to May 2011. These farmers will be guided in producing certified seed of the INERA improved cowpea varieties. Two visits by the INERA national cowpea research team will be made to each farmer's field during the June to September production season to provide practical training and advice. Breeder and foundation seed of the seven varieties will also be produced during the 2011 growing season to yield 350 kg of breeder seed of each variety and a total of 20 T. Three varieties of additional foundation seed will be produced to supply new certified seed to growers. During the 2011 rainy season three ha each of Melakh

and Yacine foundation seeds and one ha of Pakau were produced. The project team will work with ANCAR and 80 farmer organizations to train 100 to 200 farmers in seed production, harvest, and postharvest handling.

*ISRA Senegal*. To address the foundation seed bottleneck for adequate supply of seed to farmers, three varieties of additional foundation seed were produced to supply new certified seed to growers. It is estimated that 50 to 60 t of certified seeds will be produced after harvest. At least 50 ha of Melakh and Yacine were grown initially for certified seed, with the goal of scaling up in the second and third years. The target of 100 ha was attained and an additional of 50 ha was planted. During the 2011 growing season, five to six t of foundation seed was produced at the ISRA Bambey Research Station.

### **Networking and Linkages with Stakeholders**

We are working closely with national and international cowpea breeders and other scientists, including Drs. Ousmane Boukar, Christian Fatokun, and Sata Muranaka, senior scientists and cowpea breeders at IITA; Dr. Mohammed Ishiyaku of the IAR in Nigeria; Rogerio Chiulele at Eduardo Mondlane University in Maputo, Mozambique; Michael Timko at the University of Virginia; and Larry Murdock at Purdue University. We are working closely with the California Dry Bean Advisory Board and its Blackeye Council on research priorities of the industry. We are working with Inland Empire Foods, an important legume processor based in Riverside, on developing Akara (or bean tots) for inclusion in the California school program and with another major U.S. manufacturer on utilizing several products well suited to our varieties. We have provided Pulse CRSP project PIs with cowpea seed. We also worked with Drs. Jim Beaver and Tim Porch at the University of Puerto Rico on training a CRSP student from Angola and Dr. Mbaye Ndiaye at Aghrymet, Niger, for the student from Senegal. Under the CGIAR-GCP funded project Tropical Legumes 1, we are leading the cowpea improvement objective and interacting with a large international network of tropical legumes researchers.

*In Burkina Faso*, we have been working with Association FERT, a French NGO whose aim is to improve cowpea production in the northern part of the country and continue on-farm tests of improved varieties, helping produce certified seed. Linkages have also been maintained with five farmer organizations: *Song*





*Taaba* at Donsin near Ouagadougou, *Six S* at Pobe Mengao, Producteurs de Semences de Diouroum, Producteurs de Semences at Pobe Mengao, and Producteurs Semenciers Songd Woaga at Sarria.

In addition, collaboration was continued with Venegre and Famille Kabre, a seed producer and a seed entrepreneur. Linkages have been made with the former prime minister, the minister of agriculture, and the minister of research and technology to produce foundation and certified seeds.

*In Senegal*, collaboration was established with ANCAR in the Kaolack and Thiès regions and with RESOPP of EWA in the southern region of Sedhiou, for certified seed production of the variety Pakau. The Millennium Project, the private enterprise (ASRM), and ANCAR-Thiès were involved in seed production in the Louga, Mekhe, and Touba Toul regions. In 2009, the Kirkhouse Trust started supporting activities on marker-assisted backcrossing for *Striga* resistance by providing \$20,000 annually for three years.

*In Angola*, multistakeholder partnerships are increasingly becoming a common feature of agricultural research for development. We have been working with Faculty of Agrarian Sciences, Institute for Agrarian Development, AFRICARE; an American NGO; and Small Farmers Association/Community Based Organizations.

Dr. David Kiala of the Faculty of Agrarian Sciences has evaluated germplasm across agroecological zones, capturing farmers' preferences and gender considerations for selecting cowpea varieties.

José Pedro of the National Center for Phylogenetic Resources has carried out cowpea landrace characterization.

## Leveraged Funds

Scientists involved in this project have effectively used Dry Grain Pulses CRSP support and institutional linkages to leverage more than US\$3.5 million in external funding to achieve objectives related to this project.

## Publications

Barrera-Figueroa, B., Gao, L., Diop, N.-N., Wu Z., Ehlers, J., Roberts, P.A., Close, T.J., Zhu, J.-K., and Liu, R. 2011. Identification and comparative analysis of drought-associated microRNAs in two cowpea genotypes. *BMC Plant Biology* 2011, 11:127 doi:10.1186/1471-2229-11-127.

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Muchero, W., J.D. Ehlers, T.J. Close, and P.A. Roberts. 2011. Genic SNP markers and legume synteny reveal candidate genes underlying QTL for *Macrophomina phaseolina* resistance and maturity in cowpea [*Vigna unguiculata* (L) Walp.]. *BMC Genomics* 12:8.

Xu, P., X. Wu, B. Wang, Y. Liu, J.D. Ehlers, T.J. Close, P.A. Roberts, N.N. Diop, D. Quin, T. Hu, Z. Lu, and G. Li. 2011. A SNP and SSR based genetic map of Asparagus Bean (*Vigna unguiculata* ssp *sesquipedialis*) and comparison with the broader species. *PLoS One* 6: e15952.

## Contribution to Gender Equity Goal

The activities in Burkina Faso and Senegal resulted in producer-/community-based organizations of women and men receiving technical assistance. In addition, host country partner organizations in Burkina Faso and Senegal benefitted from seed systems technology. More specifically, women's organizations received technical assistance in Senegal and Burkina Faso. Also short-term training of women and men was accomplished. The technical assistance was focused on seed system processes, for growing, harvest handling, and storing cowpea planting seed (Certified Seed production).





# Biological Foundations for Management of Field Insect Pests of Cowpea in Africa

UIUC-1

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## Abstract of Research Achievements and Impacts

Our project is focused on immediate, tangible, cost-effective, and scalable integrated pest management (IPM) solutions for the largest biotic constraint on cowpea production in West Africa: six species of pest insects that attack cowpeas in the field. Pesticides are (or are fast becoming) a nonoption for many farmers; and transgenic cowpeas, if/when they become available, will only control one of the six major pest species of cowpea. Thus, there is an urgent need to develop a comprehensive IPM strategy for the insect pests that attack cowpeas using a diversity of control strategies. Three major steps are needed to achieve the goal of developing cost-effective IPM solutions:

1. Determining when and where the insect pests are located
2. The development and deployment of cost-effective and environmentally benign strategies for controlling these pests
3. The development and deployment of cost-effective and sustainable educational strategies to enable both educators and, ultimately, farmers to learn about and use these pest control approaches

To better define the insect populations, our group is developing a new paradigm for pest control—an integration of genomics tools for making integrated pest management decisions termed *Integrated Pest Management Omics (IPM-Omics)*. We have created the necessary molecular tools to understand the population dynamics and movement patterns of the legume pod borer (*Maruca vitrata*) and are creating these tools for the other major cowpea pests. These molecular tools have been and will be used with traditional field studies to identify where pest populations are originating during the dry season to determine where to release these biological control agents. We have performed field studies on the effectiveness of biocontrol agents on the control of insect pest populations and increasing yield in the cowpea crop; the results have been highly positive.

Lastly, we are developing educational deployment strategies to deploy pest control strategies on a large scale and in a cost-effective manner. We are spearheading:

1. cell-phone-ready animations to train people in pest control strategies
2. an online peer-review system for host county collaborators to share educational materials
3. working relationships with other organizations to scale up farmer education of IPM-based pest control strategies

## Project Justification and Objectives

Arguably, the greatest biotic constraints to cowpea production are insect pests. The most logical long-term options for controlling cowpea pests will be a mixture of biological control agents, cultural practices, biopesticides, and classic host plant resistance.

In the long-term, pesticides are likely to become a less viable option for control of pests on cowpea. Host plant resistance traits and cultural practices need to be complemented by

strategies that directly reduce pest populations. Transgenic *Bt* cowpea for the control of *M. vitrata* has been in development for almost two decades; however, it may be years before such varieties are available to farmers. Physical approaches have been developed and are being deployed for the control of bruchids in stored cowpeas, and many host country (HC) scientists have continued to promote the successful use of local plant extracts (*e.g.*, neem) in conjunction with host plant resistance traits to suppress pest populations. These approaches require educational materials that can be easily deployed to farmers in given regions. Biocontrol agents offer immediate, tangible, and cost-effective pest control solutions to farmers without such complications; many can simply be released to suppress pest populations over the long-term without further human intervention while others can be turned into cottage industries (*e.g.*, viral sprays).

Our HC scientists have had major successes with the use of biological control agents against other-crop pests (*e.g.*, cassava and millet) and have tested other practical control methods in the field and in farmer field schools with positive outcomes. We now have numerous biological control agents against cowpea pests and educational materials to promote other pest control strategies ready for release and testing.

One of the challenges of releasing biocontrol agents has been determining where to release these organisms for the greatest impact, the best place being where the insects are endemic and can support the biocontrol agent populations and in endemic populations that cause the most damage to cowpea fields. Thus, we needed to determine insect movement patterns and develop molecular markers to test the biocontrol agents.

Our project has aimed to combine surveys of pest populations with genomic analysis tools to determine where to release biocontrol agents for *M. vitrata*, to develop the expertise to extend these IPM-omics strategies to all other cowpea pests, and to develop the necessary capacity, institutional infrastructure, and farmer training to release biological control agents against cowpea pests strategically.

## Objectives

1. Develop cost-effective insect pest management (IPM) solutions:
  - a. Determine when and where the insect pests to cowpeas (*Maruca vitrata*) are located.
  - b. Determine when and where insect pests to cowpeas (other than *Maruca vitrata*) are located.
  - c. Survey wild alternative host plants (in- and off-season).
  - d. Develop and deploy cost-effective and environmentally benign strategies for controlling these pests.
  - e. Develop and deploy cost-effective and sustainable educational strategies to enable educators and farmers to use these pest control approaches.
2. Develop an infrastructure to release information to extension services, NGOs, and cowpea farmers.
3. Disseminate project capacity building of host countries.

4. Build capacity at host country institutions for the rearing and mass release of biocontrol agents currently ready for release.
5. Develop collections of biological control agents for the sequencing and development and of IPM-omics tools.
6. Bring new biocontrol agents into the pipeline for development and deployment.
7. Develop other institutional capacity building training activities in the rearing and release of biological control agents.

## Research and Outreach Approaches, Results, and Achievements

### Objective 1: Develop cost-effective insect pest management (IPM) solutions.

1a. Determine when and where the insect pests to cowpeas (*Maruca vitrata*) are located.

#### *Characterization of Pests of Cowpeas (Maruca vitrata) and Molecular Markers*

To deploy a biocontrol agent release program for *M. vitrata*, we need to learn when and where *M. vitrata* appears, build institutional infrastructures to monitor *M. vitrata*, and develop a better understanding of this pest in the host countries. This work has laid the base for developing an IRM plan for Bt cowpea and other IPM-based pest control strategies.

Light trapping was done over 12 months in Maradi, Kornaka, and Gaya (in Niger) and in Farako-ba and Kamboinsé (in Burkina Faso). Adults were monitored and collected from light traps daily and sent to UIUC for molecular analyses.

Our field and molecular data currently support the hypothesis that *M. vitrata* move in a northerly pattern from an endemic zone during the wet season, surviving in the southern endemic zone during the dry season; however, we have observed the endemic zone in Burkina Faso to be farther north than previously expected. Our molecular data also suggest a south to north movement pattern of *M. vitrata* in the rainy season.

#### *Implications for pest control strategy*

To establish biocontrol agents in the *M. vitrata*, biocontrol agents need to be released in Southern Burkina Faso and Northern Ghana, Togo, and Benin.

We have a large collection of *M. vitrata* from throughout Burkina Faso, Niger, and Northern Nigeria being used in molecular analysis of the populations. This activity has allowed us to:

1. build institutional infrastructures to monitor *M. vitrata* using light traps
2. develop multiple molecular approaches for studying *M. vitrata* population dynamics
3. use these genomics tools for insect management decisions for the next phase of our project
4. lay the foundation for the development of insect resistance management plans for the deployment of host plant resistant varieties of cowpeas that can be used to control of *M. vitrata*

We are using what we have learned from our combined light trapping and genomics data of *M. vitrata* to determine how to cost-effectively deploy insect control strategies for this pest. We have tested our migratory hypothesis on the movement patterns of *M. vitrata*. Based on our light trapping and molecular data, we have a better understanding of when and where biological control agents should be released to optimize impact.

#### *Molecular Tools Development.*

We have developed a series of genomics tools for integrated pest management:

1. We have discovered a series of microsatellites useful for characterizing *M. vitrata* populations across West Africa. This novel approach for microsatellite identification can now be used for other Lepidopterous pests.
2. We have used 454 sequencing technology to (a) sequence the complete mitochondrial genome of *M. vitrata*, (b) determine the exact locations in the mitochondrial genome that will and will not vary from insects found around the world, and (c) determine which genes vary locally and regionally in West Africa and across the planet. We can now easily characterize *M. vitrata* populations from distinct locations in West Africa to determine their movement patterns. Other researchers will also be able to use simple PCR tools to monitor *M. vitrata* populations in West Africa, providing our collaborators at INERA, IAR, and IITA with important information for molecular tools that can be used at their institutions to further characterize *M. vitrata* populations.
3. We have used 454 sequencing technology to determine single nucleotide polymorphisms (SNPs) across hundreds of *M. vitrata* nuclear genes and can now easily characterize *M. vitrata* populations locally, regionally, and across continents to determine where to deploy biocontrol agents that impact cowpeas. We have used these molecular tools to determine that *M. vitrata* is actually two species of insect and have determined the migratory patterns in West Africa so we can release biological control agents to have the greatest impact.
4. We have also developed diagnostic PCR-based assays for other researchers to further test details of *M. vitrata* populations. We have developed modeling strategies for minimizing resistance in the insect populations if or when the transgenic cowpea is released in West Africa. We have developed a computational model, based on our datasets, that will be critical for risk assessment associated with the potential release of transgenic Bt cowpea in West Africa.
5. All of the molecular tools we have developed, along with their applications for insect control, were applied in 2011 to the other pest insects that attack cowpea. We have collected large numbers of insects from all other pest species and performed the first steps of sequencing these populations to perform the same types of studies done with *M. vitrata*. We now have the capacity to extend these molecular marker approaches to all cowpea pests and to develop IPM-omics strategies for them.

1b. Characterization of the Other Pests of Cowpeas (other than *Maruca vitrata*) and Molecular Markers

### *Insect pests on cultivated cowpeas*

This activity will provide the basis for understanding the problems of cowpea pest insects within host countries and allow for cross training in pest insect biology across the three host countries. This work will lay the base for the development of an IRM plan for *Bt* cowpea as well as provide the base for other IPM-based pest control strategies for *Maruca* and other cowpea pests. We will also test the impact of viral and neem sprays on cowpea crops to determine if they can be used to reduce pest attack and increase yield.

The major pests of cowpea in the field in West Africa include the legume pod borer, *Maruca vitrata* Fabricius; the coreid pod sucking-bugs; the groundnut aphid; and thrips.

The data sharing from our preliminary work and the experimental design for the field studies were completed in the first six months of FY11. Based on these experimental plans, we studied the presence and detailed life-history of the five major pests of cowpea through the use of randomized complete block design experiments using multiple lines of cowpea and alternative host plants. We will also test viral (against *M. vitrata*) and neem sprays (against all the pests) on cowpea crops. Data are being tabulated.

### *Summary of findings*

1. We have collected the necessary samples of insects and have begun 454 sequencing of mixed samples (within each species) to discover polymorphisms that will be used for SNP analysis in FY12.
2. Major pest problems by region have been defined; we now have three seasons of baseline data on the pest levels of insect populations on cowpeas in test plots.
3. The good results obtained in FY10 with viral sprays against the pod borer *M. vitrata* (yield increases in the test plots [26–34 percent]) were confirmed in FY11. Detailed experiments in Benin indicated an average of 67 percent yield increase in the first cropping season compared to the untreated plot. Better, a combination of aqueous formulation of neem oil combined with the virus doubled the cowpea grain yield (108 percent). Neem sprays were effective in decreasing pest populations and increasing yield; neem sprays coupled with host plant resistant strains were the most effective in reducing the pest populations.

### *Implications for pest control strategy*

1. We have defined the regions to focus the deployment of specific biocontrol agents (to control specific pest species).
2. Viral sprays (combined with neem oil) represent a new option for the control of *M. vitrata*.
3. Neem sprays were effective, especially in combination with host plant resistant varieties, and have been used to train farmers in farmer field schools.

We performed the above experiments over the past three field seasons (summer of 2008, 2009, and 2010). A minimum of three varieties of cowpeas (early, medium, and late flowering), along with wild alternative host plants for pests of cowpeas, were

planted at each of the experimental locations in Burkina Faso, Niger, and Nigeria; we recorded all the details of which pests attacked which plants and at the specific time interval. We have analyzed the data sets and can ascertain which pest insects represent the greatest problems in the region.

We have also performed field experiments where 1. we have tested pest tolerant strains of cowpeas on their own and in combination with neem sprays and 2. tested viral sprays to control *M. vitrata*. Both strategies were successful in reducing pest numbers and increasing yield (as compared to pesticide sprays).

Other important trends emerged that will be helpful in future insect control efforts. In Niger, earlier flowering varieties did not sustain the same levels of insect attack as the medium and late flowering varieties. The plants simply mature before the pest populations reach their peak numbers and thus avoid the times of pest attack. In Niger and similar eco-agricultural zones in Burkina Faso and Mali, earlier flowering varieties may be of great benefit to farmers to avoid some pest problems.

These experiments helped us determine those regions in which certain pest insects affect cowpea crops and where to deploy certain biological control agents. For example, in Burkina Faso the major pest insects in the south are *M. vitrata* and pod sucking bugs, with aphids the third most significant pest. In central Burkina Faso, thrips and pod sucking bugs are the most significant pests, with *M. vitrata* being the third. In Northern Burkina Faso, only thrips are a major pest problem, with *M. vitrata* only a rare occurrence. IPM strategies will focus on the most significant local pests to achieve the maximum impact.

Separate experiments were performed to evaluate separate varieties of cowpeas that are tolerant to thrips and pod-sucking bugs. Our initial experiments showed these varieties to be more tolerant to insect attack. The insects collected for these experiments can be used in genomics experiments to better understand the movement of pest populations, which will be critical for the development of genomics tools to understand these pest populations and make informed decisions on where and when to release biological control agents.

### *1c. Survey wild alternative host plants (in- and off-season)*

This activity will provide a better understanding of cowpea pest insects within host countries during the growing and nongrowing seasons, which help us determine where the pest populations are when cowpea is not grown.

A standardized scouting of cowpea pests on alternative host plants will be established during and outside the cowpea-growing season in the project's first six months. The frequency and distances will depend on the costs of transportation; however, no fewer than one scouting trip will occur per country every six months.

Surveys of wild alternative hosts around and near cowpea fields will be designed in the first six months of the project and performed in each country during the cowpea-growing season. Briefly, farmers' fields will be surveyed for the numbers of insects on cowpeas in relationship to any nearby wild alternative hosts (or the lack of alternative hosts will be documented).

Insects that are observed will be collected for sequencing efforts to generate the necessary polymorphisms to study the insect populations and their movement patterns.

#### *Summary of findings*

1. Identification of important wild alternative hosts
2. Collection of insects necessary for genomics work

#### *Implications for pest control strategy*

Our results support the hypothesis that pod-sucking bugs, thrips, and aphids live during the dry season in local areas where cowpeas are grown during the wet season. If these results are supported by molecular data, i.e., that the pest populations are endemic, then biocontrol agent released locally should support local pest populations over the long run.

#### *Details of Efforts over FY11*

In keeping with these objectives, we performed a series of scouting trips in Niger, Nigeria, Benin, and Burkina Faso over the last 2.5 years, which have indicated the best locations to release the biological control agents to achieve the greatest potential impact on *M. vitrata* populations. For example, in Burkina Faso, *M. vitrata* is endemic in the southern most region of the country and moves almost directly north from these endemic areas during the growing season, affecting cowpea crops in the central areas of Burkina Faso. Based on our findings, biological control agents against *M. vitrata* should be deployed in southern Burkina Faso and in the northern parts of those countries located at Burkina Faso's southern border (e.g., northern Benin, Ghana, and Togo). Biocontrol agents for *M. vitrata* in Niger will have to be released in northern Benin and in Nigeria. The two parasitoids useful in controlling *M. vitrata* include the Hymenopteran parasitoids *Apanteles taragamae* and *Nemorilla maculosa*. As part of our Pulse CRSP Technology Dissemination Project, we are now in a position to determine where to release these parasitoids to maximize their impact.

For both pod-sucking bug species (e.g., in Burkina Faso), there are at least six local wild alternatives that support these populations during the dry season. Thrips develop in the off-season on several wild Fabaceae and other plants from the Mimosaceae, Ceasalpinaceae, and Bixaceae families. Cowpea aphids are hosted by peanuts and vegetables during the dry season.

We will continue these scouting efforts in the upcoming year to obtain more *M. vitrata* samples and further pinpoint where the endemic populations move from and to in the growing season. We will continue to extend these scouting and molecular approaches to other pests of cowpeas to determine where the biocontrol agents are most effective in initially impacting the pest populations; we also have biocontrol agents ready for deployment to control flower thrips, pod sucking bugs, and aphids.

#### **Objective 2: Develop infrastructure for release of information to extension services, NGOs, and cowpea farmers.**

The goal is to develop a long-term capacity for the large-scale release of IPM strategies for Mali, Burkina Faso, Niger, and northern Nigeria, which includes 1. an institutional human

resources infrastructure building, 2. partnerships with collaborative groups to help us deploy these approaches on a larger scale, and 3. educational tools for training host country scientists, extension educators, and farmers.

#### *To achieve these objectives:*

1. We have performed scientist, graduate student, and intra- and inter-institutional technician training.
2. IITA has developed eight videos necessary for technician and scientist training on the pests of cowpeas, including identification of the pests in the field and rearing of the pests and their biocontrol agents.
3. We have trained host country scientists and technicians on cost-effective strategies for rearing *M. vitrata* and producing biocontrol agents for release.
4. We have partnered with other organizations to deliver pest control strategies to farmers.
5. We launched the Beta version of the online information sharing system for extension materials (Sustainable Development Virtual Knowledge Interface, [susdeviki.illinois.edu](http://susdeviki.illinois.edu)).
6. We developed a series of animations that can be deployed using cell phones. We have developed videos for hermetic sealing of cowpeas for storage, solar treating of cowpeas, and proper preparation and use of neem sprays. All three videos were released in 2011 and have been translated into multiple local languages and can be found on the SusDeViKI system (<http://susdeviki.illinois.edu>). This cost-effective way to produce such material (with easy voice-overs in new languages) has resulted in the development of a UIUC-based group called "Scientific Animations Without Borders" (SAWBO) that will also be producing videos for other development projects and programs. For our CRSP project, our videos have been shared with Drs. Robert Mazur and Cynthia Donovan for use in Rwanda and Mozambique.
7. We have provided a number of governmental agencies and NGOs in Benin with information about SusDeViKI and SAWBO; video dissemination will continue in FY12.

#### *Farmer Field Schools*

We have used Farmer Field Schools (FFSs) to educate farmers about the pests of cowpeas, so they can assess, disseminate, and release improved methods for pest control (and overall production). FFSs represent multimonth half-day a week training sessions with a minimum of 20 farmers per village (10 men and 10 women). We performed a minimum of two Farmer Field Schools in each of the host countries. MP3 players and animations on cell phones were also distributed in villages for education.

As part of the FFSs, farmers set up test plots with different technologies for cowpea production (host plant resistant lines, neem sprays, and viral sprays), assessed insect attack and the impact of other production technologies, and made decisions on the outcomes of these experiments. Technologies involved:

1. insect/pest tolerant varieties of cowpeas (more than five new varieties tested)
2. local biological/botanical sprays (three technologies tested)

3. early, medium, and late flowering varieties
4. diverse fertilizer strategies (manure and fertilizer combinations)
5. intercropping approaches
6. hermetic storage of cowpeas
7. soil preparation and planting density testing
8. information on how to minimize traditional pesticide sprays where farmers typically spray their cowpea crops
9. discussions on the use of viral sprays/biological control agents to control *M. vitrata*

Feedback from these FFSs have also allowed us to identify which pest problems are of the greatest concern in various regions of each country and to give farmers the ability to identify early on which pest problems may be occurring, so they can take measures to minimize the pest populations.

Our long-term goal has been to release biological control agents into those areas with FFSs. The FFSs will continue to monitor the pest populations and the biological control agents to help us determine if the biological control agents have a practical impact on crops. We will continue to do tightly controlled experiments at INERA and INRAN to measure these same variables (pest populations and the presence of biological control agents after their release) to obtain scientifically rigorous datasets on the impact of this biological control strategy on pest populations.

To increase the impact of our program, we have held one-day sessions where other farmers can interact with the FFSs to see the impacts the various pest control strategies have on cowpea production. We have also distributed improved seed varieties to other farmer organizations.

We have focused on women's needs in cowpea production and are summarizing the critical gender differences to increase the impact of FFSs on women in the current CRSP project. We are also collaborating with Dr. Madhu Viswanathan of UIUC on our extension strategies (including assessment), especially as they relate to low literate learners.

### Objective 3: Disseminate project capacity building of host countries.

1. Low-cost/highly efficient system for mass rearing of *M. vitrata* (and its parasitoids) using cowpea sprouts adapted for HC conditions; in use in Benin, Burkina Faso, and Niger.
2. In-field rearing and slow-release delivery systems developed for the parasitoids *A. taragamae*, *T. eldanae*, and *G. fulviventris*
3. A detailed field experiment in Benin confirmed the good results obtained last year with the viral sprays. In the first cropping season, the application of aviMNPV led to an average grain yield increase of 67.2 percent, as compared to the unsprayed plot. In the same experiments, a combination of aqueous formulation of neem oil and MaviMNPV resulted in a 106.8 percent yield increase, statistically superior to the yield increase with the synthetic insecticide (66 percent).

### Objective 4: Build capacity at host country institutions for the rearing and mass release of biocontrol agents currently ready for release.

We are developing rearing and delivery systems for biological control agents against major cowpea pest infestations that can easily be implemented by HC collaborators. In particular, we are:

1. Refining and validating the recently developed mass rearing technique for *M. vitrata* using germinating cowpea sprouts. The above rearing methodology will be used to mass rear the parasitoid *A. taragamae* in HC laboratories for field inoculations. At the same time, we will be developing in-field mass rearing techniques using nurseries of the host plant *Sesbania sp.* We are using this rearing methodology for mass production of the entomopathogenic virus MaviMNPV in HC laboratories for field applications, which will lead to the development of in-field mass production techniques using nurseries of the host plant *Sesbania sp.* in the three host countries.
2. Establishing nursery plots of the host plant *Tephrosia candida* in HC for mass rearing of the thrips parasitoid.

#### Results

The technique using germinating cowpea grains to rear *M. vitrata* is currently being successfully applied for mass production of the parasitoid *A. taragamae* used for field releases in Benin. This novel rearing technique was also used for successfully rearing the parasitoid *A. taragamae*.



Figure 2: A cocoon of *A. taragamae* found on the grass *Adropogon sp.* (touching a *M. vitrata* infested inflorescence of *T. platycarpa*) in the caging device.

In addition to the above, detailed experiments were set up in Benin to monitor the short range dispersal capacity of the parasitoid *A. taragamae*. For this purpose, sentinel *Sesbania cannabina* plots artificially infested with *M. vitrata* were planted at five and 25 m distances from a release point of *A. taragamae* (an earlier planted *S. cannabina* plot artificially infested with *M. vitrata* and inoculated with *A. taragamae*) and monitored regularly for the formation of *A. taragamae* cocoons. The dispersal of *A. taragamae* from the release site was subjected to the prevailing wind direction and could be measured in all sentinel plots at



Figure 1a and 1b: The in-field mass rearing technique using *Sesbania cannabina* scheduled for years two to three was further modified and adapted in FY11 to use naturally occurring patches of a weedy legume widely available in the Savannah area in Southern Benin, an important host plant for *M. vitrata*. This was done with the intention of using this caging device in rural areas, without having to purchase galvanized pipes and planting crops of *S. cannabina*. The experimental inoculation was repeated

three times, and cocoons of *A. taragamae* could be observed on the leaves of *T. platycarpa* and on leaves of the accompanying vegetation (such as *Andropogon sp.* as in Figure 2). From these first inoculations, it appears that the density of *A. taragamae* cocoons will be less on *T. platycarpa* than previously observed on *S. cannabina* (up to 40 cocoons/sqm). However, considering the reduced costs and labor, we can anticipate having more caging devices than would be possible for *S. cannabina*.

both five and 25 m. This information is particularly important for shaping future deploying systems using caged natural vegetation as already attempted with *T. platycarpa*.

Nursery plots of *T. candida* were established at INERA Farakoba and INRAN Maradi as planned. The plants started to flower in August 2011, and the first inoculation is in progress and will be completed in November 2011.

#### Objective 5: Develop collections of biological control agents for the sequencing and development of IPM-omics tools.

Priority natural enemies for sequencing:

1. The parasitoids *Apanteles taragamae* (which attack the pod borer *Maruca vitrata*)
2. The parasitoid *Ceranisus femoratus* (which attack the flower thrips, *Megalurothrips sjostedti*)

Dr. Manu Tamò will be primarily responsible for the collection and shipping of insect samples to UIUC. Dr. Pittendrigh's laboratory will receive samples of the biological control agents from IITA, sequence populations of insects, and determine molecular markers useful in the monitoring of these pest populations. Dr. Pittendrigh will use these tools to test biological control agents that have been found in monitoring project, to determine if they are genetically similar to those insects that were initially released.

1. Results. *Apanteles taragamae* samples from our initial rearing at IITA-Benin have been processed for RNA extraction in our labs and sent to UIUC for sequencing.
2. Samples of *C. femoratus* have been collected from Benin and Ghana and have been processed for RNA extraction in our labs and sent to UIUC for sequencing.

#### Objective 6: Bring new biocontrol agents into the pipeline for development and deployment.

Against *M. vitrata*

1. The trichogrammatid, *Trichogrammatoidea eldanae*.
2. The tachinid, *Nemorilla maculosa*.

Against *C. tomentosicollis*

1. The parasitoid, *Gryon fulviventre*.

Results

1. An average egg mortality of 73.4 percent was recorded in cages using sentinel egg batches of *M. vitrata*. Under artificial infestation with *M. vitrata* adult females, however, no statistically significant differences in *M. vitrata* infestation could be observed. It must be noted that, in spite of the artificial infestation, *M. vitrata* larval population levels were quite low in both the untreated control (3.6 larvae/plant) and the *T. eldanae* inoculated cages (2.3 larvae/plant), due possibly to the unseasonably high rainfall in the region that might have interfered with the oviposition behavior of *M. vitrata*. Nonetheless, the potential of *T. eldanae* to parasitize eggs of *M. vitrata* on cowpea has been clearly demonstrated and warrants the continuation of developing field deployment systems.
2. We are currently testing an improved slow-release system using different substrates (millet-based and cowpea-based) for rearing the rice moth *Corcyra cephalonica*, whose eggs are used as oviposition substrate for *T. eldanae*.
3. A similar release device has also been developed for the release of the egg parasitoid *Gryon fulviventre*, which attacks eggs of the brown coreid bug *Clavigralla tomentosicollis*. Adults of *C. tomentosicollis* are reared inside a plastic bowl containing cowpea grains as food substrate (fig. 3). The eggs produced are parasitized by females of *G. fulviventre* previously inoculated into the device with the pod bugs.
4. Rearing of *Nemorilla maculosa* on *M. vitrata* was begun.

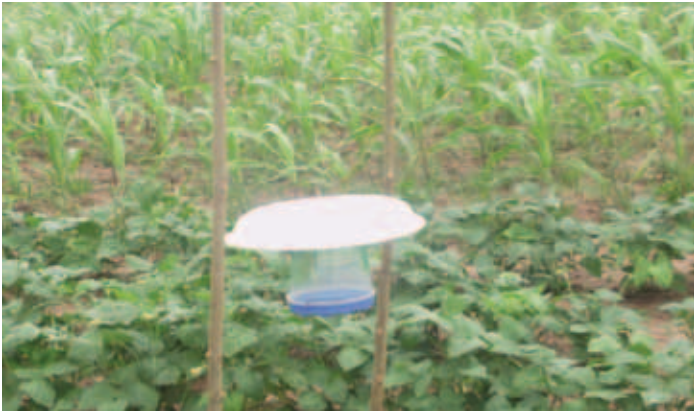


Figure 3: Improved slow release system for biocontrol agents using inexpensive local materials.

Based on the encouraging results from the viral spray experiment carried out in Benin, Burkina Faso, Niger, and Nigeria last year, a detailed experiment was carried out in Benin comparing conventional spray with an aqueous formulation of both neem oil and oil of *Jatropha curcas* mixed with the virus to control the other cowpea pests (e.g. thrips, aphids) present in the field with *M. vitrata*. In the first season, the application of MaviMNPV alone was able to control *M. vitrata*, producing a cowpea grain yield increase of 67.2 percent over the unsprayed control. However, the association of the virus with the aqueous formulation of neem oil gave by far the best yield gain—106.8 percent compared to the unsprayed plot. These results confirm literature reports about the synergistic effect of mixing aqueous oil formulation with NPVs.

**Objective 7: Develop other institutional capacity building training activities in the rearing and release of biological control agents.**

The collaborating host country scientists performed cross-training of each other and each other's staffs to develop better rearing, release, and monitoring of biological control agents through the exchange of technicians between institutions and by intra- and inter-institutional one-day and multiple-day training sessions.

**Networking and Linkages with Stakeholders**

Dr. Pittendrigh has visited both the USAID missions in Mali and Nigeria during the current CRSP grant. Dr. Tamò is continuing to work with collaborators in Ghana to ultimately request funds from a USAID mission office for a biological control program of insect pests of cowpeas in Ghana (an IITA activity). The concept note has been submitted to the USAID mission by the Ghanaian government with information provided by Dr. Tamò. If funded, our IITA collaborator will receive direct funding to work in Ghana on activities that have been made possible, in part, by our CRSP project. If funded, part of our CRSP program (at IITA) would certainly benefit, as would the country of Ghana.

In Benin, efforts are underway to work with the biggest federation of agroecological farmers (Federation Agro-ecologique du Benin) to promote biocontrol agents and biopesticides in their organic production, mainly in cowpea and horticultural crops. This will

enable IITA to make faster progress in participatory evaluation of the proposed biological control agents, biopesticides, and validate their delivery systems currently under development.

In Niger, our program is partnered with the Peace Corps to develop joint farmer field schools. Additionally, numerous farmer organizations have been engaged to help distribute pest control technologies, including seeds of cowpeas from insect tolerant lines (germplasm provided to us by the UC–Riverside DGP CRSP).

**Leveraging of CRSP Resources**

Dr. Pittendrigh and his co-principal investigators have successfully leveraged more than \$500,000 in external funding in FY11 for research and outreach projects related to insect pest biology and management in cowpeas in part due to the assistance received from the Dry Grain Pulses CRSP.

**Publications**

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# Development, Testing and Dissemination of Genetically Improved Bean Cultivars for Central America, the Caribbean and Angola

UPR-1

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## Abstract of Research Achievements and Impacts

Significant progress was made toward research and training objectives. The small red BGYM and BCMV resistant cultivar *CENTA Chaparrastique* was released in El Salvador. The small red bean cultivar *Paisano PF*, developed by PPB (participatory plant breeding) approaches in collaboration with farmers from the Yojoa Lake region, was released in Honduras. *Bentiquez*, a BGYMV, BCMNV, BCMV, and rust resistant white bean cultivar, was released in Puerto Rico in collaboration with the UPR, the USDA-ARS-TARS, and the University of Nebraska. Web blight resistant breeding lines PR0401-259 and PR0650-31 were released in cooperation with the UPR, the USDA-ARS-TARS, and Zamorano. Populations were developed from crosses between commercial seed types used in Angola and sources of resistance to BCMV, CBB, and ALS. Marker assisted selection was used for selection of CBB and BCMV resistance and phenotypic selection for ALS resistance. Yellow bean breeding lines were developed that have resistance to BGYMV, BCMV, and common bacterial blight. Bean breeding lines from Oregon State University had high levels of resistance to the common bean weevil.

Greenhouse trials conducted in Honduras identified lines with higher nodulation scores and greater root and shoot dry weights under low N conditions. The soil cylinder technique was used at Zamorano to measure the response of bean lines to specific strains of *Rhizobium*. Inbred-backcross populations were developed to study the expression of nodulation and  $N_2$  fixation traits. The most promising  $F_4$  lines derived from crosses between diverse parents having good nodulation will be recombined to form the second cycle of recurrent selection for enhanced biological nitrogen fixation. Significant *Rhizobium* strain x bean line interaction for seed yield was observed in a field trial planted at Isabela, Puerto Rico. Lines were identified that nodulated and yielded well when inoculated with either strain 899 or 1597.

USDA and UPR scientists collaborated in the identification of the dominant gene, *Xap-1*, that confers resistance to common bacterial blight. The response of common bean to ashy stem blight was evaluated in the field and greenhouse using a detached-leaf inoculation technique. ENM-Few/RVe primers showed potential for indirect selection for the *bc-3* gene. The web blight and root rot reactions of nine *Rhizoctonia solani* isolates from bean leaves and roots were studied in the greenhouse. There were significant bean line x isolate interactions for both web blight and root rot reactions. PR0401-259 had the best overall resistance to web blight.

There were significant differences among Lima bean landrace varieties for seed type, leaf and pod type, days to flowering, seed yield, and concentration of HCN in the leaves and seed. The Caribbean collection of Lima bean landraces was sent to CIAT for preservation in the germplasm collection. The diversity of Angolan cowpea germplasm, in relation to a diverse worldwide collection, was evaluated through phenotypic characterization in two field trials planted at Isabela, Puerto Rico, and for seed elemental composition in the laboratory. The initial seed elemental composition results indicate some unique nutritional characteristics of Angolan germplasm, including high protein

and iron content. Tepary breeding lines were developed from crosses between elite germplasm resulting in lines with increased seed size, improved architecture characteristics, and bacterial blight resistance. These lines are being tested in Honduras, the United States, Angola, and Puerto Rico.

Two technicians completed M.S. degrees in Plant Breeding at the University of Puerto Rico, Mayagüez, and returned to Angola to work in common bean and cowpea breeding. One student from Puerto Rico completed his M.S. degree at the University of Puerto Rico in plant breeding. Two students completed B.S. degree training at Zamorano. Short-term training was provided for Pablo Vargas (Zamorano student) at the University of Puerto Rico and at the USDA-ARS-TARS.



## Project Justification and Objectives

Common bean is an important source of protein for low income families in Central America, the Caribbean, and Angola. Increased or more stable bean yield can improve the diet and provide a reliable source of income for small-scale farm families in these countries. An increased supply of beans also benefits the urban bean consumer.

The development of improved bean varieties has proven to be an effective strategy to address biotic and abiotic factors that limit bean production in Central America and the Caribbean. During the past 10 years, however, only a limited number of black bean cultivars have been released in the region. This limited release of germplasm resulted from a lower level of investment in black bean breeding and less emphasis in Central America on the testing and on-farm evaluation of advanced black bean breeding lines by national programs. Consequently, black bean cultivars tend to have lower seed yield potential and less disease resistance than the most recently released small red bean cultivars. The most promising small red bean cultivars developed at Zamorano can be readily used to improve black beans. In fact, the lowland bean breeding project of the Bean/Cowpea CRSP initiated the development of black bean breeding lines, and a sizeable number of breeding lines have already been distributed to bean research network members in Nicaragua, Guatemala, and Haiti. Although this Dry Grain Pulses CRSP

project emphasizes field-testing of black bean breeding lines in Central American and Caribbean countries, the project also develops and releases Andean (red mottled, yellow, and light red kidney) bean lines that have resistance to BGYM, BCMNV, and other diseases of economic importance.

This research project is in the position to make significant impact in Central America and the Caribbean. Many small red and black bean breeding lines with enhanced disease resistance and tolerance to abiotic stress are already in an advanced stage of development. There is an established network of bean researchers in Central America with a proven capability of testing, releasing, and disseminating improved bean cultivars. The Dry Grain Pulses CRSP project will complement ongoing collaborative bean research in Central America. In addition, it has leveraged additional funds that will extend the potential impact of collaborative research in Haiti. The project also trains researchers in Angola based on the critical experiences and successes in Central America and the Caribbean.

Improved bean breeding lines developed by the Dry Grain Pulses CRSP bean breeding program in Central America and the Caribbean may be useful in

Angola, given the similarity in agroecological zones and production constraints. Some small red bean cultivars and breeding lines developed in Central America have

resistance to diseases (BCMNV, rust, angular leaf spot, and anthracnose) and tolerance to abiotic stresses (low soil fertility, drought, and high temperature) that are important constraints to bean production in Africa. Because there is increased interest in Africa in bean production at lower altitudes, Central American bean breeding lines with resistance to common bacterial blight and web blight may be of particular value to northeastern Angola, where small red beans are produced in hot and humid conditions. Although black beans are estimated to account for less than five percent of bean production in Africa, this seed type is often a component of mixtures grown in low fertility soils. The lowland bean breeding team has also developed Andean (red mottled and light red kidney) bean breeding lines with resistance to BCMNV and rust that may be useful in Eastern Africa. Angola, a major importer of pinto beans, may benefit from testing the BelDakMi bean breeding lines that have resistance to BCMNV and rust. We will collaborate with other Dry Grain Pulses CRSP projects and bean research networks in Africa (e.g., SABRN, ZARI, CIAT) in the evaluation of improved bean cultivars and breeding lines from the United States, Central America, and the Caribbean. Project personnel meet frequently to evaluate bean lines in nurseries and to exchange information at scientific meetings.

## Objectives

1. Development, release, and dissemination of improved bean cultivars for Central America, the Caribbean, and Angola
2. Selection of beans for adaptation to low N soils

3. Development of molecular markers for disease resistance genes
4. Evaluation of other dry pulse crops for Central America and the Caribbean

## Research and Outreach Approaches, Results, and Achievements

### Objective 1. Development, release, and dissemination of improved bean cultivars for Central America, the Caribbean, and Angola

Plant breeders focus on the combination of disease (BGYMV, BCMNV, rust, common bacterial blight, anthracnose, and angular leaf spot) resistance with enhanced resistance to pests (bruchid, leafhopper) and greater tolerance to abiotic stress (drought, low soil fertility, high temperature). Elite bean breeding lines with multiple disease resistance were crossed with sources of resistance to pests or tolerance to abiotic stress. Bean lines were screened for the selected traits in each generation in environments most likely to provide the desired abiotic or biotic stress. Regional performance trials for black, small red, and

red mottled bean lines were conducted in collaboration with national bean research programs in Latin America and the Caribbean.

Basic seed stocks of bean varieties developed and released

by the project were multiplied and small lots of seed were distributed to farmers in Latin America and the Caribbean for testing in on-farm trials. Performance in the on-farm trials provided bean breeders with valuable feedback. The project also produced basic seed stocks of the most promising bean breeding lines and made seed available to the national bean research programs and NGOs involved in the multiplication and dissemination of improved seed.

Promising bean breeding lines from Central America, the Caribbean, and the United States, primarily of medium-sized market classes, were provided to the Angolan bean research program for evaluation for local adaptation and consumer acceptance.

#### *Development of breeding populations*

Small red, black, and Andean bean breeding populations were developed and evaluated during the past year. The overall goal



is to combine resistance to diseases with drought and low fertility tolerance in improved cultivars and breeding lines. Parents used in the crosses included promising breeding lines, improved cultivars and landraces, and sources of disease resistance and tolerance to abiotic factors from Zamorano, the UPR, the USDA-ARS, and CIAT. Some of these populations were developed for greater adaptation to the highlands of Honduras, Guatemala, and Haiti, while others for the lowlands of all Central American countries and Haiti, and others for Angola. During the past year,  $F_1$  populations were developed and  $F_2$  plants were evaluated and selected for highly heritable traits. Crosses were made in Honduras to improve small red landraces carrying the Rojo de Seda bean seed type for Central America and black bean cultivars for Guatemala and Haiti. Populations derived from crosses including local landrace cultivars were developed using participatory plant breeding (PPB) approaches in collaboration with farmer groups and researchers from El Salvador, Honduras, and Nicaragua. Early generation populations were developed at the University of Puerto Rico from crosses among sources of disease, pest resistance, and tolerance to low N soils. During the past year, individual plants were selected in  $F_3$  and  $F_4$  generations based on agronomic characteristics and seed type (black, red mottled, and yellow). Lines will be screened in later generations for disease and pest resistance and tolerance to low N soils.

Breeding populations were derived from crosses between landrace x improved cultivars to improve the most common Honduran small red bean landraces. Selected lines from these populations are currently under validation in farmer fields in most bean production areas in Honduras.

During current funding, we have made a major effort to develop superior black bean cultivars for Guatemala and Haiti, which has led to the release of several black bean cultivars during the past few years. In addition, promising black bean cultivars are currently being tested in field trials in Nicaragua, where black bean production for export has increased. Breeding populations derived from crosses were tested in 2009–10 and promising lines from these populations are being evaluated in farmer fields.

Inbred backcross populations for developing small red and black bean cultivars that combine BGYMV, BCMV, and BCMNV resistance for Central America and the Caribbean were generated and advanced to early generations using the black bean cultivar XRAV40-4 and the small red breeding line PR9825-49-4 as BCMNV resistance sources.  $F_4$ - $F_5$  families have been selected for superior agronomic performance, desirable seed traits, and SCAR markers for BGYMV and BCMV resistance. The black bean lines under development should also be useful for bean production in Haiti and other Caribbean countries, where resistance to BGYMV and BCMNV in combination with other disease resistance genes and adaptation to production constraints, such as limited rainfall and low soil fertility, are necessary.

Germplasm collected in Angola representing predominant market classes (medium sized yellow, green, and white types; and large seeded cranberry and kidney types) grown in the major

common bean growing regions were evaluated in Puerto Rico. All of the Angolan landraces were found to be susceptible to CBB and BCMV, and they were largely susceptible to ALS. Several of the Angolan lines showed good BNF potential, while most were not well adapted in Puerto Rico to high temperatures. Based on testing of improved varieties and breeding lines in Angola over the past four years, genotypes were selected that are adapted to Angola and have resistance to BCMV, BCMNV, CBB, and ALS. Populations were developed in 2007, 2008, and 2009 at USDA-ARS, based on crosses between the commercial seed types used in Angola with the sources of disease resistance. Marker assisted selection and field and greenhouse evaluations were used to select breeding lines with CBB and BCMV resistance. In total, more than 400  $F_3$  lines, 130  $F_{4,5}$  lines, and 46  $F_{6,7}$  lines were sent for evaluation by Monica Martins in Huambo, Angola, in the fall of 2011.



Figure 1. Bean seed types found in the public market in Huambo, Angola.

The yellow bean is a preferred seed type in Haiti and Angola. Azufrado bean breeding lines from Mexico were obtained and crossed with red mottled lines with multiple disease resistance. Marker-assisted selection and greenhouse evaluations were conducted in Puerto Rico to identify lines that have genes for BGYMV and BCMV resistance. Lines were also selected in the field for common bacterial blight resistance. A group of the most promising yellow bean breeding lines will be tested in Haiti and Angola during the upcoming year.

The common bean weevil is a major seed storage pest. The University of Puerto Rico received black and light red kidney breeding lines that were expected to segregate for resistance to the bean weevil. Plastic, 150 ml cups containing 20 seeds were infested with 20 adult bean weevils. Date of first emergence was noted and damage to the seed was measured 100 days after infestation. The lines were evaluated in two trials during 2011. Seed of both Andean and Middle American bean cultivars were severely damaged by the bean weevil (table 1). Three light red kidney lines from the OSU populations had useful levels of resistance. Most of the seed of the resistant lines was undamaged 90 days after infestation. Seed of resistant lines had greater than or equal to 65 percent seed without holes. The only other line with a similar level of resistance was RAZ 25.  $F_2$  populations and  $F_3$  lines derived from these crosses were planted at Isabela, Puerto Rico, in July 2011. Individual plants with local adaptation will be screened in the greenhouse for reaction to the NL3 strain of BCMNV. Resistant lines will be screened for the presence of the *bgm-1* gene using the SR-2 and for the *I* gene using the SW13 SCAR markers.

Identification	Origin	Seed type	Total # of holes in the seed	Percent seed without holes	Percent seed weight lost
AO-1012-27-2	OSU	Red kidney	9.5	65.0	0.0
AO-1012-29-3	OSU	Red kidney	14.5	75.0	0.0
AO-1012-31-4	OSU	Red kidney	14.0	65.0	18.8
Badillo	UPR	Red kidney	129.0	0.0	45.0
INIAP Fanesquero	Ecuador	White kidney	122.0	0.0	25.0
RAZ 25 ( <i>Arc-1</i> )	CIAT	Red mottled	13.5	62.5	16.7
INIAP Portillo	Ecuador	Red mottled	180.5	0.0	37.5
INIAP Yungilla	Ecuador	Red mottled	132.5	0.0	37.5
INIAP Concepción	Ecuador	Red mottled	150.0	0.0	37.5
PR9745-232	UPR	Red mottled	125.5	0.0	35.8
Catarina	Angola	Cranberry	132.0	0.0	37.5
Calembé	Angola	Green	143.0	0.0	25.0
Canaria	Angola	Yellow	129.5	0.0	25.0
Verano	UPR	White	150.0	0.0	25.0
Morales	UPR	White	112.5	0.0	25.0
RAZ 75	CIAT	Small red	23.5	35.0	16.7
INTA Precoz	Guatemala	Small red	120.5	0.0	37.5
DEHORO	Zamorano	Small red	128.0	0.0	33.3
Amadeus 77	Zamorano	Small red	122.5	0.0	33.3
Carrizalito	Zamorano	Small red	106.5	0.0	33.3
CENTA Pupil	Zamorano	Small red	110.0	0.0	25.0
RAZ 50	CIAT	Black	79.5	10.0	16.7
Aifi Wuriti	Zamorano	Black	140.5	0.0	33.3
DPC 40	Dom. Rep.	Black	103.5	0.0	40.0
ICA Pijao	Colombia	Black	109.0	0.0	25.0
Mean			93.8	9.0	30.7
LSD(0.05)			18.0	8.9	12.6
CV(%)			9.7	50.1	20.7

Table 1. Damage caused by bean weevil (*Acanthoselides obtectus*) in 20-seed samples of common bean lines of diverse origin.

#### Regional performance trials

Advanced breeding lines were derived from crosses between disease resistant and abiotic stress tolerant parental lines from Zamorano, the UPR, USDA-ARS-TARS, CIAT, and NBP. These advanced lines have resistance to the major diseases and enhanced levels of tolerance to at least one abiotic factor, good agronomic adaptation, and commercially acceptable seed type.

More than 50 small red and black bean breeding line VIDAC nurseries and ECAR trials were distributed to collaborators from the National Bean Programs (NBP) from Central America and the Caribbean. In addition, more than 60 elite lines were evaluated in COVAMIN (high iron/zinc biofortified lines), PASEBAF (low fertility, drought tolerant lines), and ERMUS (web blight tolerant lines) trials. More than 200 on-farm cultivar validation trials, including improved cultivars (ICTAZAM, Aifi Wuriti, XRAV40-4, ICTA Sayaxché, ICTA Petén), promising lines from the high minerals and drought and low fertility trials, and lines from populations developed to improve landrace cultivars, were conducted in collaboration with researchers from NBP, NGO, and farmer groups in Central America and the Caribbean. Results from these trials identified several promising breeding lines for additional testing. During the upcoming year, at least three bean cultivars are expected to be released in Guatemala, Honduras, Nicaragua, and/or El Salvador.

More than 40 regional trials, including drought and heat tolerant small red and black bean cultivars and breeding lines from the CA/C region, were prepared and distributed to NBPs of Costa Rica, Honduras, Panamá, Nicaragua, El Salvador, and Guatemala, as part of the collaboration with the Red SICTA Project for climatic change adaptation. In Honduras, these trials were distributed to collaborators. In addition, the project is participating in the regional evaluation of a Bean Adaptation

Trial from CIAT to study climatic change adaptation in common beans using the DSSAT model.

Six ERMUS trials including web blight resistant lines from the first and second cycle of recurrent selection were distributed to NBPs in CA/C. The ERMUS 2011 trials included 20 advanced lines selected from the previous year that combine WB, BGYMV, and BCMV resistance, agronomic adaptation and commercial seed types and five checks.

The UPR developed red mottled bean lines that combine resistance to BGYMV, BCMNV, BCMV, and common bacterial blight. Seed of these lines was increased in Puerto Rico during the past year. The red mottled lines were also evaluated in trials planted in Haiti, the Dominican Republic, and Puerto Rico. PR0637-134 and PR0737-1 were among the highest yielding lines in the trials. These red mottled lines, which have the *hgm* gene for BGYMV and the *bc3* gene for BCMV and BCMNV resistance, also showed resistance to common bacterial blight and powdery mildew in Puerto Rico in 2011.

#### On-farm validation of promising breeding lines

On-farm validation trials were conducted in Central America in collaboration with the National Bean Research programs, Local Agricultural Research Committees (CIAL), NGOs, and other extension organizations. The PASEBAF validation trial included drought, low fertility tolerant lines developed with support from the Dry Grain Pulses CRSP, Red SICTA. The Agrosalud (COVAMIN) trials, which included small red lines with greater mineral content (iron and zinc), were conducted in Central America. During FY11, Agrosalud lines were released as cultivars in Nicaragua and El Salvador. During 2012, it is expected that at least one of these biofortified lines will be released as a cultivar in Honduras. During the past year, packages of five of the most popular improved small red cultivars from El Salvador and other Central American countries were provided to the FAO Seed Project for on-farm validation in Guatemala.

Zamorano collaborated with technical personnel from Catholic Relief Services (CRS) in the evaluation of small red bean cultivars and promising bean breeding lines for adaptation to the western region of Honduras. Two types of trials were provided for on-farm testing to CRS. The COVAZA trial included seven improved cultivars and a local check variety adapted to highland conditions of Honduras. The COVABI trials included seven small red cultivars and advanced lines adapted to lowland to intermediate conditions and a local check. Three small red cultivars were selected from the COVAZA trial by participating farmers and are currently in on-farm validation using larger plots.

Bean landraces with unique seed traits are still considered a valuable germplasm for bean export, due to preferences associated with seed color and taste. However, most landraces are susceptible to the major bean diseases and have lower yield potential than improved cultivars. Advanced bean breeding lines derived from crosses between landraces x improved cultivars are being validated in Honduras as part of the development of cultivars that combine the seed traits and earliness of the landraces with disease resistant and yield potential of the improved cultivars.

Validation trials including two to three advanced lines and the landrace check cultivar derived from the Honduran landraces Paraisito, Cincuentaño, Marciano, and Rojo de Seda, are being conducted in farmer fields at different locations in Honduras.

#### *Release of cultivars and seed multiplication*

In February 2010, the Instituto Dominicano de Investigaciones Agropecuarias y Forestales (IDIAF) formally released DPC-40. This black bean variety was developed in collaboration with the University of Nebraska and the University of Puerto Rico with support from the Bean/Cowpea CRSP. This black bean cultivar combines resistance to BGYMV, BCMNV, and BCMV and will be sold to the private sector in Haiti for multiplication and sale to farmers.

The pink bean line PR0401-259 and the black line PR0650-31 were released as improved germplasm. Both lines have the *I* gene conferring resistance to BCMV and high levels of resistance to common bacterial blight (CBB) and moderate levels of resistance to web blight. PR0401-259 also has the *bgm* gene for resistance to BGYMV. The white bean breeding line PR0634-13 was released *Beniquez* and has resistance to BCMV, BCMNV, and BGYMV.

The small red cultivar *Paisano PF* was released in 2011 in Honduras. The small red cultivar *CENTA Chaparrastique* was released in El Salvador during 2011, as a BGYM and BCMV resistant, high yield potential cultivar with commercially desirable seed type. In Guatemala, the black bean cultivars *ICTA Petén* and *ICTA Sayaxché* were released in FY10 and disseminated in FY11.

TARS-MST1 and TARS-MST2 were developed by the USDA-ARS, the University of Nebraska, and the University of Puerto Rico. These black bean lines were selected for multiple stress tolerance, including tolerance to high ambient temperature and drought stress. Both lines were found to carry the *I* gene.

#### **Objective 2: Selection of beans for adaptation to low N soils**

Inadequate soil nitrogen is a frequent seed yield constraint for common beans in the Tropics. The use of nitrogen fertilizers increases production costs and can contribute to groundwater contamination. Researchers have pointed out the need to develop integrated soil nutrient management practices for beans that would combine biological nitrogen fixation (BNF) with limited use of fertilizers, sustainable crop management practices, and the development of crop varieties better adapted to low fertility soils. Bean varieties with greater efficiency in the utilization of nitrogen should have enhanced BNF capacity, root traits such as greater root hair density that contribute to tolerance to low soil P, and healthy root systems that can take advantage of available soil nitrogen and other nutrients.

Recurrent selection (RS) has proven to be useful in the selection of quantitatively inherited traits, such as web blight resistance and tolerance to low soil P. The project has used recurrent selection to develop Mesoamerican breeding lines with greater adaptation to low soil N. Preliminary screening conducted in

Honduras and Puerto Rico has identified disease resistant bean breeding lines that were used to form the base population for recurrent selection. A few elite small red bean breeding lines from Zamorano were found to have good biological nitrogen fixation when evaluated in field trials in Minnesota. The root rot resistant black bean line PR0443-151 from Puerto Rico and the CIAT bean breeding line VAX 3 have performed well in low N soil in Puerto Rico. During the past five years, the Zamorano bean breeding program and Dr. Jonathan Lynch have collaborated in the development of small red and black bean breeding lines with greater tolerance to low P soils and drought. Some of these lines also have better yield under low N soils due to increased nodulation by resident rhizobia. Black bean lines developed at the University of Puerto Rico will serve as a source of root rot resistance. Breeding lines were evaluated in the F<sub>3</sub> and F<sub>4</sub> generations in replicated field trials. The field trials received low levels of N fertilizer. The bean lines were inoculated with recommended bean *Rhizobium* strains to create conditions favorable for biological nitrogen fixation. The most promising lines from each cycle of recurrent selection will be included as entries in regional performance trials in Central America and the Caribbean.

Greenhouse trials were conducted in Honduras to identify lines with better performance under low N conditions by expressing greater nodulation and BNF along with other mechanisms that allow beans to have greater accumulation of dry matter and seed yield under low N. A preliminary trial including 180 bean accessions from the working collection of the Zamorano breeding program was inoculated with a mixture of two *Rhizobium* strains. Significant variation among bean lines for nodulation using a 1 to 9 scale (1= none or very few, small nodules; 9= maximum number of large nodules), root, shoot, and total dry weight (DW), and root/shoot ratio were observed. The cultivars and lines with higher nodulation scores also had greater root, shoot, and total DW. The best nodulation was observed in the *Rhizobium* inoculated treatment without N; and the greatest root, shoot, and total plant DW were observed in the + N treatments; both were superior to the no-inoculation and - N treatments.

Experiments were conducted in Honduras using the soil cylinder technique containing a soil:sand (1:2) substrate low in N to study the response of selected genotypes to inoculation with *Rhizobium* strains and to identify potential parents for a recurrent selection program for high nodulation and N<sub>2</sub> fixation.

Additional BNF studies in Honduras included testing the response of 50 inbred-backcross (IB) lines to inoculation with strains CIAT 899 and CIAT 632 under low fertility conditions. These IB lines have Amadeus 77 genetic background and were developed with support from the EAP/Penn State University DGP CRSP project (PI-PSU-1) to study the adaptation of bean lines and multilines to low soil fertility.

During the current year, F<sub>4</sub> families from 25 bean populations derived from crosses between diverse parents with good nodulation ability were screened in Honduras under greenhouse conditions using a soil:sand (1:1) substrate low in total N (0.08 percent) and organic matter (1.7 percent). The 20 best F<sub>4</sub>

families from different populations will be recombined using a partial diallel crossing design to develop the populations for the second cycle of recurrent selection for nodulation.

The nodulation patterns of 20 bean lines selected from previous studies for adaptation to low N conducted in Honduras and Puerto Rico, and from a group that included the majority of small red and black bean cultivars released by the project in the CA/C region, were characterized in a low N soil using inoculation with three *Rhizobium* strains. Differences in nodulation, root traits, plant growth, and yield were observed among the cultivars and lines included in these trials. Results from these trials will be used to identify the most useful cultivars and lines for further hybridization and selection for greater nodulation and better adaptation to low N soils.

Field experiments conducted over a two-year period in Puerto Rico identified breeding lines with greater N use efficiency in low N soils. In trials planted at Isabela, Puerto Rico, the mean seed yields of the black bean line PR0443-151 and the small red lines VAX 3 and IBC 309-23 ranked no lower than 9th in the N plots. Populations were developed using PR0443-151 as a parent to develop breeding lines that combine adaptation to low N soils with resistance to BCMV and BGYMV. Four black bean lines were identified that combine the disease resistance with superior performance in low N soils. Seed of these breeding lines were sent to Haiti and Honduras for further evaluation.



*Phaseolus vulgaris* is naturally nodulated by different *Rhizobium* strains. The most important species are *R. tropici* and *R. etli*. Promiscuity of the host and unfavorable environmental conditions can limit inoculation response. The successful introduction of inoculants depends on an efficient interaction between the *Rhizobium* strain and *Phaseolus* genotype. Bean cultivars and USDA-TARS and UPR bean breeding lines were evaluated for their efficiency to nodulate with *R. tropici* and *R. etli*. In 2011, two field experiments consisting of 15 lines and strains, *R. tropici* CIAT 899 and *R. etli* UMR 1597, were conducted at the Isabela and Fortuna Substations. At the Fortuna Substation, there were significant differences among lines and strains in the nodule position at 20 days after planting. Soil rhizobia plant infection counts were considered high at 10<sup>4</sup> rhizobia per gram of soil. Among the genotypes evaluated, PR9745-232 (Andean) nodulated with CIAT 899 in the upper 1 cm of the tap root had greater number of nodules when

compared to strain UMR 1597. In contrast, lines PR0401-259 and 10IS-2421 had greater nodule numbers in the upper one cm of the tap root with UMR 1597. Cultivars *Verano* and *DPC-40* nodulated between one and two cm of the upper part of the tap root and had between 10 to 20 nodules, which compared to the best performing cultivars. Root and shoot dry weights were not different between strains and lines. However, there were significant differences among lines for seed yield. The highest yielding lines were Beniquez, DPC-40, Verano, and PR0401-259. The small red line 10IS-2423 produced a mean yield > 2,000 kg/ha. Verano, Morales, and Beniquez also performed well. The red mottled line PR0737-1 had the best overall performance for an Andean bean line. The pink bean line PR0401-259 nodulated well with CIAT 899 and UMR 1597.

### Objective 3: Development of molecular markers for disease resistance genes

Marker-assisted selection has proven to be a very useful tool for bean breeders. Unfortunately, molecular markers are not available for some important genes and the use of other molecular markers is often limited to either the Andean or Middle American gene pools. The development of new molecular markers for valuable traits or markers with greater versatility would benefit the entire bean research community.

Resistance to charcoal rot caused by *Macrophomina phaseolina* has been associated with drought tolerance and it has been recommended that breeding for terminal drought tolerance should include breeding for resistance to charcoal rot. The charcoal rot resistance in the breeding line BAT 477 was found to be controlled by two dominant complementary genes. The utility of these markers has not been confirmed because the presence of the markers has not been surveyed in susceptible lines and in other sources of resistance to charcoal rot. The Dry Grain Pulses CRSP project will evaluate the usefulness of the putative molecular markers. If proven to be useful, Dr. Tim Porch will convert these RAPD markers to SCAR markers.

Common bacterial blight is a broadly occurring and significant disease of common beans. QTL for this trait have been identified but single genes have not. To facilitate breeding for this trait, scientists collaborated in the evaluation of its genetics using bi-parental populations and molecular marker analysis to identify tightly linked markers to a newly identified single dominant gene.

Bean common mosaic virus and the necrosis virus are significant diseases for common bean production. The *bc3* gene is an important recessive resistance gene, but molecular markers are not yet available for this locus. In a recent study, a eukaryotic translation initiation factor (eIF4e) marker was found to co-segregate with BCMV resistance. Thus, PveIF4E appears to be associated with *bc-3* resistance and thus has potential for testing as a molecular marker. The ENM-FWe/RVe primers will be tested for association with the *bc3* gene in known genotypes of common bean through a CAPs assay, using a restriction enzyme, RsaI, after PCR amplification. If the CAPs marker is associated with *bc3*, then it will be used in segregating populations for verification of this new marker.

The RAPD markers previously reported to be linked to genes for charcoal rot were screened with a set of susceptible and resistant genotypes. Seven susceptible genotypes and eight resistant genotypes were tested. RAPD B386<sup>900</sup> (coupling) was not amplified in BAT 477 nor in other resistant genotypes, while B459<sup>1600</sup> (repulsion) was not amplified in any susceptible genotypes. Bands of other sizes were amplified with each RAPD marker but were not associated with resistance. The PCR cocktail and PCR amplification conditions were then modified to optimize amplification and to reproduce the reported bands, but they were not reproducible. Consultation with another group working with *Macrophomina phaseolina* in common bean confirmed that B386<sup>900</sup> and B459<sup>1600</sup> do not have utility for charcoal rot (Mayek, pers. comm.).

Because the putative RAPD markers were proven to be ineffective, recombinant inbred lines (RILs) from crosses between BAT 477 and susceptible bean lines were pursued for the development of novel markers.

A detached leaf technique for *Macrophomina phaseolina* evaluation has been implemented for screening the BAT 477 x DOR 364 RIL population. Significant differences were found among RILs in the population and some lines were identified in which seed yield and detached leaf score corresponded. The experiment is being replicated to attempt QTL analysis for detached leaf response in common bean. A new RIL population is also being generated for the evaluation of *Macrophomina* using superior lines from the BAT 477 x DOR 364 RIL population as resistant parents. The generation of a population from highly differential parents will facilitate phenotypic and genotypic screening. USDA and UPR scientists collaborated in the identification of the dominant gene, *Xap-1*, which confers resistance to common bacterial blight of beans the first report of a gene for resistance to this disease. The ENM-FWe/RVe primers, linked to the *bc3* gene, were optimized for amplification at the USDA-ARS. The primers were found to be associated with the *bc3* gene in known genotypes of common bean through a CAPs assay. Preliminary results suggest that this marker has potential for use in marker-assisted selection.

*Rhizoctonia solani* (*Rs*) is a widespread soilborne pathogen of common bean. This pathogen is a species complex classified in 14 anastomosis groups (AG). Some AGs can cause web blight (WB), one of the most important diseases of bean plants in the Caribbean, while others are responsible for root rots (RR). Knowledge of these subgroups and their interactions with plant hosts contributes to a better understanding of virulence patterns of the pathogen and may lead to more effective strategies to breed beans with resistance to WB and RR. Nine *Rs* isolates from bean plants expressing WB or RR symptoms were compared by measuring differential reactions among bean lines using a detached-leaf inoculation method. The same *Rs* isolates were also used to inoculate the roots of the differential bean lines using a mycelia solution. The *Rs* isolates obtained from the bean roots at the USDA-ARS Research Farm near Isabela, Puerto Rico, were AG 4. *Rs* isolated from bean plants expressing WB symptoms were able to induce RR symptoms and vice versa. Significant line x isolate interactions were observed for the

detached-leaf inoculation and root rot inoculations for the three planting dates suggested a differential response of the host to the pathogen. In general, the WB 2 (AG 4) isolate had the most severe and the RR1 (AG 4) isolate had the least severe WB readings. The pink bean breeding line PR0401-259 had the best overall resistance to web blight.



#### Objective 4: Evaluation of other dry pulse crops for Central America and the Caribbean

The Lima bean (*Phaseolus lunatus* L.) is a heat and drought tolerant dry grain pulse crop produced and consumed throughout the Caribbean and in certain regions of Africa. Because Lima beans grow well in fence rows or on walls, the crop is well suited for urban agriculture. We collected and characterized the agronomic traits of 50 Lima bean landrace varieties from Puerto Rico and Haiti. Passport data were collected so that the germplasm can be included in the CIAT and USDA germplasm collections. Seed of superior Lima bean accessions will be increased for further evaluation and possible release in the country of origin.

Cowpeas are produced on a limited scale in the Caribbean. Potential areas of adoption of new cowpea lines are the semi-arid regions in northern Nicaragua and southern Honduras, where the crop is used as an alternative to common beans during the postrera season.

Tepary, a desert native species, has high levels of heat and drought tolerance and common bacterial blight resistance. However, small seed size, prostrate growth habit, and poor palatability have reduced its acceptance in areas outside of its center of origin. As a result of global warming, there is increased need for and interest in abiotic stress-tolerant legumes. To increase possible adoptability of this species, USDA-ARS-TARS, in collaboration with the UPR, initiated breeding of tepary for increased seed size and improved architecture.

Morphological, phenological, and agronomic traits of 55 Lima bean landrace varieties from Haiti, the Dominican Republic, and Puerto Rico were evaluated at Isabela, Puerto Rico. Collaborators at the University of Puerto Rico studied the genetic diversity of the landrace varieties using molecular markers. Another collaborator from the University of Delaware evaluated the Lima bean varieties for HCN concentration in leaves and seed. Photoperiod insensitive Lima bean germplasm having low HCN concentration in the seed were identified.



Seventeen lima bean accessions from the UPR collection were screened for adaptation in Honduras. When planted in Honduras, four landraces flowered less than 60 days after planting, suggesting that these varieties could be planted in Central America and the Caribbean throughout the year.

Nineteen cowpea lines from UC, Riverside, were screened for adaptation in Honduras and seven relatively short season lines were selected for further evaluation in Central America. Seed of the most promising accessions was increased during the primera planting season at Zamorano, and a yield and adaptation trial (ERCAUPI) was distributed for testing during the postrera season of 2011.

## Networking and Linkages with Stakeholders

Interspecific lines, originally developed in Puerto Rico for web blight resistance, were screened at the University of Idaho for white mold resistance. Four lines were identified that had high levels of resistance to white mold. Seed of these lines was increased in Puerto Rico and sent to Dr. James R. Steadman at the University of Nebraska for evaluation in the W-2150 Regional Hatch Project White Mold Nursery.

The UPR bean breeding program collaborated with Dr. Graciela Godoy-Lutz, Instituto Dominicano de Investigaciones Agropecuarias y Forestales (IDIAF) plant pathologist, in the preparation of a proposal that was submitted and approved by the Consejo Nacional de Investigaciones Agropecuarias y Forestales (CONIAF). Although the project will not provide any additional funding for research in Puerto Rico, it provides an opportunity to continue to test the most promising lines from the DGP CRSP breeding programs in the Dominican Republic. This collaboration is expected to result in the release of additional disease resistant black and red mottled bean cultivars. The project received seed of five black bean lines from Ing. Julio Cesar Nin, IDIAF bean breeder in the Dominican Republic.

BGYMV and BCMV are important production constraints for snap bean producers in Costa Rica. The UPR bean breeding program provided Ing. Juan Carlos Hernández, Ministry of Agriculture bean researcher in Costa Rica, with seed of snap bean breeding lines that have resistance to these viral diseases. The lines were evaluated in Costa Rica during 2010 and 2011 using participatory plant breeding techniques and selected by farmers for additional testing.

## Leveraged Funds

Scientists involved in this project have effectively used Dry Grain Pulses CRSP support and institutional linkages to leverage more than \$3.3 million in external funding in 2011 to achieve objectives related to this project.

## Publications

Beaver, J.S., M. Zapata, M. Alameda, T.G. Porch and J.C. Rosas. 2011. Registration of PR0401-259 and PR0650-31 dry bean germplasm lines. *J. Plant Reg.*

Mbui Martins, M. 2011. Desarrollo de líneas de frijol (*Phaseolus vulgaris* L.) con mayores niveles de resistencia a las enfermedades. M.S. Thesis. University of Puerto Rico, Mayaguez Campus.

Ndengoloka David, A. 2011. Estudio de la diversidad genética de germoplasma de caupí [*Vigna unguiculata* (L.) Walp.] de Angola y un grupo de germoplasma que representa otras regiones del mundo. M.S. Thesis. University of Puerto Rico, Mayaguez Campus.

Porch, T.G., C.C. Urrea, J. S. Beaver, S. Valentin, P. A. Peña, and J. R. Smith. 2011. Registration of TARS-MST1 and SB-DT1 multiple-stress-tolerant black bean germplasm. *J. Plant Reg.* doi: 10.3198/jpr2010.08.0501crg; published online 8 August 2011.

Ruíz Quiles, L.A. 2011. Desarrollo de líneas de habichuela (*Phaseolus vulgaris* L.) con resistencia a BCMV, BGYMV y mejor adaptación en suelos bajos en nitrógeno. M.S. Thesis. University of Puerto Rico, Mayaguez Campus.



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

ISU-2

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## Abstract of Research Achievements and Impacts

This BNF CRSP project is designed to identify production systems that enhance biological nitrogen fixation (BNF), develop germplasm that benefits most from symbiotic inoculation, and aggressively share this 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 nonnodulating 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 a 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
- completion of a draft survey tool to assess farmer knowledge, practices, and attitudes (KPA) about bean seed inoculation
- hiring field staff to monitor and coordinate extensionist and farmer trainings on inoculant technology

## Project Justification and Objectives

Common beans are the most important legume crop in Uganda, Rwanda, and Tanzania, occupying a large proportion of land devoted to legumes. More than 45 percent of the protein intake by Ugandans comes from beans providing 25 percent of dietary calories. Likewise, more than 75 percent 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 approximately 38 percent of

utilizable protein and 12–16 percent of daily caloric requirements. Improved bean production in Uganda, Rwanda, and Tanzania offers 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 that promote plant growth and overall plant health.

Although common bean has the potential for BNF, it is reported to have the lowest percent N<sub>2</sub> 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 would 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 project also contributes directly to USAID Mission's "Feed the Future" strategic development objectives. Activities are focused in three of the five 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 smallholder farms. The Uganda Mission in particular identified "Beans for Nutrition" as a priority value chain to reduce poverty and increase the sustainability of livelihoods and the economic empowerment of women. The outreach component of this project contributes to USAID's mission of strengthening producers' organizations by working with individual farmers and farmers groups.



## Objectives

1. 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.
  - a. Evaluate the effectiveness of biological inoculants on local and improved germplasm.
  - b. Quantify genotype by environmental interactions and constraints to enhancing BNF of inoculated plants.
2. Examine the inheritance of genetic and environmental variation in BNF in common bean, and identify molecular markers associated with QTL conditioning for enhanced BNF.
  - a. Identify parental materials for inheritance studies of BNF.
  - b. Phenotype existing mapping populations for BNF response, populate with molecular markers, and conduct QTL analysis.
3. Improve the productivity, profitability, and sustainability of agricultural systems on degraded soils through effective dissemination of new information and technologies to smallholder farmers.

- a. Improve farmer awareness of inoculation technologies.
- b. Prepare for on-farm demonstrations comparing inoculant strategies.
- c. Strengthen farmers' collective capabilities to purchase inoculants and incorporate them into a profitable and sustainable system for small landholders.

## Research and Outreach Approaches, Results, and Achievement

**Objective 1: 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.**

- 1a. Evaluate the effectiveness of biological inoculants on local and improved germplasm.

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 nonnodulating lines of common bean and increase seed for field trials, quantify yield responses to inoculation in field trials established at U.S. collaborating institutions and host country sites, and establish a website for posting, storing, and sharing protocols and results.

- 1b. Quantify genotype by environmental interactions and constraints to enhancing BNF of inoculated plants.

### Field studies

- Complete analysis of plant/soil/weather data.
- Establish a weather database for all field sites.
- Calculate seasonal and long-term (five-year) soils temperatures 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 at all U.S. 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.
- Analyze nodule DNA for indigenous strains.

*Results: Host Countries.* Field experiments involving NaCRRRI and Makerere University continued in Namulonge (central Uganda), Mbarara and Kabale (southwestern Uganda). Treatment remained since three rhizobia types were sourced from the United States (Becker Underwood) and from the Universities of Nairobi and Makerere. A control without inoculation was also included to represent the indigenous

strains. Other study factors included six bean varieties (three bush and three climbing). As the most constraining nutrient in the soils of east Africa yet crucial to effective BNF, Phosphorus was considered as a treatment (0 and 40 kg P ha<sup>-1</sup>) to evaluate the extent the imported rhizobia could withstand the limited P supply in the soil. There were 72 treatment plots.

Soil and weather data were collected from each research site. 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 for a range of chemical and mineral parameters. While most soils were low in N and P, a potentially more challenging concern in these soils is Boron (B) deficiency. 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.

Data were collected and are being processed for leaf area index, 100 seed weight, and nodulation score. The nodulation score computation involves assessing plant vigor, number of nodules with leghemoglobin pigment, and nodule position.

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 for evaluation in other countries. Treatments included P application (P+ and Po), Rhizobia 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. Phosphorous application was the main plot, rhizobia inoculation as subplots, and genotypes as subsub 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 pods per plant, number of seeds per pod, and seed yield and nodule weight

All data except for seed N and nodule weights (which is still being processed) were analysed. For the Tanzania HC trials, there was a slight yield advantage from using the local inoculant (NitroSUA) at one location; 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; the control plot had the highest yield at both locations.

Although we are currently in the process of interpreting these field results, it is clear the varieties varied considerably in response to P+ and inoculation in grain yield, yield components, and leaf N. Uneven stands and irrigation are possible causes of 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.

*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, with the number of genotypes tested expanded to 36. The 2011 plots have been harvested and yield data are being analyzed.

Eleven nonnodulating lines representing Andean and Middle American origins and tropical, subtropical, and temperate adaptation were obtained from CIAT in August. Twenty seeds of each line were obtained. Five seeds of each were sent to Karen Cichy, six seeds to Susan Nchimbi, five seeds 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 2011, we collected three representative plants from each plot (~400 plots total). 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<sup>14</sup>/N<sup>15</sup> and total N analysis. For each plot, seed size, yield, 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; yield data are currently being analyzed.

Soils were collected from field sites tested for indigenous rhizobia strains and sent to Washington State University for analysis. Testing is underway. DNA has been collected from nodules of A-55 and F-122, five plants (reps) per variety x site. A variable section of the 16SrDNA has been amplified in preparation for deep sequencing (454 analysis). 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 one mL soil extract diluted at 10<sup>-2</sup>, 10<sup>-4</sup>, and 10<sup>-6</sup>. Roots were inspected after two weeks and very few nodules had formed. There were not enough nodules to give a reliable population estimate or to collect a community for study. After two more rounds of soil inoculation using increasing amounts of soil (five g per plant in the end), good nodule populations were obtained. These samples are now being analyzed.

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 three 16S sequences we have analyzed. All three sequences are characteristic of Enterobacteriaceae. Genera *Serratia*, *Klebsiella*, and *Pantoea* give the closest matches (98 percent+) 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, since 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  $^{15}\text{N}$  dilution. Nodules were counted, and above- and below-ground dry biomass was measured. Samples are in queue for  $^{15}\text{N}$  measurement, from which we will determine the proportion of plant N fixed from atmospheric  $\text{N}_2$ .

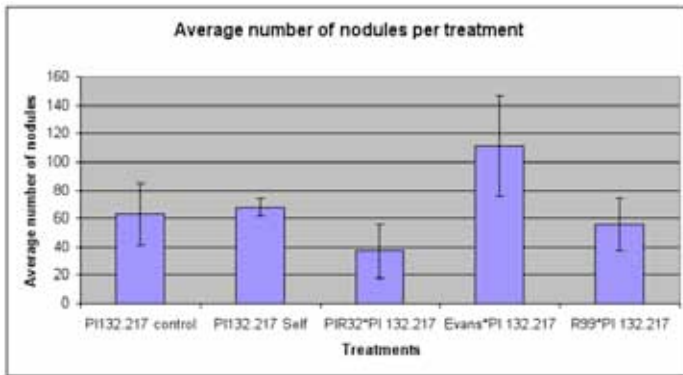


Figure 2a. Nodule number on roots of grafted plants using the soybean line PI132.217 as the scion grafted onto various rootstocks. PIR32 and R99 are bean lines. R99 is a nonnodulating line under normal condition. Evans and PI132.217 are soybean lines varying in seed N. Note the large number of nodules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

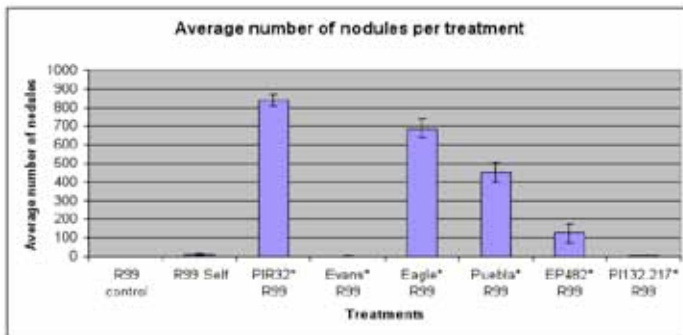


Figure 2b. Nodule number on roots of grafted plants using the non-nodulating line (R99) as the scion grafted onto various rootstocks. PIR32, Eagle, Puebla, and EP482 are bean lines varying in capacity for BNF. Evans and PI132.217 are soybean lines varying in seed N. Note the large number of nodules formed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

Preliminary studies were undertaken at ISU to test the potential to increase nodulation and capacity for  $\text{N}_2$  fixation by grafting shoots of aggressive  $\text{N}_2$  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 nongrafted controls. Plants were harvested at flowering/podfill to assess nodule formation, total

plant N, and ureide concentrations. A portion of the data on nodule formation is included in this report (figures 2a and 2b). Nodules were observed on the nonnodulating line when cross-grafted with a soybean scion. Surprisingly, nodules also were formed on several bean lines when R99 (a nonnodulating 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.

#### Summary

- 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 U.S. and HC partners.
- Analyses of plant N, biomass, nodule classes, and 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 nonnodulating 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 has been completed.

#### Objective 2: Examine the inheritance of genetic and environmental variation in BNF in common bean, and identify molecular markers associated with QTL conditioning for enhanced BNF.

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

- 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 nonnodulating 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.
- Advance RIL populations currently available.

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

- Phenotype selected populations (Bean CAP and South American Core) for BNF response in U.S. 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 and MSU to identify and collect bean lines for a BNF diversity panel. Thus far, 333 bean lines have been collected for the panel. 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. 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 two Middle American checks including a nonnod 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. Vast diversity for BNF response in this subset of the Andean Diversity Panel is a very positive indication of the potential 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, Washington. 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 to analyze these Andean Diversity Panel lines with the available SNP chip(s), once the composition of Diversity Panel has been finalized.

Greenhouse screening trials for BNF response was initiated on selected lines at 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; shoot and root biomass and shoot N concentration were measured. As expected, the nonnodulating 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.

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 N<sup>14</sup>/N<sup>15</sup>. Significant

genotypic differences in seed protein and percent N from fixation were detected. 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 being processed to determine seed yield, yield components, and seed N.

A number of selected RILs have been advanced to the F<sub>3</sub> generation. F<sub>2</sub> seed of Puebla 152 x G08263 were planted in Frankenmuth, MI and F<sub>3</sub> seed were harvested to be advanced to F<sub>4</sub> 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. These genes were originally identified in *Arabidopsis* and then as *Phaseolus vulgaris* homologs by Dr. Carol Vance (University of Minnesota), who 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.

### Summary

- 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, and U.S. 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 have been identified for support of future studies to distinguish superior BNF lines.

### **Objective 3: Improve the productivity, profitability, and sustainability of agricultural systems on degraded soils through effective dissemination of new information and technologies to smallholder farmers.**

3a: Improve farmer awareness of inoculation technologies.

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

3b: Prepare for on-farm demonstrations comparing inoculant strategies.

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

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

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

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 to support HC evaluation and training activities and is being tested for

suitability on a subset of the 1200 farmers in the Uganda ISU/VEDCO rural livelihoods program.

### Summary

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

### 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 percent 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 U.S. institutions are female.

### Networking and Linkages with Stakeholders

All 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 the 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

Scientists involved in this project have effectively used Dry Grain Pulses CRSP support and institutional linkages to leverage US\$700,000 in external funding in 2011 to achieve objectives related to this project.



Bean seed production—Malawi.



# Improving Nutritional Status and CD4 Counts in HIV-Infected Children Through Nutritional Support

MSU-3

## Principle Investigator

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*Elizabeth Ryan, Colorado State University, USA*

*Reuben Kadigi, Sokoine University of Agriculture, Tanzania*



## Abstract of Research Achievements and Impacts

Much of the activities planned for FY11 were not completed because of difficulties related to ordering and receiving cell counting reagents and the vitamin/mineral premix required for the study. Once the reagents and premix were received, the study began. Activities that were scheduled for FY11 but not completed due to the above difficulties will be completed in FY12.

### Project Justification and Objectives

The overall goal of the research is to determine if eating beans will improve the immune status of children who are not being treated with antiretroviral drugs. The global theme addressed by this research is “to increase the utilization of bean and cowpea grain, food products and ingredients so as to expand market opportunities and improve community health and nutrition” and the topical area that will be addressed is “Achieving Nutritional Security for Improved Health of Target Populations.” HIV has caused an estimated 25 million deaths worldwide in just 27 years; there are approximately 33 million people in the world infected with HIV. Around two million children under 15 years of age have HIV and 90 percent of the children living with—and dying from—HIV live in sub-Saharan Africa. Further, about 140,000 of these children live in Tanzania. It is well known that insufficient intake of macronutrients and some micronutrients leads to a decrease in immune function and an increase in infectious diseases. Infections in turn cause nutrient loss that quickly leads to greater malnutrition and a vicious cycle is set in motion. Since the human immunodeficiency virus destroys CD4 cells (immune cells), opportunistic infections are commonplace among those living with HIV. In addition, most young children (not infected with HIV) in resource poor countries are undernourished or have marginal nutrition status. Since the insults of malnutrition and HIV on the immune system are synergistic, it is not surprising that young children with HIV are 2.5 to four times more likely to die than their counterparts who are not infected. We previously showed that providing HIV-positive children with a bean-maize supplement containing minerals and vitamins could reverse malnutrition, if present, and improve the immune system (increased CD4 counts), even if the children were not receiving highly active antiretroviral (HAARV) drugs. This is an extremely important finding since 50 percent of HIV-positive people do not have access to HAARV drugs and consuming the bean-based supplement could be an important stop gap until more people are able to obtain HAARV drugs. Children receiving HAARV treatment also benefited from the bean-based supplement in a second study we have done, and so, the bean-based supplement would also be useful to children who have access to HAARV medicine. Consuming a bean-based supplement could improve the lives of millions of HIV-infected people and simultaneously benefit the entire bean value-added chain from farmers to consumers.

## Objectives

1. Determine if HIV infected, HAARV naïve, two- to 15-year-old children and adolescents eating a bean-maize or cowpea-maize supplement will maintain higher CD4 percent than HIV infected, HAARV naïve, two- to 15-year-old children and adolescents eating a fish-maize supplement.
2. Determine the relative costs of three dietary treatments compared to HAARV drug treatment.
3. Determine if eating the bean-based supplement improves the integrity of the mucosal barrier in the gut and leads to reduced gut permeability and release of pro-inflammatory cytokines.

### Research and Outreach Approaches, Results, and Achievements

**Objective 1: Determine if HIV infected, HAARV naïve, two- to 15-year-old children and adolescents eating a bean-maize or cowpea-maize supplement will maintain higher CD4 percent than HIV infected, HAARV naïve, two- to 15-year-old children and adolescents eating a fish-maize supplement.**

1. Enroll 205 additional subjects (anticipated total number of subjects for the 30-month project is 540).
2. Purchase ingredients, cook and package food supplements, transport and distribute food supplements to 540 subjects.
3. Collect and analyze blood samples for CD4, CD8, CD3, and total lymphocyte counts.
4. Train six M.S. students to assist in research.
5. Provide field practical training in community nutrition and health for 16 undergraduates.

Seventy-three children were enrolled and began the feeding intervention late in FY11. An additional 465 HIV-infected, HAARV-naïve children have been identified and will begin the study in FY12. Two-hundred-sixty-six children live in five rural villages in the northern region of Rombo. Two-hundred-seventy-two children live in eight rural villages within a 150 km radius of SUA. Additional subjects will be identified in the Morogoro



Rural District during FY12, so that we will have adequate statistical power to determine if a particular nutritional supplement will elicit a difference in immune recovery (an increase in CD4 counts). Our goal is to have 540 subjects complete the study in the absence of HAARV. However, the availability of HAARV drugs is increasing, and we anticipate some of the 538 subjects will start HAARV treatment during the study period. Therefore, we will enroll more than the planned 540 subjects that are HAARV-naïve to allow for the subjects that will begin ART treatment.

Food processing and packaging began in earnest in September 2011. Four full-time technicians are required for this task. Approximately 17,000 individual packages of supplement will need to be produced each month once we have all subjects receiving nutritional intervention.

No CD4, CD8, or CD3 cell count data were obtained prior to the end of FY11. Funds committed to these activities in FY11 will be used in FY12 to complete them.

Field practical training in community nutrition and health was provided for 16 undergraduates. Two M.Sc. students will utilize data collected for this study for their theses. They received support for their research activities related to objective one.

**Objective 2: Determine the relative costs of three dietary treatments compared to HAARV drug treatment. (This will complete the data gathering begun in FY10).**

1. Determine costs associated with cooking beans in a pot and preparing Ugali (corn-based local food).
2. Determine costs associated with preparation of the bean-maize supplement and thin porridge from the supplement.
3. Determine costs associated with preparation of the fish-maize supplement and thin porridge from the supplement.
4. Determine costs associated with HAARV drug treatment.

Activities related to objective two will be completed in FY12.

**Objective 3: Determine if eating the bean-based supplement improves the integrity of the mucosal barrier in the gut and leads to reduced gut permeability and release of pro-inflammatory cytokines.**

1. Analyze dried blood samples shipped from Tanzania for HIV load, selected pro-inflammatory cytokines, and R16s.
2. Conduct studies with rats to ascertain the extent of bacterial translocation across the gut and the release of pro-inflammatory cytokines. This will be a continuation of the work begun in FY10 and the exact experiments will depend upon the results obtained from those studies.



**Research**

Activities related to objective three will be completed in FY12.

**Networking and Linkages with Stakeholders**

M. Bennink participated in a four day meeting of partners involved in Feed-the-Future activities funded by the USAID mission in Tanzania. The meeting took place in Udzungwa Falls Lodge near Kilosa, Tanzania, May 23 to 27, 2011. This was an excellent opportunity to inform NGOs of our work activities in Tanzania and to learn of their program activities. In addition, valuable contacts with USAID mission people were established. Bennink also maintains informal contact with the U.S. Dry Bean Council and several regional Dry Bean Commissions. He participated in two workshops involving scientific experts conducting research in the area of chronic disease reduction through ingestion of dry beans.

T. Mosha and M. Bennink met with nutritionists at the World Food Program office in Dar es Salaam to discuss our research and potential interaction with the broad initiative of WFP to initiate a national food fortification program in Tanzania. Both Mosha and Laswai have subsequently participated in food fortification activities. Dr. Laswai has chaired several of these meetings and was involved in similar activities involving neighboring countries.

Drs. Mosha and Laswai often meet with a variety of local groups and individuals as they learn of the activities related to nutritional support of HIV-infected individuals. These discussions inform local communities' leaders of the project's efforts. One noteworthy outcome was the establishment of a linkage with Tunajali (an NGO) that will share some of the costs associated with obtaining CD4 cell counts for our study.

**Leveraged Funds**

No leveraged funds directly related to this project were received during FY11.



# Impact Assessment of Bean/ Cowpea and Dry Grain Pulses CRSP Investments in Research, Institutional Capacity Building and Technology Dissemination in Africa, Latin America and the U.S.

MSU-4

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*Emmanuel Prophete, Haiti,*

*Mathew Blair, CIAT*

*Ndiaga Cisse, ISRA, Senegal*

*Issa Drabo, INERA, Burkina Faso*

*Phil Roberts, University of California,  
USA*

*Jeff Ehlers, University of California,  
USA*



Bean trials at ICTA Chimaltenango, Guatemala.

## Abstract of Research Achievements and Impacts

The project team compiled two databases: 1) A database of improved varieties of beans and cowpeas in countries where the Bean/Cowpea CRSP was involved in crop improvement research and 2) the database of socioeconomic studies and impact assessments conducted by the DGP (and its predecessor, the Bean/Cowpea) CRSP, to date. Both these databases have been developed in MS Access with reports generated and available in MS Word and Excel. The database on improved varieties and the impact studies will be further scrutinized as part of the meta-analysis planned in FY12

The impact pathway analysis for each of the phase two and three projects was completed in FY11 and results presented to the Pulse CRSP Management Office (MO). Based on this analysis, the ten CRSP phase two and three projects can be grouped into three types:

1. projects for which the prognosis for achieving development impacts is positive, contingent upon successfully scaling up the application of outputs
2. projects for which the potential for long-term impacts is low, based on their current scope and scale
3. projects for which the potential for long-term impacts is uncertain because the realization of the vision of success depends on many factors outside the control of researchers or because the scale at which the research is conducted may not generate a critical mass of knowledge/evidence needed to influence major policy decisions



Seed samples taken from Honduran markets for molecular markers analysis

Field research and analysis towards two ex post impact assessment studies were conducted as planned. This included the adoption and impact study on bean improvement research in Central America and Ecuador and cowpea improvement research in Senegal. Past and current research conducted by the Bean/Cowpea and Pulse CRSP on value addition, food science, and human nutrition was reviewed with the aim of documenting all the outputs, outcomes, and impacts from such investments.

The lead PI of this project interacted with several CRSP project PIs to explore the opportunities and feasibility of conducting impact evaluation research with the goal of implementing an integrated impact evaluation strategy as part of the CRSP project design. Four such opportunities for data collection and investigative research, with the aim of addressing questions of what works, where, why, and why not, were identified.

## Project Justification and Objectives

Impact assessment is essential for evaluating publicly funded research, capacity building and outreach programs, and for planning future research. Organizations that implement these programs should be accountable for showing results, demonstrating impacts, and assessing the cost-effectiveness of their implementation strategies. It is therefore essential to document outputs, outcomes, and impacts of public investments in research for development (R4D) activities.

Impact assessments perform two functions—accountability and learning. Greater accountability (and strategic validation) is seen as a prerequisite for continued support for development assistance. Better learning is crucial for improving the effectiveness of development projects and ensuring that the lessons from experience—both positive and negative—are heeded. Accountability and strategic validation have long been core concerns for ex post impact assessments and learning has been primarily a concern of impact evaluation. The primary focus of this project is on ex post impact assessment; however, attention is also devoted to finding opportunities to include impact evaluation as part of CRSP projects to be implemented in phases two and three. In addition to measuring and evaluating impacts of past research investments, this project is also concerned with increasing impacts from current investments by examining the impact pathways of research projects and inculcating an impact culture within the Pulse CRSP research community.

## Objectives

1. To build an inventory of past documented outputs, outcomes, and impacts of investments by the Bean/Cowpea CRSP and to develop a trajectory of outputs and potential types of impacts of investments made by the Dry Grain Pulses CRSP.
2. Conduct ex post impact assessment of Bean/Cowpea and Dry Grain Pulses CRSP investments in research, institutional capacity building, and technology dissemination in Africa, Latin America, and the United States.
3. Investigate opportunities to integrate baseline data collection and impact evaluation strategies as part of the CRSP project design.

## Research and Outreach Approaches, Results, and Achievements

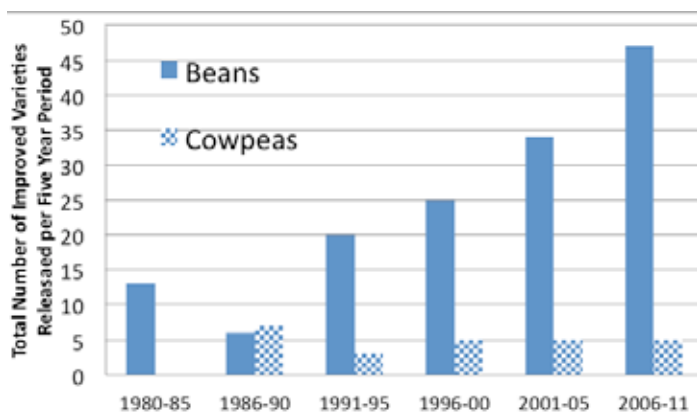
**Objective 1: To build an inventory of past documented outputs, outcomes, and impacts of investments by the Bean/Cowpea CRSP and to develop a trajectory of outputs and potential types of impacts of investments made by the Dry Grain Pulses CRSP.**

### 1a. *An Inventory of Past Outputs and Documented Impacts.*

The project team has completed the compilation of two databases: 1. a database of improved varieties of beans and cowpeas in countries where the Bean/Cowpea (B/C) CRSP has been historically involved in crop improvement research, and 2. the database of socioeconomic studies and impact assessments conducted by the DGP (and its predecessor) CRSP, to date.

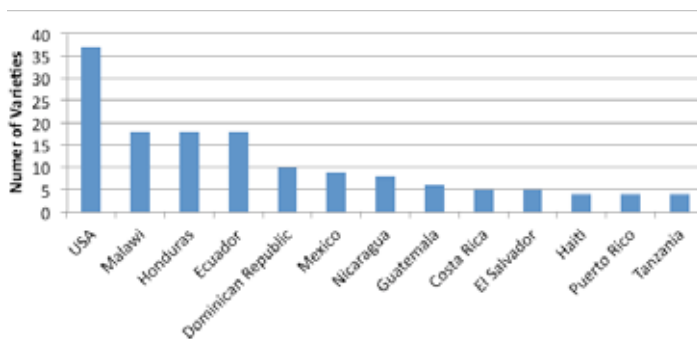
Figure 1 presents the number of bean and cowpea improved varieties released from 1980–2011 per five-year period by host country partners that received CRSP funding. Over the past 30 years, a total of 145 improved bean varieties and 25 improved cowpea varieties released in the United States and many CRSP partner countries can be attributed as outputs of CRSP funded research projects. For beans, the number of varieties released at an aggregate global level shows an upward trend, increasing from an average of about two varieties per year in the 1980s to almost 10 per year from 2006 to 2011. A major source of this upward trend in CRSP supported bean varietal release is from Central America and the Caribbean. Figure 2 shows the breakdown of the 145 bean varieties by country of release.

Compared to beans, the number of cowpea improved varieties attributed to CRSP support has remained stable, at about five varieties per five-year period (or an average of one per year) since the mid-1980s (figure 1). Over the past 25 years, CRSP can be credited for the release of eight cowpea varieties in Senegal, seven in Burkina Faso, four in the United States, three in Ghana, two in Cameroon, and one in Sudan.



Source: Variety database compiled by CRSP IA team, 2011

Figure 1. Trend in the number of improved bean and cowpea varieties released in CRSP partner countries (including USA) by breeding programs that received CRSP funding, 1980–2011



Source: Variety database compiled by CRSP IA team, 2011

Figure 2. Number of CRSP supported improved bean varieties released in different countries, 1980–2011

The impact database contains 40+ studies on the quantitative assessment of impacts. A majority of studies assess the ex post adoption or farm level benefits of varietal outputs of CRSP research. There are also a few studies that examine the impact of cowpea storage technology in Africa and IPM research.

### 1b. Trajectory of Outputs and Potential Outcomes/Impacts of Ongoing Investments by the Pulse CRSP.

This activity was similar in scope to activity 1a but was a forward-looking exercise, since the focus was on ongoing CRSP projects in phases two and three. Table 1 gives a summary of the impact pathway analysis for each of the phase two and three projects based on the information provided by individual project teams in a spreadsheet developed to capture the project's outputs, outcomes, and impact. The impact pathway analysis gives a synopsis of the types of outputs generated by different research projects by the end of FY2012, potential scale or impacts envisioned by the research team in the next five years, impact pathway and indicators along that pathway to achieve developmental outcomes (in the form of impacts at the beneficiary/adopter level). The last column of this table presents our subjective assessment of the potential for realizing the long-term impacts in the form of aggregate level benefits to society.

The impact pathway analysis and the prognosis for development impacts of current projects presented in table 1 highlight that the CRSP portfolio comprises research projects on different trajectories in terms of outputs, outcomes, and impacts and are at varying distances from achieving development impacts. Some research supported by the Pulse CRSP is basic research and thus farther away (in time) from the vision of achieving large-scale development impacts. This research is critical in advancing the knowledge frontier in small increments, which cumulatively may lead to applications, technologies, practices, and policies that positively impact people's lives.

### Objective 2: Conduct ex post impact assessment of Bean/Cowpea and Dry Grain Pulses CRSP investments in research, institutional capacity building, and technology dissemination in Africa, Latin America, and the United States.

2a. *Synthesis and Update Study on the Adoption and Impact of CRSP's Bean Improvement Efforts in the LAC Region* The general objective of the study was to assess the ex post impact of B/C and Dry Grain Pulses CRSP investments in research, institutional capacity building, and technology dissemination in Latin America, focusing on Honduras, Guatemala, El Salvador, Nicaragua, Costa Rica, and Ecuador. To achieve these objectives, a rapid appraisal methodology was used:

1. Five types of bean sector key informants were interviewed and secondary data were collected from FAOSTAT and National Statistical Offices (NSO) in each country during 2010.
2. A total of 67 key informants were interviewed and the data were analyzed using Excel and STATA.
3. Adoption levels were estimated using estimations of bean experts and seed distribution/sales data.
4. To estimate the effect of improved varieties (IVs) vs. traditional varieties, key informants provided estimates of yield loss averted by farmers planting improved seed. Further, experimental data are being analyzed to estimate yield gains over time.

As part of the impact evaluation methodology, several bean samples from three markets in Tegucigalpa were collected and sent to CIAT to evaluate the presence of the *bgm-1* gene, which would confirm that the seed came from an IV. In addition,

Project (Name of Lead PI)	Types of outputs	Potential Scale of impacts envisioned by PI in the next 5 years	Impact indicators and pathway to achieve developmental outcomes	Potential for long-term impacts (per USAID's expectations) (subjective analysis)
PII-MSU-1 (Mazur)	New methods and approaches	Small—District level (Kamuli, Uganda)	Higher yield→increased income	Yes—if the methods/approaches are scaled up by the NGO (currently this vision is not explicit)
PII-MSU-2 (Bernsten / Donovan)	Information systems	Small—pilot scale (regions within a country)	Increased access to market at higher price→increased income	Yes—if the system is scaled up by partners (currently this vision is not explicit) and if research shows evidence of impact indicators and pathways
PII-PSU-1 (Lynch / Findes)	New materials for breeding programs  Knowledge on seed system	Not specified  Local impacts in bean growing region (Mozambique)	Improved materials→adopted by breeders→new varieties with root traits→increased yields by adopted farmers→increased income and production  Changes in national policy→reduction in constraints to low-P bean seed diffusion	Yes—if breeders integrate the materials in bean breeding program. But PI expressed frustration on the lack of interest from breeders  Low—Difficult to change policy with one small scale study
PII-UCR-1 (Roberts)	New materials (advanced lines and varieties)	Medium—sub-regions in multiple countries (Senegal, BF, Mali, Niger)	Increased productivity→increased income and production	Yes—if breeders play an active role (as a partner) in seed multiplication and dissemination efforts
PII-UIUC-1 (Pittendigh)	New materials released (Biocontrol agents)  New strategies, information systems and extension materials	Medium—thousands of farmers across multiple countries (BF, Mali, Niger, Nigeria)	Biocontrol agents→decrease pests→increased yield→increased income and production  New information→adoption of new on-farm practices→decrease in yield loss→increased income and production	Yes—if research shows evidence of impact indicators identified in the pathway
PII-UPR-1 (Beaver)	New materials (markers, parental lines and varieties)	Large (100,000 farmers in multiple countries)	Increased adoption→increased yield→increased income and production	Yes—if the FIF technology transfer project in Central America is successful
PIII-MSU-2 (Westgate)	New knowledge, recommendations and materials (QTLs and germplasm)	Large (multiple countries, 15% of farmers)	Adoption of recommendations and materials→increased productivity→increased income/production, environmental impacts	Yes—if research shows evidence of impact indicators and pathway (and adoption occurs as predicted)
PIII-MSU-3 (Bennink)	New knowledge on how pulse consumption reduces markers of chronic disease  New knowledge on how pulse consumption reduces markers of chronic disease improved nutritional and immunological status	None (PI sees this project as achieving impacts over long-term—30-35 years)	Consumers and commodity advocates instigate change in nutritional recommendations by national and international agencies→ recommendations are implemented at grassroots→ Changes in food choices→improved health and reduction in health costs  Donors provide funds for large scale multi-national testing of nutritional intervention for PLHA→ policy change and commitment of resources for nutritional support and not just for drugs→ changes in food choices and increased consumption of pulses and essential nutrients → improved health and reduction in health costs	Uncertain—it depends on changing behavior and attitudes of many players along the pathway. Also, not sure if the knowledge generated by one study at such a small scale can influence national and international policies; Need a critical mass of 'knowledge' pool to influence change in policy and consumer behavior
PIII-KSU-1 (Amanor-Boadu)	New information, knowledge and ideas (on bean and cowpea supply chains and adoption protocols to enhance the relationship between value accretion and gender)	None	New information→ improved governance system in supply chain → higher value accretion → higher income accruing to female producers→ improved household food and nutrition security	Uncertain—not sure about the realization of the pathway from outputs to impacts
PIII-TAMU-1 (Awika)	New knowledge (effect of food processing on cowpea bioactives)  New materials (cowpea lines, improved varieties with bioactivity traits)	None  None	Community outreach targeting consumers, policymakers and farmers→increase in demand and use of cowpeas with enhanced health attributes→ Improved health and food security among vulnerable groups and improvement in income for cowpea farmers  Development of varieties with high phytochemical content and enhanced health benefits → adoption by farmers → increased production and consumption of nutritionally enhanced cowpeas	Uncertain—it depends on many factors outside the control of researchers. Also, not sure if the knowledge generated by this one study can influence consumers.

Table 1. Impact Pathway Analysis of the Dry Grain Pulses CRSP Phase II and Phase III Projects—Prognosis for Development Impacts

control samples were included in the sample to test the accuracy of the results, which suggest that more research needs to be done to successfully use this technique.

Bean production and trade data from two sources were analyzed. The differences between FAO and NSO data were small for bean planted area, production, and exports; however, for El Salvador, Guatemala, and Ecuador, the differences between these two sources of data were statistically significant for bean imports. The data suggest that among the six countries, only Nicaragua (the largest) and Ecuador were net bean exporters.

Although all breeding programs have the same research priorities they had 20 years ago, the work has intensified and the programs have incorporated new research priorities. In Central America, new priorities include selecting for resistance to other biotic (e.g., Web Blight, ALS) and abiotic (e.g., droughts) stresses

and high nutritional value. In Ecuador, new priorities include research on climbing beans, additional market classes, and processing properties. In 2010, a little more than half of the bean programs used molecular markers during the breeding process. Further, more than two-thirds were implementing participatory breeding that demonstrated the increased collaboration between scientists and producers.

The bean programs had between two and four sources of funding. The program with the most funding was Zamorano's, followed by INIAP's and CENTA's. DICTA's bean program had the least funds available for bean research. Further, while INTA-CR's three sources of funding have increased over time, all of DICTA's funding sources have decreased. On average, each bean program was collaborating with six institutions in research-related activities. However, several factors threaten future collaboration, including institutional instability regarding the continuity of staff and the focus of the research, a lack (discontinuity) of funding, a high dependence on Zamorano for germplasm, and donors' lack of interest in the region.

In 2010, there were 124 people working on bean-related activities in these programs; approximately 90 percent were male and devoted 72 percent of their time to bean-related activities. The programs with the highest percent of female staff were Zamorano's (25 percent) and INIAP's (19 percent). None of CENTA's or DICTA's staff was female. Among all staff working on bean research, almost 54 percent had earned a B.S. degree.

Although 99 IVs were released in the six countries between 1990 and 2010, several varieties were released in more than one country. Thus, 85 unique bean IVs were released during the period, most of which were small red (46), followed by black (13), and red mottled (10) varieties. Furthermore, 53 percent of the 85 IVs were developed using direct or indirect CRSP funding.



While farmers could access certified seed in most countries, in Ecuador and El Salvador, alternative seed types exist because the cost and requirements needed to certify seed make it impractical to produce. Since purchasing seed is expensive (regardless of the type), alternative ways of making high-quality, low-cost seed available to farmers are necessary. In Costa Rica and Nicaragua, the bean programs are already promoting the production of alternative (low cost) types of seed.

In four of the six countries, the governments have implemented free or subsidized seed-distribution programs that have greatly contributed to increasing adoption rates. However, some of these programs are distributing low-quality seed and have transparency problems. Thus, there is a need to invest resources guaranteeing seed quality.

Bean experts estimated that in the 09/10 AY, on average, 54 percent of the bean area was planted to IVs. In contrast, seed production data suggest that, on average, only 19 percent of the bean area was planted to IVs. Both the expert's estimations and seed data suggest that Guatemala had the lowest adoption rates. In contrast, while expert estimates suggest that Nicaragua had the highest adoption rate (80 percent), seed data suggest that Honduras has the highest adoption rate (37 percent). It is suspected that experts' estimates of the adoption rates in Nicaragua are overestimated. Seed production data suggest that *Amadeus 77* was the most widely-planted IV in the region.

Bean experts estimated that the most important biotic stresses affecting the bean crop were Web Blight (WB), Angular Leaf Spot (ALS), Bean Golden Yellow Mosaic Virus (BGYMV), and Common Bacterial Blight (CBB). The former two are important in all countries and the latter two are important in four of the six countries. For Web Blight, bean researchers estimated that in a typical year, on average, farmers could lose 77 percent of their production if they planted susceptible varieties vs. only 40 percent if they planted resistant IVs, representing a 37 percent production loss averted by planting resistant IVs. For ALS, on average, farmers could lose 33 percent of their production if they planted susceptible varieties vs. only 23 percent if they planted resistant IVs. Similarly, on average, farmers could lose up to 78 percent of production to BGYMV if they planted susceptible varieties vs. zero percent if they planted IVs. Finally, farmers could lose up to 29 percent of their production to CBB if they planted susceptible varieties vs. 21 percent if they planted resistant IVs.

Although there were no abiotic stresses common to all countries, key informants reported that intraseason drought was the most important factor affecting the bean crop in three of the six countries (i.e., Ecuador, Guatemala, and Nicaragua). In contrast to biotic stresses, outbreaks of abiotic stresses are more common. For example, intraseason drought was a serious problem, on average, in one of the last 10 years. Bean researchers estimated that in a typical year, on average, farmers could lose up to 64 percent of their production due to intraseason droughts if they planted susceptible varieties vs. only 17 percent if they planted resistant IVs, representing a 47 percent production loss averted by planting resistant IVs (table 4).

Currently, experimental data are being analyzed to estimate yield gains associated with IVs released since 1999. Furthermore, adoption curves are being estimated for the most widely-adopted IVs in each country.

Most important stress	Average production lost (%) with [...] varieties		Production loss averted (%) when planting IVs <sup>1</sup>
	Susceptible	Resistant	
<i>Biotic:</i>			
Web Blight	77	40	37
ALS	33	23	10
BGYMV	78	0	78
CBB	29	21	8
<i>Abiotic:</i>			
Intra-season Droughts	64	17	47

Table 4. Production lost (%) to the most important biotic and abiotic stresses when planting susceptible vs. resistant varieties. 2010.

Sources: DGP CRSP Key Informant Interviews (2010a).

<sup>1</sup> Difference in production loss by planting susceptible vs. resistant varieties.

## 2b. Global Contribution of CRSP to Genetic Improvement of Common Bean (Including the United States, LAC, and SSA)

The CRSP varietal database described under objective 1a was assembled to make an inventory of varietal outputs in major bean producing countries around the world. This database includes 525 varieties, the name of the variety, country and year of release, releasing institution, parental line, characteristics, and other observations/notes.

Due to early and pre-matured departure of the graduate student from MSU who was going to lead this study, this study has not progressed as planned. We plan to use the data and information gathered thus far to do a descriptive analysis of the database and use the information as an input in the meta-analysis study planned in FY12.

## 2c. Benefits of Genetic Improvement of Cowpea in Senegal and West Africa Study in Senegal: Two main activities were commissioned during the reporting period:

1. A field survey to identify the current extent of adoption by farmers in Senegal of improved cowpea varieties developed under the Bean/Cowpea (now Dry Grain Pulses) CRSP.
2. Collection of information on improved cowpea seed production and dissemination systems, the costs of seed distribution activities, and the advantages (enhanced yield, quality, reduced yield variability, etc.) of improved cowpea varieties relative to traditional varieties to estimate potential economic benefits of adoption of CRSP varieties.

Both of the above activities were focused on the three principal regions and departments in which CRSP-produced cowpea varieties have been disseminated—Diourbel, Louga, and Thiès. The survey sample consisted of 1. farmers interviewed during the 2010 nationwide DAPS survey who indicated that they used improved seed for cowpea (781) and 2. an additional sample of 584 households drawn randomly from the same survey enumeration areas, sufficient to give a total of seven households per local enumeration area. Questions asked of both sets of farmers included:

1. What is the name of improved cowpea variety planted?
2. What was the source of the seed?
3. Why was this variety used?
4. When was the first time the farmer used that variety?
5. Was cowpea planted in pure stand or intercropped? If intercropped, what percentage of the field is in cowpea?
6. What do you believe are the advantages (drought resistance, disease or pest resistance, yield increase) of the improved variety relative to unimproved or traditional varieties?
7. How much cowpea did the farmer harvest from the plot (where improved variety was grown) last season in a. green pods?, b. as grain?, c. any other form (e.g., fodder for animals)?
8. What variety or varieties of cowpea do you intend to plant next season? Why?





Seed storage facility in Nicaragua

The second part of the study was implemented in May and June. The terms of reference (TOR) for the draft report were:

**TOR 1:** To gather information on the production and dissemination of improved cowpea seed and the costs of these activities. Specifically:

1. Describe the cowpea seed system in Senegal
2. For the three main CRSP varieties—Melakh, Mouride, and Yacine—document the seed multiplication and distribution efforts in the past five years (or more, if possible).
3. Interview each of the organizations identified to collect the following information:
  - a. Name, location, type of organization
  - b. Length of time they have been involved in the cowpea seed production/dissemination system
  - c. Whether they produce/distribute seeds of other crops (if so, list)
  - d. The role they play in cowpea seed production and dissemination
  - e. Amount and variety of cowpea seed(s) they have produced/disseminated in the past five years
  - f. The type of cowpea seed produced/multiplied, e.g., foundation, certified, quality declared, registered, etc.
  - g. How they produce seed, e.g., contract seed growers or other NGOs, on their own farms/fields, etc.
  - h. To whom (i.e., geographic locations, farm communities) they distributed the seeds
  - i. The method used to distribute the seed (e.g., free distribution, sold to a private seed trader, or sold directly to farmers, etc.)
  - j. Price at which seeds were sold
  - k. The organization's costs of producing and disseminating cowpea seeds
  - l. Name of other organizations/groups/companies producing cowpea seed in Senegal

m. Opinions:

- i. What factors have contributed most to farmer adoption of improved cowpea varieties?
- ii. Are there any weaknesses in the cowpea seed distribution system that limit making high quality seed available to small farmers?
- iii. What could be done to increase farmer adoption of improved cowpea varieties?
- iv. What factors could contribute most to strengthening the cowpea seed production system in Senegal?

**TOR 2:** To gather information on the advantages (in the form of enhanced yield, grain quality, reduced yield variability, etc.) of improved cowpea varieties relative to traditional varieties to estimate potential economic benefits of adoption of CRSP varieties. Specifically:

1. Information from reports on experiments conducted on research stations or in farmers' fields
2. General information on cowpea seed planting practices at farm level.

*2d. Review and Assessment of Bean/Cowpea and Pulse CRSP Investments in Value Addition and Food Science Research*

Over the past 20 years, the Bean/Cowpea CRSP has made substantial investments in food science research to develop new value-added products to benefit producers of beans and cowpeas in market opportunities and consumers in convenient and nutritious food products based on beans and cowpeas. To a lesser extent, investments in this line of research have also continued in the new Pulse CRSP. The only study conducted on this topic in the CRSP program was done a few years ago. The results of that study confirmed the lack of significant impact of research on value-addition in Ghana, which raises the question, What is the value of investments by a CRSPs program on food science and nutrition research?

To address this question, we reviewed past and current research conducted by the Bean/Cowpea and Pulse CRSPs on value addition and food science, and documented all the outputs, outcomes, and impacts from such investments. A comprehensive list of all the CRSP research activities and outputs generated from investments in food science, food technology, and human nutrition research since 1997 that fall in the four categories described in table 5 was compiled by the project team. Principal investigators of relevant projects were contacted about the status of any outputs their research had generated in the past.



Bean researchers and seed producers visiting a seed production field in Nicaragua

This investigation confirmed the lack of documented evidence of adoption, uptake, and utilization of outputs of food science research by participants in the bean and cowpea value chains in host countries (or even in the United States). We will identify factors that were present or absent in the pathway of identified research outputs to better understand why past investments were not successful in generating impacts to derive lessons for guiding future investments by the CRSP in this line of research.

Categories	Examples of research outputs
Analysis of chemical, functional, and nutritional characteristics of processed bean/cowpea products	<ul style="list-style-type: none"> <li>Determine protein quality and protein digestibility-corrected amino acid score and the iron and zinc bioavailability of black bean-rice-based products processed by either microbial fermentation or germination</li> <li>Determine the extent of decrease in oligosaccharide content of dry beans achieved with fermentation of the beans and the acceptability of bean-rice weaning food made with fermented beans</li> <li>Development of a method for evaluation of cooking properties of cowpeas</li> <li>Effect of the hard-to-cook phenomenon on cooking and physicochemical characteristics of cowpeas</li> </ul>
Improvements in the technology of bean/cowpea processing and storage	<ul style="list-style-type: none"> <li>Artisanal processing of cowpeas in Nigeria</li> <li>Hydrothermal processing of dry (unsoaked) cowpeas</li> <li>Hydrothermal treatment of whole seeds—its impact on storage stability and food quality</li> <li>Effect of tempering/preconditioning in a solution of monovalent cations and micronization on cooking characteristics of hard-to-cook cowpeas</li> </ul>
Development of processed bean/cowpea products	<ul style="list-style-type: none"> <li>Developing and evaluating consumer acceptability of cowpea-fortified gari and cowpea-fortified fermented corn flour</li> <li>Creation and testing of weaning foods</li> <li>Develop a bean-based food with a stable shelf life to be eaten in a nontraditional way</li> <li>Develop nutritious, highly acceptable bean-based granola bars and cereal</li> <li>Developing extruded /expanded snack/ convenience foods</li> <li>Low-cost, fortified supplementary foods from locally available ingredients</li> <li>Modifying/adapting traditional cowpea-based foods</li> <li>Nutritious convenience/snack foods</li> </ul>
Consumer/producer and demand analysis of processed bean/cowpea products	<ul style="list-style-type: none"> <li>Assessment of consumer acceptance of bean ingredients and products</li> <li>Assessment of potential demand for cowpea-based processed products in West Africa</li> <li>Bean use patterns and preferences of farmers</li> <li>Consumer acceptability of cowpea-fortified gari and cowpea-fortified fermented corn flour at the institutional level</li> <li>Consumer acceptance, nutritional value, and economic potential of bean-based ingredients and products</li> <li>Cowpea flour production and use in Benin</li> </ul>

Table 5: Major categories of food science and human nutrition related research conducted by the Bean/Cowpea CRSP and examples of research outputs generated

### Objective 3: Investigate opportunities to integrate baseline data collection and impact evaluation strategies as part of the CRSP project design.

As described under the impact pathway analysis activity described in 1b, CRSP investments in the research for the development spectrum of activities range from basic/fundamental research to applied/adaptive research to technology transfer. Since resources to conduct research are scarce, many CRSP projects on the applied end of the R4D spectrum are pilot scale initiatives and programs designed to test the efficacy and effectiveness of a



Bean seed production field in Nicaragua

science-based intervention in a developing country, with the aim of deriving lessons on what works and what does not. Such applied field-based research initiatives are undertaken and supported by the CRSP with the goal of identifying the most effective strategy/models that can then be scaled up to achieve developmental impacts. For a research project to be successful in achieving this goal requires some forethought on the design of field activities and a strategy for collecting appropriate data or making use of available data to make sure that an opportunity to assess the cause-effect relationship between a research project and indicators of outcomes/impact is not lost.

Towards implementing an integrated impact evaluation strategy, the lead PI of this project interacted with several PIs directly related to technology transfer interventions to explore opportunities and the feasibility of conducting impact evaluation research. Four such opportunities for data collection and investigative research with the aim of addressing questions of what works, where, why, and why not were identified and included as part of the workplan for FY2012. The research underlying these four proposed activities will be conducted in close collaboration with the respective CRSP project PIs. These activities include:

1. Baseline assessment of the economic effects of pest problems on cowpea growing areas in Burkina Faso (with PII-UIUC-1)
2. Impact evaluation to test the effectiveness and impacts of methods of extension to disseminate materials for IPM of cowpea pests (with PII-UIUC-1)
3. Benefit/Cost analysis of the bean-based nutrition intervention in Tanzania (with PIII-MSU-3)
4. Case study of the bean seed multiplication and distribution system in Central America (with host country partners in Nicaragua participating in the CRSP Associate Award)

### Leveraged Funds

Dr. Maredia and her collaborators successfully leveraged \$6,000 from MSU's College of Agriculture and Natural Resources to support a Dissertation Completion Fellowship for a Ph.D. student, in part due to the assistance received from the Dry Grain Pulses CRSP.

# Increasing Utilization of Cowpeas to Promote Health and Food Security in Africa

TAMU-1

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## Abstract of Research Achievements and Impacts

Evidence indicates that legumes may contain compounds that have health benefits against such diseases as cancer and cardiovascular disease; however, not much information is available on the type of compounds—and their bioactive properties—in cowpea. Based on our screening studies in year one, we conducted a detailed characterization on ten diverse cowpea lines to determine phytochemical composition and properties using spectroscopy advanced UPLC-MS<sup>n</sup>. We then tested select line for anti-inflammatory and oxidative stress reduction properties using nonmalignant colonic myofibroblasts CCD-18Co cells. We also tested antiproliferative activity of select lines against human colon cancer cells *in vitro*. The major phenolic compounds in all cowpea varieties were flavonoids. Important phenotypic differences were observed; light brown (cream) lines generally contained mostly flavan-3-ols; red lines contained mostly flavonols (especially quercetin glycosides); black varieties contained mostly anthocyanin pigments; the white varieties lacked flavan-3-ols but had modest quantities of flavonols. Thirty-eight new compounds were identified in cowpea, in addition to 18 previously reported. Light brown cowpea lines were the most effective oxidative stress inhibitors, while the black and red lines were most effective against various anti-inflammatory markers, indicating that the type of compounds in the seed have an impact on specific potential health benefits. Most samples tested were effective at low concentrations (2ug/mL), which suggests possible relevance to human consumption levels. Additional investigations on minimum effective dose and effect of cooking are underway. Our research was recognized by peers and received a highly competitive award at the American Association of Cereal Chemists International as the best graduate research presentation and paper at the 2011 annual meeting. The data reported involved collaborative effort of the HC Institutions and Texas A&M, which indicates that the training and equipment available to HC Institutions in year one were put to good use. A total of 24 HC personnel were also trained.

## Project Justification and Objectives

Poor families, especially children, in sub-Saharan Africa suffer high rates of malnutrition, and diet-related chronic diseases have become a common phenomenon among urban African populations. A recent survey reported that stunting and overweight due to malnutrition coexisted and were rampant among school-age children in poor communities of western Kenya, affecting up to 70 percent of the children. Moreover, evidence indicates that childhood malnutrition may lead to increased risk of chronic diseases, such as cancer, in adulthood. In fact, nutrition-related chronic diseases are becoming increasingly common in Africa, especially in urban areas, putting a large strain on the limited health infrastructure and imposing economic burdens on the poor. For example, recent data indicate that obesity among urban Kenyan women is approaching 30 percent, with similar trends in other African countries.

Research shows that regular consumption of dry beans and other legumes may reduce serum cholesterol, improve diabetic therapy, and provide metabolic benefits that aid in weight control

as well as reduce the risk for coronary heart disease and cancer. Thus, in addition to alleviating protein malnutrition, grain pulses have the potential to contribute to chronic disease prevention.

In Africa, malnutrition is closely linked to food insecurity, meaning the most vulnerable people are those in marginal rainfall rural areas and the urban poor. Cowpea is one of the most drought-tolerant crops and has big potential as a food security crop for many African subsistence farmers. Additionally, cowpea has high quality proteins that compare favorably with soybean proteins when substituted in diets at equivalent protein contents. A limited number of studies have also demonstrated that cowpeas have high antioxidant capacity and that the antioxidant properties may be improved by heat processing or fermentation. Recent evidence suggests that whole cowpea is effective at binding cholesterol and lowering blood cholesterol in hamsters; however, information on how cowpea and its constituents may impact human health is lacking. Additionally, how variations in cowpea genetics affect their composition of potentially beneficial compounds is unknown, which makes it difficult to promote cowpea as a healthy grain, dampening its demand and utilization.

The image of cowpea as a healthy food lags behind other commodities, partially due to lack of scientific data on its health and nutritional benefits. In many parts of East and southern Africa, the common perception that beans, cowpeas, and other pulses are poor man's food has also been a major impediment to broader consumption of these grains. Thus, most of cowpea use is still restricted to low-income populations, which leads to weak demand and depressed economic value of the crop and limited incentive to invest in an efficient cowpea production and utilization infrastructure. In the United States, lack of nutritional information limits incentive to promote cowpeas as a main part of the diet.

Reliable scientific evidence is essential to make educated dietary recommendations on type of cowpea, level of consumption, and design of food processing strategies that maximize the beneficial effects. The evidence will also provide a basis for genetic and agronomic improvement aimed at optimizing composition of beneficial compounds. Sound scientific evidence is essential for consumer buy in. It is a first step in transforming cowpea into a



primary food to address malnutrition in poor populations and promoting cowpea as a mainstream part of a healthy diet. This will lead to increased demand for cowpea and improvement in the economic well being of producers and the overall health of consumers.

## Objectives

1. Identify cowpea lines with a high content of health enhancing compounds and their relationship to seed color and other seed traits.
2. Establish how the phytochemical profiles of cowpeas affect bioactivity by measuring key markers/predictors of protection against chronic diseases.
3. Elucidate the mode of heritability of selected bioactive traits in cowpea and the genetic association between physical and bioactive traits.



## Results, Achievements, and Outputs of Research

### Objective 1: Identify cowpea lines with a high content of health enhancing compounds and their relationship to seed color and other seed traits.

The goal was to determine genetic variability in cowpeas for the types and levels of key bioactive components as well as protein content and quality. Association between these traits and seed color and seed characteristics was determined.

Based on our previous data, in which we determined how phenotype (particularly seed coat color) influences phenol content of cowpea, we selected a set of lines that represent the broad spectrum of cowpea phenotypes for more focused investigation. Selected samples from Kenya, Zambia, South Africa, and the United States were analyzed for phenolic content to confirm previous findings. A select subset of ten (table 1) were used for flavonoid profiling and bioactives screening.

#### HPLC and Mass spectrometry analysis

*Extract purification.* The cowpea phenolics extracted with 70 percent acetone acidified with one percent formic acid were fractionated on Sep-Pak Solid Phase Octadecylsilane (C18) cartridges into ethyl-acetate and methanol fractions.

Variety	Seed coat property
IT95K-1105-5	Black, smooth, white-eyes
IT98K-1092-1	Black, smooth, white-eyes
IT82D-889	Red, smooth, white eyes
IT97K-1042-3	Red, smooth, white eyes
TX2028-1-3-1	Green, freckled, black-eyes
IAR-48	Light brown, rough, white eyes
09FCV-CC-27M	Light brown, smooth, white eyes
IFE BROWN	Golden brown, rough, white eyes
IT84S-2246	Golden brown, smooth, white eyes
EARLY ACRE	White, freckled, brown eyes

Table 1: Description of cultivars selected for flavonoid fingerprinting

*UPLC-ESI/MS Analysis.* A Waters-ACQUITY UPLC/MS system equipped with a binary solvent manager, sample manager, column heater, photodiode array eλ interfaced with a Mass Spectrometer, a tandem quadrupole (TQD) electrospray ionization (ESI) detector was used. Mass spectrometric data of the column eluant were acquired in positive mode for anthocyanins and negative mode for flavonol glycosides and flavan-3-ols. The spectra were monitored at 280 nm (nanometers) for phenolic acids and flavanols; at 360 nm for flavonols and isoflavonoids; and at 520 nm for anthocyanins. The mobile phases were two percent formic acid in H<sub>2</sub>O (solvent A) and acetonitrile (solvent B) for anthocyanin analysis; and 0.05 percent formic acid in H<sub>2</sub>O (solvent A) and acetonitrile (solvent B) for other phenolics. Data acquisition and processing were performed using Empower 2 software. Optimization of ionization conditions was based on the intensity of the mass signals of protonated/deprotonated molecules and aglycones fragments and was performed for each individual peak/compound detected.

#### Quantification of anthocyanins, flavan-3-ols, and flavonols

Quantification was based on LC peak area; authentic standards were used to obtain standard curve. Where standards were not available, molar absorptivity for the compound was assumed to be equivalent to that of the aglycon or closest glycoside structure. The flavonoids were expressed as micrograms of flavonoid per gram of cowpea flour (µg/g) on a dry weight basis.

#### Determination of total phenols content (TPC) and condensed tannins content (CTC)

The total phenols content (TPC) was determined by a Folin-Ciocalteu assay using gallic acid (GA) as the standard and expressed as µg GAE/g on dry weight basis.

#### Protein content and quality

These tests were conducted on elite cultivars selected for crossing and their selected progeny. To obtain relevant data from this procedure, samples were cooked in boiling water for 75 minutes and then dried at 45° to 50°C. The protein content was measured; the complete amino acid profile was measured; the available lysine was measured and in vitro protein digestibility was determined by pepsin/pancreatin digestion method.

#### Results

##### Flavonoid composition of cowpea

The UPLC-MS system, a newly available separation and chemical characterization system, has enabled us to provide a

detailed characterization of cowpea flavonoids. We identified a host of new compounds that should improve our understanding of how cowpea bioactives' composition influences human health.

- a) **Anthocyanins.** A total of 8 anthocyanin compounds were detected in cowpea. Anthocyanins were only detected in black and green cowpea varieties. From this study, petunidin-3-O-galactoside was found for the first time in the black and green cowpeas.
- b) **Flavonols.** The majority of flavonol compounds identified in cowpea were quercetin glycosides. Myricetin glycosides were only found in black, red, and green varieties, while kaempferol glucoside was only detected in the black, golden brown, light brown, and white Early Acre varieties. Total flavonol content was lowest in light brown and higher in the red variety. In general it is apparent that the red cowpea phenotypes accumulate the highest levels of flavonols, while the green and white varieties accumulate the lowest. Another interesting observation was that among the light brown varieties, accumulation of flavonols was inversely proportional to accumulation of flavan-3-ols. The light brown variety that accumulated exceptionally high levels of flavan-3-ols also had the lowest level of flavanols.
- c) **Flavan-3-ols.** The flavan-3-ol compounds identified in cowpea were mainly catechin/epicatechin, catechin-3-O-glucoside, procyanidin dimer B-type, procyanidin dimer-3, 7-diglucoside, procyanidin trimer C1, procyanidin trimer T2, and procyanidin tetramer A-type. Four of the isolated flavan-3-ols are still unidentified. Among all the cowpea varieties, the light brown had the highest flavan-3-ols, followed by golden brown Ife Brown and red. The light brown had especially high levels of oligomeric flavan-3-ols that were present in the other samples at low levels. Moderate levels of flavan-3-ols were found in the black and light brown cowpea varieties. The golden brown variety contained the least amount of total flavan-3-ols. There were no flavan-3-ols detected in the green and white Early Acre cowpea varieties. Among the flavan-3-ol compounds, catechin-3-O-glucoside was the most dominant compound detected.

- d) **Other flavonoids.** Examples of major flavonoid aglycones identified in cowpea included flavononols, flavanone, dihydrochalcone, and phenolic acids.
- e) **Total flavonoid content.** Among all the samples, the light brown had the highest total flavonoid content contributed by higher levels of flavan-3-ols in this variety, followed by the black, which contained mostly anthocyanins. The white and green variety had the least flavonoid content.

#### *Gross phenol and condensed tannins content*

The light brown variety had the highest TPC and CTC values, while the white Early Acre and green varieties had significantly lower TPC and CTC values. The differences in TPC and CTC values between varieties from the same phenotype suggest the observed differences in phenolic profiles subsequently affect their total phenolic compositions. In general, the TPC and CTC values for the cowpea varieties analyzed had the following trend: light brown > black > red > golden brown > green > white.

#### *Summary*

We were able to structurally characterize previously unidentified compounds in cowpea using the new sensitive UPLC-TQD MS system. From the results, it is apparent that seed coat color has a major influence on the type of phenolic compounds accumulated by cowpea. Even though most lines investigated had a mix of most of the compounds identified, important patterns were observed. For example, anthocyanins were exclusively found in black and green varieties, whereas the white and green varieties had no flavan-3-ols. The dominant flavonoids in major cowpea phenotypes can be summarized as follows:

- *Light brown* – flavan-3-ols (catechin and condensed tannin family)
- *Red* – flavonols (quercetin family)
- *Black* – anthocyanins (pigment family)
- *White* – flavonols (with no flavan-3-ols)

These compositional variations will allow for studying specific biochemical pathways influenced by cowpea polyphenols, which will eventually lead to selection of cowpea for health attributes.

Darker-colored grains have been shown to contain higher levels of phenolic compounds than lighter-colored grains; however, the association of dark colors in cowpea with higher phenolic content may be misleading since the light brown cowpea variety is not dark colored but contained the highest concentrations of total phenolics compared to the black seed coated varieties.

We are currently investigating the effect of thermal treatment on the structure and levels of the compounds identified to extrapolate the effect of traditional cooking on cowpea phenolics and design strategies to optimize their levels in processed products.

**Objective 2: Establish how the phytochemical profiles of cowpeas affect bioactivity by measuring key markers/predictors of protection against chronic diseases.**



The aim is to establish how the phytochemical profiles affect the ability of cowpeas to influence metabolic, cardiovascular, and chemoprotective health predictors *in vitro*. The select samples were screened for predictors of bioactivity.

#### *Determination of Antioxidant Capacity*

The scavenging activity of 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) radical cation (ABTS•+) was determined. Oxygen Radical Absorbing Capacity (ORAC) assay was performed.

#### *Glycemic properties*

Procedures were used to measure the rate of *in vitro* starch hydrolysis in selected cowpea lines. Hydrolysis index and estimated glycemic index were calculated from area under curve (30 min intervals to 180 min digestion), using fresh white bread as a control.

#### *Cell culture studies*

**Cell Lines.** Non-cancer colon CCD18Co cells were cultured using high glucose Dulbecco's Modified Eagle Medium. The cells were incubated at 37°C in a humidified 5 percent CO<sub>2</sub> atmosphere. For cell culture assays, freeze-dried cooked cowpea extracts were redissolved in DMSO and then diluted to known concentrations of total soluble polyphenolics ranging from 0 to 20 mg GAE/L.

#### *Oxidative stress studies*

**Generation of Reactive Oxygen Species (ROS).** The production of ROS was performed. A FLUOstar Omega plate reader was used to monitor the fluorescence signal at 520 nm emission and 480 nm excitation. Relative fluorescence units (RFU) were normalized to control cells not treated with cowpea extracts.

#### *Anti-inflammatory studies*

**RNA extraction and Real-time PCR analysis of mRNAs.** Cells seeded ( $8 \times 10^5$  onto a 12-well plate) and incubated for 24 hours to allow cell attachment were pretreated with varying concentrations of crude cowpea polyphenolic and stimulated with a lipopolysaccharide, LPS before lyses, followed by mRNA extraction and analysis. Total RNA was isolated for mRNA analysis. Samples were evaluated for nucleic acid quality and quantity. Isolated RNA was used to synthesize cDNA. PCR-RT was carried out with the SYBR Green PCR Master Mix on an ABI-Prism 7900 Sequence Detection System. Three independent cell culture replicates were analyzed for gene expression (n=3).

#### *Anti-cancer effects*

**Phase II detoxifying enzyme assay.** This method is based on the fact that enhanced activity of enzymes that detoxify potential carcinogens will lead to prevention of cancer initiation. Murine hepatoma cells were incubated with various concentrations of cowpea extracts and NQO enzyme activity and cytotoxicity were measured. Sulforaphane were used as a positive control.

**Anti-proliferation assays.** These methods measure how the various cowpea extracts affect growth of preformed cancer cells. We used the widely studied HT-29 and Caco-2 human colon carcinoma cells for this assay following the viable cell (MTT) and DNA (PicoGreen) procedures. Various concentrations of the cowpea extracts were incubated with the cells for 48 hours after

which the viable cell population was measured. Double stranded DNA were measured. *Apoptosis* were assessed in cells by analyzing PARP-cleavage.

#### *Results*

- a) **Antioxidant capacity.** The raw light brown variety had significantly higher ORAC and ABTS values than the other varieties, while the black, red, and golden brown varieties had intermediate levels of ORAC and ABTS values. The green and white Early Acre cowpea varieties had the lowest antioxidant activity, which correlated with lower TPC and CTC values reported in these varieties, suggesting that different phytochemical profiles might have different degrees of contribution to the overall antioxidant activity. These results indicate that in addition to their traditional role of preventing protein malnutrition, intake of cowpea may have greater potential in managing and/or preventing degenerative diseases associated with oxidative damage.
- b) Boiling generally had modest effect on ORAC and ABTS values; however, the degree of reduction in antioxidant capacity values depended on individual cowpea variety. A boiling process that preserves the health benefits associated with the compounds identified in raw cowpeas is therefore recommended.
- c) **Protective effects against Reactive Oxygen Species (ROS).** Oxidative damage by ROS is crucial in the initiation of cancer; therefore, the potential of cowpea extracts in protecting colon cells against ROS was investigated. At the lowest concentration, the flavonol-rich red and anthocyanin-rich black had the highest inhibitory effect on ROS, followed by the light brown (28.4 percent reduction). At the highest concentration tested, the black had the highest effect on ROS inhibition, followed by red and light brown. Thus, the differences in structural features of the flavonoid present in these cowpea varieties have a significant effect on their capacity to abrogate the generation of ROS. Cancer cells' proliferation and tumor progression increase with increase in ROS generation. Therefore, inhibition of ROS generation may contribute to slowing down tumor progression. A similar study showed that flavonols, especially quercetin and kaempferol, had favorable inhibition of TNF- $\alpha$ -induced ROS generation in noncancer human embryonic kidney HEK 293 cells.
- d) **Anti-inflammatory properties.** In general, cowpea has good anti-inflammatory properties even at low concentrations of 2  $\mu$ g/mL and may lower the risk of chronic inflammatory conditions.
- e) **Antiproliferation.** Two samples from Africa, Agrinawa (red) and Blackeye, were used for this investigation. In general, a dose response was observed for cancer cell growth inhibition by both extracts, indicating the compounds in these cowpea varieties may contribute anticarcinogenic properties. Cooking the samples modestly reduced the potency of the extracts against cancer cell inhibition, which may be related to reduction in polyphenol content. The red cowpea sample had lower IC<sub>50</sub> (was more potent) against cancer cell inhibition. The effect of simulated enzyme digestion on bioactivity of these samples is currently under investigation.

### Objective 3: Elucidate the mode of heritability of selected bioactive traits in cowpea and the genetic association between physical and bioactive traits.

This objective will help determine the mode of inheritance and the extent of genetic associations of key bioactive traits in cowpea, which will open opportunities for genetic selection and improvement efforts and modern molecular techniques to develop specific specialty cowpea lines for targeted health benefits.

The parents used in the greenhouse crosses were Early acre, 889, 2028, 1042-3, 205-8, and GEC. Each cross involves a parent with one low and one high antioxidant activity. The crosses that were made are:

- Early Acre (low antioxidant) x 889 (high antioxidant)
- 2028 (low antioxidant) x 1042-3 (high antioxidant)
- 205-8 (low antioxidant) x 1042-3 (high antioxidant)
- GEC (low antioxidant) x 1042-3 (high antioxidant)

All the F2 seeds were planted in greenhouses with a set of parents and F1 hybrids in spring 2011 at Texas A&M University. Initial greenhouse crosses to obtain F1 hybrids were conducted in fall 2010. Hybridization to obtain F1 seeds was done meticulously by emasculating the anthers of the female parent and dusting the desired pollen collected from the flower of the male plant. The dusted flower was secured with a tape and tagged. Higher night temperatures are not conducive to flowering and seed set, hence cooler months of the year were chosen for such experiments. Crossing experiments were done in the mornings before 10 a.m. since after this time the pollen becomes sticky and the crosses show low success.

The traits that were recorded include the seed coat pattern: speckled/dotted, patched, and solid colored seed coat. Seed size was also considered and was recorded using the length of seed using a vernier calipers. A size scale of < 5 mm for small, 5 mm to 8 mm for medium, and >8 mm for large seeds was used to group the seeds.

#### Results

Results indicate that in all the seeds of F1 hybrids, the eye color restricts the seed coat color of either parents in seeds of F1 hybrid, and the seeds take the color of the eye of the parent. However, all the seed coat colors existing in the parents segregate in the F2 generation (F3 seeds) obtained by selfing F1 hybrids. In every cross, the seed coat color segregates into patterned seeds and solid color seeds.

In general, the preliminary investigation reveals that seed coat color and texture (rough vs. smooth) are reliable indicators of antioxidant activity, thus heritability of these seed coat traits explains most of the variability in the antioxidant. This was observed in both African and TAMU crosses. We are currently studying phenolic profiles of selected progeny to determine if accumulation of major compounds changes.

Among light brown varieties, flavonoid composition and antioxidant activity vary significantly; hence, there is a need to investigate the pathways involved in flavonoid accumulation in

these varieties and the genetics involved. We have observed that light brown lines that accumulate very high levels of flavan-3-ols have very low flavonol content and vice versa

### Networking and Linkages with Stakeholders

During the year, we visited the USAID mission in Nairobi and had a fruitful discussion with the USAID country director for Agriculture, Business and Environment. Also in attendance were the regional agricultural advisor, a private sector specialist, the regional food security coordinator, and a food aid specialist. The discussions concentrated around how to leverage our project to contribute to ongoing USAID country endeavors and the Feed the Future program. The CRSP project was well received, especially for its potential to contribute to economic and nutrition security in semiarid regions of Kenya.

We also had meetings with various Government of Kenya officials, including Ms. Veronica Kirogo, head of nutrition programs under the Ministry of Agriculture. She emphasized that sound scientific evidence was essential to effectively influence government policy. She said cowpea had a big potential as a complement and substitute for beans, which are always in short supply in the country.

### Leveraged Funds

Scientists involved in this project have effectively used Dry Grain Pulses CRSP support and institutional linkages to leverage more than US\$125,000 in external funding to achieve the objectives related to this project.

### Contribution to Gender Equity

Currently two women (from Kenya and Zambia) working on their Ph.D.s are directly funded on this project. In addition, of the 24 people who received short-term training in Kenya this year, 13 were women. We will continue to actively engage women throughout the project.

### Publications

Archana Gawde, B. B. Singh, J. Ehlers, J. M. Awika. 2011. Associating *Vigna unguiculata* phenotypes with composition of bioactive compounds. *CFW*, 56, A41-A42.

Leonnard O. Ojwang and Joseph M Awika. 2011. Anti-inflammatory properties of cowpea phenotypes with different phenolic profiles. *CFW*, 56, A3-A4.





# Pulse Value Chain Initiative— Zambia (PVCZ)

KSU-1

## Principle Investigator

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## Collaborating Scientists

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## Abstract of Research Achievements and Impacts

In the first year of operation we have developed systems for operations: protocols, bank accounts, invoicing systems, etc. and have completed the producer-level data collection. Additionally, six undergraduate students completed their programs successfully, with a couple receiving merit awards and graduating with distinction. These students have produced six theses on various dimensions of beans and cowpeas in Zambia. One of the sponsored MS students has also graduated and is currently working with the World Food Program in Lusaka. The MAB students sponsored by the project have already started making impacts in their organizations, implementing solutions to problems using the concepts and tools they are acquiring in their classes. Finally, PVCI-Z has successfully developed a relationship with the Zambia National Farmers Union (ZNFU) and with the Southern Africa Bean Research Network (SABREN) to work on improving understanding and operation of the bean and cowpea value chains. The second year of the project is devoted to the completion of the research component of the project objectives.

## Project Justification and Objectives

Pulses are important in concentrated locations in Zambia. Zambian Central Statistics Office (CSO) data show that while the Northern Province accounts for the majority of bean production (62 percent), the Southern Province accounts for the majority (58 percent) of cowpea production. The remainder of the top four producing provinces for beans includes Northwestern (8 percent); Central (7 percent); and Luapala (6 percent). For cowpeas, the remaining top-four producing provinces are Central (11 percent), Northern (9 percent), and Lusaka (6 percent). Despite this concentration, pulses are also important to the Zambian food economy because they are planted in all provinces.

Despite their importance in the country, knowledge about bean and cowpea value chains is overwhelmingly anecdotal. It is particularly unclear what effect internal and transborder trade arrangements for beans and cowpeas have on the efficiency of current supply chains, the value they create, and the distribution of that value against the costs incurred across the different supply chains used by producers to get product to market. Additionally, it is unknown how much value is created at the different stages of the production process, i.e., leaves, green pods, dry grain, and the relative contributions of these stages to the overall profitability of production activities. Securing better information about these would contribute to better understanding of these crops in the welfare of Zambian producers and others in their value chains. This information should contribute to the development of better policies aimed at poverty alleviation and reduction in food security risks as well as enhancing producer incomes.

The Pulse Value Chain Initiative—Zambia (PVCI-Z) vision is to contribute to poverty alleviation and improve food and nutrition security through research, education, and engagement. This project aims to achieve this vision by 1. conducting research to address the identified knowledge gaps about bean and cowpea value chains in Zambia; 2. determine the most efficacious

value chains, given producer and partner characteristics; and 3. work with industry to develop and construct value chains that help increase producer incomes, improve food and nutrition security, and improve efficiency along the supply chain. This ensures that all partners along the chain are beneficiaries of improvements in chain performance and that the distribution of new value is done in an equitable and fair manner. The foregoing is in line with the Dry Grain Pulses CRSP overall goal of alleviating poverty and enhancing food and nutrition security. The lessons and tools emanating from this research will be applicable to other countries in the region and to other crops within Zambia and across southern Africa.

The project's success, therefore, contributes to the Global Hunger and Food Security Initiative of USAID through two principal components: research and human capacity development.

## Objectives

1. Development of Baseline Knowledge and Benchmark Metrics.
  - a. Identify the different supply chains used by the Zambian pulse industry and describe the characteristics of those using them at the different loci of the supply chains.
  - b. Identify and estimate the effects of stakeholder characteristics on producers' supply chain participation decisions.
  - c. Describe and estimate the pecuniary and nonpecuniary value for different supply chain participants.
  - d. Identify the institutional and policy issues influencing value creation and determine if any differences exist by crop, location, gender, and stage of the chain.
  - e. Based on the results from the foregoing, develop and deliver education and outreach programs targeting specific stakeholders and provide policy recommendations to facilitate solutions.
2. Exploration of Effects of Alternative Governance Systems on Value Creation, which involves conducting supply chain management experiments to identify the factors that influence success in value creation.
  - a. Work with specific industry stakeholders to pilot different governance systems to identify the factors and participant characteristics influencing performance.
  - b. Use the results of the experiment to develop outreach programs, program advocates, and program advisory support systems to help producers and their partners develop appropriate governance systems to improve their economic well-being.

It is expected that upon achieving these objectives, the PVCI-Z project will contribute knowledge and understanding of the structure, conduct, and performance of supply chains in the Zambian bean and cowpea industry, specifically providing knowledge about how the different players in the market—farmers, traders, retailers, customers, etc.—define and create value and identify how that value is distributed along the chain. One of its major contributions will be identifying and valuing



the nonmarket components of the bean and cowpea chain in Zambia to develop a more complete economic picture of the industry. From a policy development and implementation perspective, the achievement of the foregoing objectives would facilitate targeting the appropriate policies to achieve the highest payoffs by producer characteristics. Additionally, the preferred value chain governance mechanisms will be identified and policy makers could be encouraged to support producers to engage in those that are most preferred to minimize governance-related transaction costs and maximize total value accruing to chain participants. This knowledge should help collaborating institutions and organizations—government and public policymakers, industry and trade associations, and NGOs and others seeking to help improve the well-being of African smallholder producers—develop a better appreciation of how to organize value chains to achieve their desired objectives.

## Results, Achievements and Outputs of Research

Primary data is used to achieve the first category objectives. A two-stage stratified cluster sampling procedure was used. The first stage involved probability sampling of the standard enumeration areas (SEAs) of Zambia with weights designed to reflect bean and cowpea growing regions. The second stage used systematic sampling of households from each selected SEA. Additionally, commercial producers were targeted and included in the sample to ensure the different types of producers were covered in the data collection process. The sampling processes also ensured that female producers were adequately represented.

### Objectives a through e under Category 1

The primary producer surveys were completed at the end of August 2011. Those data are being collated, cleaned, and coded. Additionally, downstream buyers' information is being extracted from the primary data to facilitate conducting the downstream interviews this fall. We expect these to go quickly since most of them will be conducted by phone in Lusaka and a few central marketing places.

Finally, interviewing public policy makers as well as NGO agents involved in beans and cowpeas industries is expected to be completed to facilitate completion of Category I activities by November 2011.

#### *Project Website*

The project website (<http://valuechains.k-state.edu>) was developed and launched at the end of November 2010. Populating the site

is ongoing. This will accelerate as the project develops content from its own research activities.

We proposed to conduct one policy study. We have completed seven of them under the project. These are student theses that focused on both understanding the pulse value chain and providing policy direction on how to address the challenges that were identified. These studies are available on the project website (<http://valuechains.k-state.edu>).

The University of Zambia has benefited directly from the project by the support provided to students in both undergraduate and graduate programs. Additionally, SABREN has benefited from the collaboration it has developed with the project. Our three MAB students are all working in local organizations. Because of the structure of the program, these students bring work-related challenges to their classes and receive direct support from faculty in addressing these problems.

## Contribution to Gender Equity Goal

Of the six undergraduate students supported by the project, five are women. Of the six graduate students, two are women. The sampling process was specifically weighted to ensure representation of equitable proportion of women in the sample.

## Networking and Linkages with Stakeholders

- Strategic planning session with collaborating organizations: ZNFU, Central Farmers Association, and Central Statistics Office, September, 2010
- Visiting USAID Mission officials, October, 2010
- Worked with USAID Mission to help MAB students arrange for their U.S. Visas, December 2010
- Worked with SABREN in collecting primary farm-level data during survey instrument development as well as sampling process
- Numerous meetings with Zambia National Farmers Union (ZNFU) and with the Southern Africa Bean Research Network (SABREN) on collaboration.

## Leveraged Funds

SABREN provided in-kind support in the form of human resources to help with the data collection as well as evaluation of survey instruments and testing of the instruments.



Dry Grain Pulses CRSP  
Institutional Capacity Building and  
Human Resource Development  
FY 2011 Summary Report



# Dry Grain Pulses CRSP

The Dry Grain Pulses CRSP seeks to build host country institutional capacity through three mechanisms— support for long-term degree training, short-term non-degree training and the purchase of equipment to enhance research capacities. The status of activities planned and undertaken under these three categories of capacity building activities is included in the annual technical progress reports of the individual Phase II and III projects. In this section we provide a summary of these FY 2011 activities for the entire Pulse CRSP program.

### A. Degree Training

Pulse CRSP degree training is closely linked to research activities and aligned with CRSP project research and outreach objectives. By integrating graduate students into the research and outreach activities, their dissertation research problem has relevance and application to the Host Country context plus they contribute much to the technical quality of Pulse CRSP research activities. The graduate students’ research both contributes to the development of technologies as well as enhances understanding into the socio-economic, agronomic, environmental, political, cultural, etc. realities in the Host Country.

Nearly all graduate degree students are under the guidance and supervision of Pulse CRSP Principal Investigators (PIs). If a CRSP PI is not the “major professor”, the PI is certainly a member of the guidance and thesis research committees of the student. When a trainee is pursuing an advanced degree at a university in the Host Country, the Host Country PI will typically serve as the major professor. As a consequence, the research and teaching activities of CRSP trainees form an integral part of the annual workplans of each project.

The Dry Grain Pulses CRSP is continuing to make human resource development and institutional capacity building a priority objective for all projects awarded. There is an expectation that all Pulse CRSP projects will include an institutional capacity building objective for the partner host

countries and support an average of two to three degree training activities.

Annex 1 provides data on all the degree trainees financially supported by the Dry Grain Pulses CRSP from October 1, 2007 through September 30, 2011. A total of 53 students were either fully or partially supported in graduate or undergraduate degree programs in FY 2011. A descriptive summary of the degree training activities supported by the Pulse CRSP is provided in Table 1.

An estimated 6 graduate students at U.S. universities in 2011 were “indirectly” supported by the Dry Grain Pulses CRSP. These are students with research assistantships who are conducting their research in the host countries in collaboration with the HC PIs. CRSP funds therefore are only used to compensate them in the form of salary to conduct the research activities as outlined in the workplans. CRSP funds were not used to cover traditional academic expenses such as tuition, and the purchase of text books and computers. Since these graduate level degree students are not “Participant Trainees”, they are therefore included in the Pulse CRSP Trainee data base (Anex 1.) Subcontracted U.S. universities supporting graduate students on research assistantships are providing 25% match on their salaries and research expenses as they are viewed as a cost to complete the Phase II and III CRSP research projects.

It is noteworthy that 33 of the total 53 degree students supported by the Pulse CRSP in FY 2011 were enrolled in universities either in Host Countries or in academically advanced institutions in other countries (e.g., South Africa, Brazil) than the U.S. By supporting graduate training at partner HC universities, HC PIs are able to assume a greater role in the advising and monitoring of the academic formation and research activities of Pulse CRSP trainees. Moreover, by supporting graduate degree students at HC universities, the CRSP is contributing to the ongoing strengthening of academic graduate programs at these institutions. U.S. PIs frequently provide guest lecturers as well as serve on the guidance committees of graduate students. Finally, economies are achieved by supporting the training of USAID sponsored at universities in countries in Africa and Latin America. The hope is that the quality of instruction is not compromised.

### B. Non-Degree and Short-term Training

Non-degree training and short-term training are also considered to be vitally important for attaining CRSP institutional capacity building goals. This includes training through organized workshops, group training, short-term individualized training at CRSP participating institutions, and participation in networking activities with peers working on pulses in their region or internationally. Training activities typically last only a few days training programs (e.g., workshops) or involve a highly structured learning experience extending from a few weeks to several months or a year with individualized instruction in a lab/field setting. Like degree training, all non-degree training is integrated with research activities and is incorporated into the annual research workplans of each research project.

	No. of Trainees
<b>Training Status</b>	
▪ Active (in FY 11):	53
▪ Delayed/Pending	4
▪ Discontinued/cancelled:	5
▪ Training Completed:	12
<b>Profile of “Active” trainees (31)</b>	
<b>Gender</b>	
▪ Male	22
▪ Female	31
<b>Region of Origin</b>	
▪ East Africa	10
▪ Southern Africa	28
▪ West Africa	10
▪ Latin America/Caribbean	3
▪ United States	2
<b>Degree program</b>	
▪ M.S.	27
▪ Ph.D.	16
<b>A. B.S.</b>	10
<b>Training Location</b>	
▪ U.S.	20
▪ Host countries	30
▪ Third countries	3

Table 1: Summary of Degree Training by the Dry Grain Pulses CRSP as of September 30, 2011.

In FY 2011, an estimated 920 individuals benefitted from short term training subcontracted through Phase II and III projects in the Dry Grain Pulses CRSP. Of these short term trainees, over 48% were female. Table 2 presents a listing of some of the short-term training activities completed in FY 2011. Experience has shown that short term training is an effective strategy to build the capacity of technical staff at NARS and agricultural universities. These individuals do not require an advanced degree to conduct their analytical work or technology dissemination activities. Technical staff also find it difficult to obtain release time for educational purposes for extended periods from their institutions. Thus short term training is an attractive option for HC institutions. Moreover, short term training is highly cost effective and provides opportunities for the U.S. and Host Country PIs to join forces in the design and implementation of training activities.

**Table 2: Examples of FY 2011 Short-term Training Activities Supported by the Pulse CRSP**

**Pulse CRSP Project: PII-MSU-1  
Rwanda**

- Training on seed multiplication (for 28 participants including researchers and technicians from Rwanda Agriculture Board (RAB) research and extension programs) was conducted in order to build their capacity in seed multiplication, increase the qualities of seed produced, and build a common understanding of some guidelines in the domain. These technicians are supposed to train farmers and other technicians from collaborators institutions and Community Based Organization (CBOs).
- A bean stakeholder meeting was organized in Rwanda as a starting point in the establishment of an innovation platform for bean producers and traders. Participants from different organizations attended the meeting including privates sectors working in seed production and commercialization (RWASECO and Win-Win), International and local NGOs (Africare, DERN, DRD, CSC). Farmer associations and individual farmers were also represented. Discussions were focused on the following themes: Updates on bean breeding in Rwanda, seed system, role of ISAR seed program in promoting improved seed uptake and linkages with stakeholders in the seed industry; updates on Rwanda and regional markets; small packs in dissemination of improved bean varieties; and update on Agriculture Extension and policies in Rwanda by Raphaël Rurangwa (MINAGRI, Planning Director General). Three working groups were formed to elaborate recommendations: Group1: Roles and responsibilities in technology development and dissemination; Group 2: Accessibility of information on improved bean production and commercialization technologies to potential users; and Group 3: Seed increase of improved varieties and wide dissemination.

**Pulse CRSP Project: PI-MSU-2  
Mozambique**

- Type of Training: Analysis of market price data  
Description of Training Activity: Participants will work with data from SIMA and complete analysis to understand analytical methods and research issues related to market prices  
When the Short-Term Training Activity occurred: November 2011  
Location of Short-Term Training: Maputo, Mozambique  
Benefactors of the Short-Term Training Activity: 12 staff (2 from UAN/Angola, 5 from IIAM, and 5 from Directorate of Economics/MINAG, which includes SIMA  
Numbers of Beneficiaries by Gender: M-6, F-6, Total-12
- Type of Training: Camtasia screen recording software  
Description of Training Activity: Taught participants how to use Camtasia software  
When the Short-Term Training Activity occurred: May 2011  
Location of Short-Term Training: IIAM, Maputo, Mozambique  
Who benefitted from this Short-Term Training Activity? IIAM and UJES  
Number of Beneficiaries by Gender: M-2, F-0, Total-2

**Pulse CRSP Project: PI- UIUC-1  
Benin**

- Type of Training: Internship  
Description of Training Activity: Biocontrol of cowpea pests  
Status of this Activity as of September 30, 2011: on-going  
When the Short Term Training Activity occurred: July – October 2011  
Location of Short Term Training: IITA Benin  
Number of Beneficiaries by Gender: Male-2, Female-3, Total-5
- Type of Training: Training of technicians  
Description of Training Activity: Biocontrol of cowpea pests  
Status of this Activity as of September 30, 2011: on-going  
When did the Short Term Training Activity occur? FY11  
Location of Short Term Training: Burkina Faso and Niger  
Number of Beneficiaries by Gender: M-2, F-2, Total-4
- Type of Training: Training of technicians  
Description of Training Activity: Farmer field flora  
Status of this Activity as of September 30, 2010: August-October  
When the Short Term Training Activity occurred: FY11  
Location of Short Term Training: Burkina Faso, Mali, Nigeria, and Niger  
Number of Beneficiaries by Gender: M- >250, F- >250, Total- >500

### **Burkina Faso, Mali, Nigeria and Niger**

- Type of Training: Online Video Materials on SusDeViKI, SAWBO site, e-mail distribution, in country distribution, media exposure, and YouTube views

Description of Training Activity: Individual and groups wishing to access video-based training materials on the Internet (some of these are just views on the web and some are actual use of cell-phone ready videos).

Status of this Activity as of September 30, 2011: February-ongoing

When did the Short Term Training Activity occur? FY11

Location of Short Term Training: Burkina Faso, Mali, Nigeria, and Niger, and other countries across West Africa (and countries beyond West Africa including Rwanda)

Number of Beneficiaries by Gender:

The PII-UIUC-1 project team was not able to record the gender of participants but believed that there were nearly equal females as males.

SusDeViKI views of CRSP videos across the world - >10,000

SusDeViKI downloads of cell phone ready SAWBO/CRSP videos - >1200

YouTube views of various Scientific Animations Without Borders Videos - >16,757

SAWBO views and download of videos (only up since the beginning of September) - >200 views and downloads

The SAWBO and SusDeViKI sites have been shared online (via e-mail introductions) with over 300 NGOs, governmental agencies, universities, and other potentially interested organizations.

Voice of America has released several articles on SAWBO including YouTube videos – the “hits” to date include 4,747 views in the English version and 1,159 views for the Vietnamese version.

An incomplete list of the all the media articles on SAWBO can be found at <https://sib.illinois.edu/pittendrigh/sawbo/news>

### **Pulse CRSP Project: PI-UPR-1**

#### **Honduras**

- Type of training: Workshop for technicians in NARS bean research programs in Central America

Description of training activity: Participatory Plant Breeding-Common beans.

Location: EAP-Zamorano, Honduras

Duration: 3 days

Participants/Beneficiaries of Training Activity: 15

Numbers of Beneficiaries (male and female): M-9, F-6

### **Pulse CRSP Project: PIII-TAMU-1**

#### **Zambia**

- Type of Training: Short term workshop and hands on laboratory training

Description of Training Activity: Workshop explaining preliminary findings and what they mean to cowpea promotion strategy; hands on laboratory training on methods of phytochemical analysis.

When the Short Term Training Activity occurred: May 24 – 27, 2011

Location of Short Term Training: Nairobi & Egerton, Njoro, Kenya

Institutions benefitting from Short Term Training Activity: Government officials, NGO representatives, academic and extension staff of Egerton and KARI

Number of Beneficiaries by Gender: M-11, F-13, Total-24

### **C. Equipment for Host Country Capacity Building**

The Dry Grain Pulses CRSP recognizes that National Agriculture Research Systems (NARS) and agriculture universities need to acquire and maintain cutting edge research and extension capacity to effectively address the challenges facing the pulse (bean, cowpea and related edible legume crops) sectors and to contribute to economic growth and food and nutritional security in their respective countries. This requires investments in human resource development, scientific equipment, laboratory and field facilities, computer technology, and infrastructure.

The Management Office of the Dry Grain Pulses CRSP budgets, and competitively awards funds to Host Country institutions for capacity building. The intent is that these funds be utilized to address critical needs of Host Country (HC) collaborators which exceed the budgetary limits of the current projects, or to respond to needs of agricultural research institutions in USAID priority countries which may be future collaborators.

In FY 2011, the Management Office (MO) in consultation with the Technical Management Advisory Committee (TMAC) approved the award of 4 supplemental activities totaling \$175,000 that would enhance the capacity of host country institutions in strategic areas (e.g., production of Rhizobium inoculants to enhance BNF, development and deployment of IPM instructional videos that can be sent to cell phones, audio-visual equipment for training of extension agents, etc.) and provide direction to future productivity enhancing research investments in Pulses (See Annex 2).

Annex 1: Status of degree training planned and executed in FY 2011

Project	Given name	Last name	Country of citizenship	Gender	Training institute	Degree	Discipline	Training status as of 09/30/11	Start date	Anticipated completion date	Type of CRSP support
PI-CU-1	Crispus Mugambi	Njeru	Kenya	M	Moi University	M.S.	Soil Science	Completed	Feb-08	Feb-10	Full
PI-CU-1	Belinda Akinyi	Weya	Kenya	F	Egerton University	M.S.	Soil Science	Completed	Aug-08	Feb-11	Full
PI-CU-1	Jane Francisca	Lusweti	Kenya	F	University of Nairobi	M.S.	Plant Protection	Completed	Oct-07	Oct-09	Partial
PI-CU-1	Silvester	Odundo	Kenya	M	Moi University	M.S.	Soil Science	Completed	2009	Feb-11	Full
PI-CU-1	Eunice	Onyango	Kenya	F	Moi University	M.S.	Applied Environmental & Social Science	Completed	2009	Sep-10	Full
PI-CU-1	Roselyne	Juma	Kenya	F	Moi University	M.S.	Plant Breeding/Evaluation	Completed	2010	Mar-11	Full
PI-CU-1	Stanley	Onyango	Kenya	M	University of Nairobi	M.S.	Food Technology & Nutrition	Completed	2010	Apr-11	Full
PI-CU-1	Caren	Oloo	Kenya	F	University of Nairobi	M.S.	Plant Protection	Withdraw	2009		Full
PI-ISU-1	Cyrille	Syanobe	Rwanda	M	Makerere University	M.S.	Food Science & Technology	Withdraw	Aug-08		
PII-ISU-1	Gerald	Sebuwufu	Uganda	M	Iowa State University	Ph.D.	Agronomy	Active	Aug-08	Aug-12	Partial
PII-ISU-1	Geoffrey Arijole	Nyakuni	Uganda	M	Iowa State University	Ph.D.	Food Science & Human Nutrition	Canceled			
PII-ISU-1	Martin	Matambuka	Uganda	M	Iowa State University	Ph.D.	Food Science & Human Nutrition	Active	Jan-09	May-12	Partial
PII-ISU-1	Aisha Nakitto	Musaazi	Uganda	F	Makerere University	M.S.	Food Science & Technology	Active	Aug-08	Dec-10	Partial
PII-ISU-1	Simon	Okiror	Uganda	M	Makerere University	M.S.	Agricultural Economics/Agribusiness	Completed	Aug-08	Dec-10	Partial
PII-ISU-1	Catherine	Ndagire	Uganda	F	Makerere University	M.S.	Food Science and Technology	Active	Aug-09	Aug-11	Partial
PII-ISU-1	George	Jjagwe	Uganda	M	Makerere University	M.S.	Ag. Extension & Education	Active	Aug-10	Aug-12	Partial
PII-ISU-1	Rose	Kambabazi	Uganda	F	Makerere University	M.S.	Food Science and Technology	Delayed/Not Enrolled yet			Partial
PII-ISU-1	Marie Grace	Nkundabombi	Rwanda	F	Makerere University	M.S.	Food Science & Nutrition	Active	Aug-11	Aug-12	Partial
PI-MSU-1	Gerardine	Mukeshimana	Rwanda	F	Michigan State University	Ph.D.	Plant Breeding and Genetics	Active	Aug-08	Aug-12	Full



Project	Given name	Last name	Country of citizenship	Gender	Training institute	Degree	Discipline	Training status as of 09/30/10	Start date	Anticipated completion date	Type of CRSP support
PII-MSU-1	Krita	Iaacs	USA	F	Michigan State University	Ph.D.	Ecology and Nutrition	Active	Aug-08	/avg-12	Partial
PII-MSU-2	Maria da Luz	Quinhentos	Mozambique	F	Michigan State University	M.S.	Agricultural Economics	Canceled			
PII-MSU-2	Ana Lidia	Gungulo	Mozambique	F	University of Pretoria, South Africa	M.S.	Agricultural Economics	Active	Feb-09	Dec-11	Full
PII-MSU-2	Estaveo	Chaves	Angola	M	University Federal Vicoso, Brazil	M.S.	Agricultural Economics	Completed	Apr-09	Jul-11	Full
PII-PSU-1	Samuel	Camilo	Mozambique	M	Penn State	M.S.	Agronomy	Active	May-11	?	Full
PII-PSU-1	Virginia	Chesale	Malawi	F	Penn State	M.S.	Plant Nutrition	Active	2009	2012	Partial
PII-UPR-1	Ronald	Dorcinvil	Haiti	M	University of Puerto Rico	M.S.	Soil Sciences	Completed	Aug-06	May-09	Partial
PII-UPR-1	Monica	Mbui Martins	Angola	F	University of Puerto Rico	M.S.	Plant breeding	Completed	Aug-09	Aug-11	Full
PII-UPR-1	Jorge	Diaz	Peru	M	EAP-Zamorano	B.S.	Crop Science	Active	Jan-11	Dec-11	Partial
PII-UPR-1	Luis	Moncaño	Ecuador	M	EAP-Zamorano	B.S.	Crop Science	Active	Jan-11	Dec-11	Partial
PII-UCR-1	Manuel	Costa	Angola	M	University of Puerto Rico	M.S.	Plant Breeding/Pathology	Canceled			
PII-UCR-1	Mame Penda	Sarr	Senegal	F	University of Dakar	Ph.D.	Breeding/Pathology	Active	Oct-10	Oct-13	Full
PII-UCR-1	Marti	Portorff	USA	M	U. California-Riverside	Ph.D.	Plant Breeding/Pathology	Active	Oct-08	Mar-12	Full
PII-UCR-1	Antonio	David	Angola	M	University of Puerto Rico	M.S.	Plant Breeding	Completed	Aug-09	Aug-11	Full
PII-UIUC-1	Traore	Fousseni	Burkina Faso	M	University of Ouagadougou	M.S.	Entomology	Active	Sep-08	Aug-12	Full
PII-UIUC-1	Sanou	Appoline	Burkina Faso	F	University of Ouagadougou	Ph.D.	Entomology	Active	2010	Jul-14	Partial
PII-UIUC-1	Hermann	Somakpon	Benin	M	IITA-Benin	M.S.	Entomology	Active	Jul-09	Jul-11	Partial
PII-UIUC-1	Joelle	Toffa	Benin	F	IITA-Benin	Ph.D.	Entomology	Active	Jul-10	Jul-12	Partial
PII-UIUC-1	Elie	Dannon	Benin	M	IITA-Benin	Ph.D.	Entomology	Active	Sep-09	Mar-11	Partial
PII-UIUC-1	Laouali	Karimou	Niger	M	University of Niamey	M.S.	Entomology	Active	Sep-10	Aug-11	Partial
PIII-ISU-1	Mercy	Kabahuma	Uganda	F	Iowa State University	M.S.	Crop Production/Physiology	Active	Aug-10	Aug-12	Full
PIII-ISU-1	Martha	Abwate	Uganda	F	Makerere University	M.S.	Soil Science	Active	Sep-10	Aug-12	Full

P111-ISU-1	Peter	Ssenyonga	Uganda	M	Makerere University	M.S.	Soil Microbiology	Active	Sep-10	Aug-12	Full
P111-ISU-1	Charles	Komba	Tanzania	M	Sokoine U. of Agriculture	M.S.	Agronomy	Active	Sep-10	Sep-12	Full
P111-ISU-1	Beata	Khafa	Tanzania	F	Sokoine U. of Agriculture	M.S.	Plant Breeding	Active	Sep-10	Sep-12	Full
P111-MSU-3	Amos	Nyangi	Tanzania	M	Sokoine U. of Agriculture	M.S.	Food Science	Active	Sep-09	Sep-11	Partial
P111-MSU-3	Sarah	Johnson	Tanzania	M	Sokoine U. of Agriculture	M.S.	Food Science	Active ?	Aug-09	Aug-11	Full
P111-MSU-3	Rosemary	Marealle	Tanzania	M	Sokoine U. of Agriculture	M.S.	Nutrition	Active ?	Aug-09	Aug-11	Full
P111-MSU-3	Sharon	Hooper	Jamaica	F	Michigan State University	Ph.D.	Food Science	Active	Aug-09	Aug-12	Partial
P111-MSU-3	Sacred	Jacob	Tanzania	M	Sokoine University of Agriculture	M.S.	Nutrition	Active	2011	Feb-12	Partial
P111-TAMU-1	Twambo	Hachibamba	Zambia	F	University of Pretoria	Ph.D.	Food Science	Active	Aug-10	Jun-13	Full
P111-TAMU-1	Alice	Nderitu	Kenya	F	University of Pretoria	Ph.D.	Food Science	Active	Aug-10	Jun-13	Full
P111-TAMU-1	Leonard	Ojwang	Kenya	M	Texas A&M University	Ph.D.	Nutrition/Food Science	Active	Jan-10	Dec-11	Partial
P111-TAMU-1	Archana	Gawde	?	F	Texas A&M University	Ph.D.	Molecular/Env. Plant Sci	Active	Jan-09	Dec-12	Partial
P111-TAMU-1	Billy	Kiprop	Kenya	M	Egerton University	M.S.	Biochemistry	Active	Jan-10	Dec-11	Partial
P111-KSU-1	Esther	Zulu	Zambian	F	University of Zambia	B.S.	Agriculture Economics	Completed	2010	2011	Full
P111-KSU-1	Agness	Myece	Zambian	F	University of Zambia	B.S.	Agriculture Economics	Completed	2010	2011	Full
P111-KSU-1	Natasha	Chilundika	Zambian	F	University of Zambia	B.S.	Agriculture Economics	Completed	2010	2011	Full
P111-KSU-1	Chimuka	Samboko	Zambian	M	University of Zambia	B.S.	Agriculture Economics	Completed	2010	2011	Full
P111-KSU-1	Edna	Ngoma	Zambian	F	University of Zambia	B.S.	Agriculture Economics	Completed	2010	2011	Full
P111-KSU-1	Chalwe	Sunga	Zambian	F	University of Zambia	B.S.	Agriculture Economics	Completed	2010	2011	Full
P111-KSU-1	Susan	Chiona	Zambian	F	University of Zambia	M.S.	Agriculture Economics	Completed	2010	2011	Full
P111-KSU-1	Cornard	Chilala	Zambian	M	University of Zambia	M.S.	Economics	Active	2010	2013	Full
P111-KSU-1	Martin	Mwansa	Zambian	M	University of Zambia	MBA	Business Administration	Active	2010	2013	Full
P111-KSU-1	Sosthenes	Mwansa	Zambian	M	University of Zambia	MBA	Business Administration	Active	2010	2013	Full
P111-KSU-1	Lydia	Msocha	Zambian	F	University of Zambia	MBA	Business Administration	Active	2010	2013	Full

### **PII-UPR-1: Building Capacity for Enhancing Biological Nitrogen Fixation in Bean Production Systems in Central America, Haiti, Angola and Mozambique**

Amount Awarded: \$27,700

Participants received training in small-scale *Rhizobium* inoculant production and inoculation. This was a hands-on training with practical exercises of isolating *Rhizobia* bacteria from nodules and soil, selection of *Rhizobia* strains for speed of nodulation and efficiency to fix nitrogen. The most probable number count method was taught to determine the soil *Rhizobial* numbers to predict the response to inoculation. Hands-on instruction was provided on small-scale peat-based inoculant production starting with increasing *Rhizobia* in culture media, control of contamination, peat preparation and sterilization, inoculation of *Rhizobia* in a peat carrier, quantifying *Rhizobia* in an inoculant and quality control.

Activities: Two workshops were funded: (1) One for NARS bean researchers from Central American and the Caribbean region at the Escuela Agrícola Panamericana (Zamorano) in Honduras (scheduled for November 2011); (2) the second workshop for NARS bean program scientists from IIA-Angola and IIAM-Mozambique to be hosted by IIAM in Maputo, Mozambique in late 2011.

### **PII-MSU-2: Video Equipment for IIAM, Mozambique**

Award Amount: \$25,300

Simple video equipment, including a web camera and a camcorder, along with a laptop computer and required software were purchased. This equipment is needed by IIAM scientists to train technicians, extension agents and NGO staff at IIAM zonal agriculture research centers. Cost of travel from Maputo to the regional centers limits critical training which could be arranged if audio-visual equipment were available.

Local specialists will be used to train the participants on using this simple technology to link video/audio equipment to Power Point and other presentation methods, creating low cost communication tools. This activity is designed as a combined activity, lead by IIAM-Mozambique with participation by UAN (Universidade of Augustine Neto) staff conducted in Maputo, using specialists from the University of Eduardo Mondlane's Technology Center. UAN will send two participants and IIAM with the MINAG Documentation Center will also participate in the 10 day intensive training.

### **PII-UIUC-1: An assessment of the availability of cell-phones among extension agents, NGO staff and farmers and of their skill sets and abilities to use the video and Bluetooth capacities of their phones in Burkina Faso and Niger to receive and deploy IPM messages for management of insect pests in cowpea.**

Award Amount: \$22,000

Insect pest attack on cowpea crops in West Africa represents the biggest biological constraint

on this crop. The PII-UIUC-1 project is focused on the development and the deployment of practical technologies that can be used by small-holder farmers to optimize their cowpea production by minimizing insect attack. A deployment strategy that utilizes simple IPM video messages that can be received and viewed by extension agents and farmers with cell phones that have video/Bluetooth capability has the potential to be a highly cost effective way to get this information quickly to large numbers of rural farmers when there is risk of intense insect infestation. It also represents a strategy that can be expanded into other countries, in many cases by doing additional language voice overlays.

Five new videos (dealing with pest control strategies – viral and neem-based sprays as well as procedures for safe handling of pesticides) will be developed and provided to host country collaborators at INERA, Burkina Faso. These will be completed in the first six months of the project (FY11). A survey of women's access and use of cell phones will occur over the first year and a half (FY11 and FY12). Pending the outcome of the assessment studies, workshops will be planned and held to train extension agents, NGOs, and INERA staff/collaborators during FY 2012 in the most effective strategies to enable maximal deployment of these videos.

### **PII-PSU-1: Workshop on “Enhancing Pulse Productivity on Problem Soils by Smallholder Farmers- Challenges and Opportunities”, The Pennsylvania State University, August 14-18, 2011.**

Award Amount: \$100,000

This workshop was hosted by Dr. Jonathan Lynch at the Pennsylvania State University with co-sponsorship by the Dry Grain Pulses CRSP, CIAT, IITA and ICRISAT and financial support from the Bureau of Food Security, Office of Agriculture, Research and Technology, USAID.

The goal of this workshop was to formulate recommendations on future research foci to achieve major increases in pulse productivity under edaphic and abiotic stress conditions in small-holder farm settings. Over 50 leading scientists, experts and stakeholders from both the U.S, and developing countries with interest in and knowledge of global grain legume production (primarily common bean, cowpea, etc.) were assembled to contribute to this research visioning and priority setting exercise. The recommendations resulted from the workshop were articulated in written Executive Summary) that was presented to USAID and USDA/ARS for consideration in making future investments in grain legume research under Feed the Future.

*Specific Workshop Objectives:*

- 1) Discuss and describe the imperative of increasing grain legume productivity to achieve food and nutritional security, sustainability of cropping systems and resilience to climate change. Priority consideration will be given to focal developing countries under Feed the Future in Africa, Latin America and Asia ([www.feedthefuture.gov/](http://www.feedthefuture.gov/)) where grain legumes are commonly grown and consumed.
- 2) Discuss and describe edaphic, abiotic and production costs/ returns constraints to achieving high yields of grain legumes by small-holder resource-poor farmers on degraded/problem soils. Specific attention will be given to the following cropping systems in developing countries; lowland cereal-bean production system with high rainfall and temperatures; hillside bean production systems irregular rainfall and degraded soils, highland bean production systems, cowpea production in the Sahel and Savanna regions, etc.
- 3) Explore the potential to increase productivity and reduce risk afforded by technologies and current knowledge of (1) plant biology, (2) environmental stress physiology, (3) molecular genetics and crop breeding, (4) soil fertility and water management, (5) biological nitrogen fixation, and (6) integrated crop management. Identify knowledge gaps and bottlenecks to technical progress in each area.
- 4) Formulate a list of research approaches in each of the 6 areas that have high potential to effectively and successfully contribute to substantial gains in productivity of grain legumes under the small holder farm situations identified in point (2). Prioritize these approaches for their potential to substantially increase grain legume productivity with adequate sustained research investment.
- 5) List the high priority training and institutional capacity building needs that may be concomitantly required for institutions in the countries listed in point (1) to achieve these research goals.
- 6) Identify technologies and management practices which may be ready for deployment with adaptive research and which could contribute to grain legume productivity gains and the amelioration of edaphic/abiotic stresses in the production systems identified in point (2).
- 7) Identify research and training capacities, timelines, and resources required as well as coordination by public and private partners (U.S. and Host Country agriculture research institutions (NARS and universities), IARCs (CIAT, IITA, etc.), and private sector) to achieve the research listed in point (4) in a timely and efficient manner.

The Executive Summary of the Workshop can be accessed from the Pulse CRSP's website [www.pulsecrsp.msu.edu/](http://www.pulsecrsp.msu.edu/).

**Total FY 2011 Investment by Dry Grain Pulses CRSP in Institutional Capacity Building: \$175,000**