



Dry Grain Pulses
Collaborative Research Support Program (CRSP)

2012

Technical Highlights

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Preface

Dry Grain Pulses Collaborative Research Support Program (CRSP)

FY 2012 Technical Highlights Report
(October 1, 2011, to September 30, 2012)

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 builds 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 seek to address the root causes of hunger and forge 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 United States to access these markets. Concurrently, many developing countries continue to face food and nutritional insecurity, and smallholder pulse 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 that addresses the challenges of these smallholder farmers. Pulse breeding for high yield potential and resistances to abiotic and biotic stress factors, integrated insect pest management, improved storage methods and technologies, and enhancing soil fertility and/or improving a plant’s ability to utilize soil nutrients are among the Pulse CRSP projects.

Pulse crops represent an important group of staple foods that contribute to addressing household food security, generating income, enhancing soil quality—and thus the sustainability of agricultural systems—and providing nutrients (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 its Global Themes:

1. To reduce pulse production costs and risks for enhanced profitability and competitiveness
2. To increase the utilization of pulse 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 pulse sectors and developing country agricultural industries

Under the five-year Dry Grain Pulses CRSP, a two-phase technical program was 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, from which 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, which were subcontracted mid-fiscal-year 2010, address strategic technical gaps in the Pulse CRSP research program, including biological nitrogen fixation, human nutrition, and value chain research.

The 12 projects presented in the FY 2012 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 these projects.

This report highlights the technical progress and achievements made by Pulse CRSP projects during FY2012. 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 2012 Technical Highlights Report* is only a one-year snapshot. Moreover, these highlights are condensed versions of more comprehensive technical reports that subcontracted U.S. universities provide annually to the Management Entity and USAID. These technical progress reports are valued and utilized for assessing Pulse CRSP program performance and reporting by USAID to the U.S. Congress on Title XII and Feed the Future achievements and impacts.

I want to encourage you to read the *FY 2012 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 United States. 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 whether a bean-based diet for HIV-positive children will maintain higher CD4 counts than a fish–maize based diet.

- Detailed, analytical studies on the correlation between bean consumption and chronic disease prevention

Global Theme 3: To improve pulse value chain performance

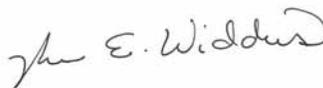
- Improved storage technologies to increase the quality of grain available for sale in the market apart from harvest times, when prices are generally low
- Identifying the different supply chains used by the Zambian pulse industry and determining which are most efficacious for grain legume producers

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

- Eighty students from host countries enrolled in training associated with the Pulse CRSP
- More than 30 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.

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 final year have produced important achievements in regard to project research and development goals. Efforts to improve bean quality and yield involved continued research on complex interactions of bean variety and fertilizer. Participatory research with farmers enabled us to develop extension materials using print and video media that have been refined and translated into local languages. These have been utilized in training nearly 1,000 farmers (70 percent women) in 60 demonstration sites and providing seed for two improved varieties. Training covered germination testing; plant spacing; manure application; pest and disease management; harvesting, threshing, drying, and moisture testing; solarization and triple bagging; and sorting and seed selection. Capacity building continued for six groups involved in community-based production and sale of quality seed to ensure success and sustainability.

To enhance nutritional value, appeal, and consumption of beans, appealing bean-based products have been developed and farmers have been taught nutrient-enhancing ways to prepare beans at home. Farmer groups have also been taught to prepare more shelf-stable, value-added, bean-based products. As a result, rural microenterprises have been created, selling snacks to school children and at/for special occasions in the community. Research was performed to determine the influence of preprocessing methods on starch and protein digestibility, micronutrient bioavailability, and the sensory acceptability of a bean-based porridge for supplementary feeding. Tests were also performed to screen the culinary and sensory characteristics of seven local and 18 improved bean varieties in Uganda and 16 improved varieties in Rwanda. 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.

Project households have increased the area planted in beans, market participation, and income. The project strengthened the collective marketing capabilities of farmer groups through enhanced understanding of market price variations, skills to obtain higher prices resulting from improved postharvest grain handling, better coordination of grain bulking and storage at the community level, and negotiation skills. The project provided assistance to farmer groups to develop business plans, improve record keeping and analysis, and promote gender equity and better group dynamics. A multistakeholder bean value chain forum has been established to enable participants to identify key constraints and solutions for broad-based, successful market participation.

Project Justification and Objectives

Agriculture in East Africa is characterized by women and men working in small-scale, rain-fed fields of poor soil fertility, averaging two hectares per household. Erratic bimodal rainfall

patterns in recent years further challenged cropping results. Farmers have limited access to extension training for improved agronomic practices, quality seed, technologies to improve yields and reduce postharvest losses, and access to 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. Availability and use of processed products remains very modest. Hunger and poverty are widespread.

The lack of value-added bean products with reduced cooking time 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, germination, and extrusion cooking) can enhance digestibility and nutritional value by reducing phytates and polyphenols that limit iron uptake, and create value-added, bean-based products.

Prospects of marketing increased quantities of beans and new agro-processed bean products within the Ugandan and regional markets require careful examining of production and marketing constraints (increased farm productivity, producer incentives, and access to better markets). It is equally important to

Participatory research with farmers enabled us to develop extension materials using print and video media that have been refined and translated into local languages.

understand the prospects for increasing demand for beans and agro-processed products through collaboration with private sector businesses.

Our efforts to introduce new agronomic practices and technologies show encouraging progress. Ongoing collaboration since 2004 among Iowa State University (ISU), Makerere University (MAK), and Volunteer Efforts for Development Concerns (VEDCO) in Uganda's Kamuli District using a sustainable livelihoods (SL) approach increased food security and market readiness from nine to 77 percent among 800-plus farm households. Most (90 percent) of participating households produce beans, but, initially, only a few (20 percent) sold beans. The SL approach focuses on understanding and supporting individual and community capabilities, assets (natural, physical, human, financial, social, cultural, and political capital), goals, strategies, and activities. Diversification of livelihood opportunities and activities is crucial to sustainability. 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 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, providing food security and income to the most vulnerable group—women and children.



Village taste test

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.
4. Increase the capacity, effectiveness, and sustainability of agriculture research institutions that serve the bean sector in Uganda and Rwanda.

Research and Outreach Approaches, Results, and Achievements

Objective 1: Improve harvested bean yields and quality.

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

Based on significant local variation in soil fertility conditions, overall bean productivity remains well below genetic potential. Soils in the test sites were low in phosphorous and often unresponsive to supplemental Nitrogen fertilizer. We tested the hypothesis that incorporating varying amounts of inorganic phosphorous into the soil prior to planting would help identify the level required to generate a profitable return on seed and N-fertilizer investment.

The genotypes tested were Kanye bwa, a local variety common in southeast Uganda, and NABE4, an improved variety released by NaCRRI. Phosphorus application rates were 0, 60, 120, and 180 kg/ha. Increasing rates of phosphorus were used to test whether an increased supply of phosphorus would lead to better agronomic performance on low nutrient soils common in Uganda. Phosphorus was applied in bands along the rows at planting.

The experiments were carried out on stations at Nakabango Variety Testing Center in southeastern Uganda, at NaCRRI's main fields in Namulonge in central Uganda, and at the Mbarara Zonal Agricultural Research and Development Institute (ZARDI) in southwestern Uganda. The experiments were organized in split plot design with the varieties as the main plots and the phosphorus rates as subplots. At physiological maturity, data on yield and its components were collected. Data were subjected to analysis of variance in PROC GLM using SAS statistical package.

In Nakabango, results showed that there were significant differences in yield, seeds per pod, and plants harvested per square meter for all phosphorus rates. The number of pods per plant was significantly higher with addition of phosphorus than the control for Kanye bwa.

Phosphorus fertilizers did not have significant effects on 100 seed weight for Kanye bwa. The 100 seed weight for NABE4 at 120 kg/ha was significantly higher than the other phosphorus rates. There were no significant effects of phosphorus on the harvest index for NABE4; however, the harvest index for Kanye bwa at 60 kg/ha was significantly higher than that for the control.

At NaCRRI-Namulonge, increasing rates of phosphorus did not have significant effects on yield, pods per plant, seeds per pod, 100 seed weight, and plants harvested per square meter; there was no significant effect of phosphorus on the harvest index for Kanye bwa. For NABE4; the harvest index with the addition of phosphorus fertilizer was significantly higher than the control.

At Mbarara ZARDI, results showed that for Kanye bwa, yield at 120 kg/ha of phosphorus was significantly higher than that of the control and for 180 kg/ha of phosphorus. There were no significant effects of phosphorus on the yield of NABE4. There were no significant effects of phosphorus on the number of pods per plant for Kanye bwa; however, for NABE4 the number of pods per plant at 120 kg/ha of phosphorus was significantly higher than that at 180 kg/ha of phosphorus. The number of seeds per pod was significantly higher at 120 kg/ha of phosphorus than the other phosphorus rates. There were no significant effects of phosphorus on the 100 seed weight and plants harvested per square meter for both varieties. The harvest index for Kanye bwa at 180 kg/ha of phosphorus was significantly higher than that of 120 kg/ha of phosphorus. For NABE4, no significant effects of phosphorus on harvest index were observed.

At Nakabango, results showed that there were no significant effects of variety, phosphorus rates, and on variety X phosphorus interaction on yield and all the other measured yield parameters. Yields, however, tended to increase with higher phosphorus rates. Yields at 180 kg/ha were 44 percent and 82 percent higher than the control. For NABE 4, the number of plants harvested at 120 and 180 kg/ha of phosphorus was significantly higher than that at 60 kg/ha.

At NaCRRI, results showed that phosphorus fertilizers did not have a significant effect on all measured traits except the number of seeds per pod for Kanyebwa; the number of seeds per pod at 120 and 180 kg/ha of phosphorus were higher than those at 0 and 60 kg/ha of phosphorus. For NABE4, yield at 180 kg/ha of phosphorus was significantly higher than at lower phosphorus rates. Phosphorus addition further led to significantly higher number of pods per plant than the control for NABE4. The number of seeds per pod at 60 kg/ha of phosphorus was significantly higher than that at 120 kg/ha. Phosphorus addition did not significantly affect the 100 seed weight and number of plants harvested per square meter for NABE4. The harvest index for NABE4 at 180 kg/ha was, however, significantly lower than that of other phosphorus rates studied.



Dissemination workshop

Summary and Implications

Responses to phosphorus fertilization were mixed; 2012A was the third season for repeated application of phosphorus. The low yields observed indicate that phosphorus levels in the soil may still have not built up sufficiently and/or other factors still limit productivity even under seemingly better management of the beans on station. At Nakabango, yields tended to increase with increase in phosphorus for both Kanyebwa and NABE4. At NaCRRI, P fertilization effects were muted and yields were lower than at other trial sites. At Mbarara ZARDI, results showed that yields increased for Kanyebwa while NABE4 had a slight decrease in yield with increasing phosphorus levels. Comparison of seasonal yields shows that yields in 2012A were higher than 2011B at several P levels. At Nakabango, yields for Kanyebwa in 2012A were higher than in 2011B. At the same time, yields for both varieties at NaCRRI in 2012A were generally at least three times higher than those in 2011B. Phosphorus application did not have a significant effect on plant population (plants/m²). Generally, the harvest population was lower than the optimum of 20 plants/m². The low yields observed indicate that phosphorus fertilization alone may not be the answer to the low yields. Further, most of the applied phosphorus may be fixed by the soil, thus making it unavailable for plant growth.

Variety Performance and Farmer Acceptability

Important qualities that farmers consider in accepting varieties include adaptability to a range of soil fertility conditions,

tolerance/resistance to heavy rainfall or drought, early maturity, yield, marketability, quick cooking, and taste. Farmers in Kamuli prefer red to pink mottled varieties similar to the local variety (Kanyebwa) that is commonly grown but prone to diseases. NaCRRI tested eight early maturing red and pink mottled varieties. These varieties were not widely accepted by farmers in Kamuli due to low tolerance to drought and/or high levels of rainfall.

Kanyebwa remains farmers' preferred bean variety. Of the improved varieties, K132 is the best accepted, followed by NABE and K131, the variety most tolerant to disease and water stress. K132 is the most preferred improved variety because of its high yielding potential and higher grain weight. The NaCRRI bean program recently released seven bush bean varieties that are all early maturing with tolerance to drought.

Support Community-based Seed Production by Farmer Groups and Associations

Community-based seed production (CBSP) is being used by farmer groups in Kamuli to create viable agriculture-based enterprises and to help other farmers access improved seeds at lower cost. To establish systems for community-based production of quality seed, we have trained and supported six farmer groups committed to bean production in accordance with established seed quality standards.

We have been using participatory methods to engage farmers in this process and establish viable and sustainable protocols for seed production, quality control, and storage. Seed producers have been trained in record keeping regarding production costs to determine appropriate selling prices and timing of sale to achieve a profit margin. To ensure sustainability in seed production, refresher trainings in institutional development, collective marketing, and the establishment of linkages to other existing seed producers and buyers have been done. The training and extension guide for CBSP is complete, with modules on agronomy, farm record keeping, and institutional development. Local capabilities have been further strengthened through supervision by the technical specialists from NaCRRI and by information sharing about performances of bean varieties.

Six farmer groups have been multiplying two preferred improved bean varieties, NABE4 and K132, during five successive growing seasons. They have increased the acreage for group fields and their household fields. Upon harvest, they retain seed for subsequent planting on both group and household fields, sell some to VEDCO for new demonstration gardens and distribution to other farmers, and sell to institutions, traders, and at the market.

Reducing Postharvest Losses through Solarization and Hermetic Storage (Triple Bagging)

Solarization and triple bagging have been proposed as effective remedies to maintain grain quality postharvest. In this study, the effects of triple bagging storage and solarization on beans and maize postharvest losses and germinability were studied. Maize and beans obtained from local markets were dried to 12 to 13 percent moisture content; 20 kg of grain were inoculated with 20 live insects obtained from badly damaged beans and maize.

Beans and maize were triple bagged and stored for seven months. Moisture content, broken grains, foreign material, insect damage, number of live/dead insects, and germinability were determined periodically. Triple bagged bean and maize samples retained greater than 80 percent germinability and grain quality after three months of storage. After four months, germinability reduced to 75 percent, but grain quality remained constant. Triple bagging was even more effective for maize, maintaining grain quality and germinability (greater than 90 percent) even after seven months of storage.

Solarization of beans and maize was achieved by placing a 1 cm thick layer of grain on black polythene, covered by transparent polythene, under the sun. The effects of solarization were studied through hourly determination of dead and live insects in the grain over a period of six hours. Solarization led to death of inoculated insects after six hours, more so in beans than in maize.

Evaluate Adoption of Improved Integrated Crop Management Practices and Technologies

Nearly 1,000 farmers have participated in on-farm training sessions for many of the integrated crop management practices and technologies. Extension materials (posters, PowerPoint slides, locally shot and animated video clips) have been finalized and translated into Luganda for the integrated crop management practices that have proven effective and feasible for smallholder farm production in Uganda. These included improved field site selection, incorporation of compost and manure into soils, optimizing plant and row spacing, scheduling of weeding, integrated pest management, grain harvest/threshing, postharvest drying on tarpaulins, grain moisture monitoring and grading, and hermetically-sealed triple-bag storage. Training involved interactive discussion, multimedia tools, and on-farm demonstrations.



Village food preparation

Nearly all households received training in production and postharvest management practices. Practices that demonstrate the highest level of current implementation include field preparation, line planting, timely weeding, timely harvesting, drying on tarpaulins, gentle threshing, cleaning, and hermetic storage. Approximately two-thirds of farmers received training in processing (soaking and malting, flour making, and making snacks/cakes for sale). Collective marketing activities (participation in group sales, active role in leadership, and packaging and labeling) are similarly characterized.

Strengthen Learning and Sharing of Innovative Practices

The project team has organized three meetings (in Jinja and Kampala and at a Farmer Field Day) to share results from our research and development efforts and to strengthen the learning network. In Jinja, the objectives were:

1. to characterize the bean value chain
2. to engage key stakeholders in the identification of current functioning, opportunities for improvement, and constraints
3. to develop a realistic action plan delineating the way forward in chain development

Forum participants were farmers, input dealers, traders, credit providers, institutional consumers, NGOs, local government, research and academia, processors, nutritionists, and sales outlets.

In Kampala, a project dissemination workshop was held with key stakeholders, who expressed interest in building on the project's successes in the following ways:

- promoting value-addition processing
- enhancing the nutritional content of bean-based products
- including other bean varieties in future experiments
- investigating expanded markets for beans
- broadcasting project findings through Farm Radio Africa
- facilitating access to microfinance among farmer groups

The third sharing activity was the final Farmer Field Day to demonstrate and disseminate recommended management practices and technologies for integrated crop management, with the theme "Enhancing Sustainable Agriculture Technologies for Food and Nutrition Security through Knowledge Transfer." Training modules and materials have been compiled for production, postharvest handling, processing, and marketing. Participants will include farmers and district agriculture and community development officers.

Scaling up dissemination and adoption of recommended practices and technologies has already begun in Uganda. Specific practices that are already incorporated into VEDCO project activities in some other districts, funded by various donors, are establishing direct links between project communities and NaCRRRI to obtain high quality seed and varieties for farmer-based experimentation, use of training materials developed through the CRSP project, community-based seed production systems, and hermetic storage of grain and seed.

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

During phase I, our preprocessing methods developed bean flour with significantly reduced cooking times (15 minutes). In phase II, we sought to promote increased bean consumption among farming communities and urban consumers who could realize the nutritional and health benefits as well as benefit from reduced cooking time and less monotony in their diets. The final component sought to enhance children's daily nutrient intake through increased consumption of beans and bean products;

we subsequently developed products that are practical, useful in school settings, and acceptable to students. Institutional buyers such as schools, hospitals, and humanitarian agencies have been identified as potential markets for beans and bean products.

Modeling Iron Bioavailability in Beans and Establishing Effect of Extrusion Cooking

Beans are high in iron content (30–90 ppm) and are an important source of iron in developing countries; however, the iron has very low bioavailability due to the high content of polyphenols and phytates, factors that inhibit iron bioavailability. Strategies to improve beans' contribution to iron nutrition include increasing iron concentration, improving bioavailability, or increasing both. Our research screened for iron bioavailability in Ugandan bean varieties and established the effects of extrusion cooking. There were significant differences in the chemical composition and iron bioavailability among bean varieties. The relative biological availability (RBA) of white seed coat varieties was significantly higher than in colored seed coat varieties.

The effect of extrusion cooking on iron bioavailability of NABE6, a Ugandan white seed coat bean variety, was also determined. The optimal combination of extrusion variables was determined to be 15 percent moisture content, 120°C die temperature, and 3 kg/h feed flow rate.

Address Nutrition and Health Problems among Vulnerable Individuals through Increased Consumption of Beans, Bean Products, and Complementary Foods

We hypothesized that alternative ways of preparing beans would result in increased bean consumption. We hypothesized that providing bean breeders with critical information on expectations of consumers and processors would lead to the development of sustainable and consumer acceptable varieties.

Key Phase II activities involved developing and utilizing appropriate extension information, education, and communication approaches (e.g., nutrition, processing of bean-based products) for rural community nutrition and health workers. In Uganda and Rwanda, manuals and posters on improved methods of bean preparation to enhance cookability and nutrient bioavailability were developed. A manual on feeding children aged 6 to 59 months was developed to increase awareness of adequate feeding practices and the benefits of adequate nutrient and energy intake. In Uganda, a brochure explaining improved methods of cooking beans was prepared. Another manual, for mothers, outlined how to prepare porridge with bean-based composite flour.

In Rwanda, 60 Community Health Workers were trained on nutrition and its relationship to health, bean flour pretreatments and processing, bean-based soup processing, and utilization of simple cold extrusion technology using hand-operated presses.

Analyze Culinary Properties, Sensory Characteristics, and Consumer Acceptability of Improved Varieties

Linkages between academic research institutions and national agricultural research institutions responsible for bean breeding are important to increase the likelihood that breeding program efforts correspond to traits and characteristics that consumers accept and prefer. Toward this end, project researchers analyzed culinary properties of improved bean varieties and their sensory characteristics (color, texture, taste, flavor, etc.) and assessed consumer acceptability of improved bean varieties in Uganda (from NaCRRI) and in Rwanda (from ISAR/RAB).

Culinary and sensory properties of 18 improved varieties were screened to inform the breeding programs regarding consumer acceptability of new varieties, including weight gain after soaking and cooking, solids content of the soaking water and cooking broth, degree of pigment leaching into soaking water, number of split seed coats and cotyledons after cooking, and cooking time. The local varieties studied were White, Masavu, Kahura, Nambale-long, Nambale-short, Kanye-bwa, Kankulyemaluke, Yellow-short, and Yellow-long.

In Rwanda, 60 Community Health Workers were trained on nutrition and its relationship to health, bean flour pretreatments and processing, bean-based soup processing, and utilization of simple cold extrusion technology using hand-operated presses.

Overall, the local varieties were significantly preferred in terms of color, texture, and flavor compared to the improved varieties. Generally, improved beans cooked in a shorter time than local varieties, with the majority

cooking within 60 minutes; the local varieties required more than 60 but less than 120 minutes. Protein, zinc, and iron content of local and improved varieties were also determined. Overall, improved varieties showed higher protein content but lower zinc content than local ones, although differences were not statistically significant.

A Memorandum of Agreement was signed in September 2012 between CIAT/PABRA and Makerere University, College of Agricultural and Environmental Sciences to implement activities leading to improved food and nutrition security for vulnerable communities, specifically to:

- Develop value-added products consumable by vulnerable groups, such as bean flour and bean-based flour from high iron bean varieties
- Assess the effect of processing on aflatoxin levels in bean products
- Evaluate the nutritional and physical–chemical properties of bean-based products to determine macro- and micronutrient retention
- Develop bean recipes incorporating the developed bean and bean-based flours for promotion in schools and to NGO partners
- Test the acceptability of foods from the developed recipes among target consumers
- Assess culinary and sensory properties of pre-released and new improved bean varieties

Incorporate Insights from the Analysis of the Private Food Processing Industry Regarding the Development and Commercialization of Bean-based Products

The project team established a partnership with the private business sector to promote adoption of value addition to increase bean consumption and create new market outlets. Bean varieties that are high yielding and stress resistant but have low consumer acceptability and are prone to being hard-to-cook were selected for value addition and product development in phase I. In phase II, processing protocols were refined and upscaled through the Technology Business Incubator (TBI) model to promote technology transfer to the private sector. Through project activities, private sector partners are now better linked to farmer organizations for mutual benefit in value-addition activities.

Bean flour production was piloted in Makerere University's FTBIC in partnership with Nutreal Ltd. Six-month shelf-life studies of the bean-based flour were completed, and bean-based flour was test-marketed in the Food Parlour at Makerere University. Sales are growing steadily as consumers try the bean-based flour for sauce and porridge. Appropriate packaging materials have been developed and tested, including bi-layer packaging to reduce moisture pick-up and lipid oxidation during storage, and printability to brand the product and reduce counterfeiting. Two distribution systems for the products have been tested. Bean soup and porridge were prepared and served to potential customers for tasting. Information leaflets were distributed and customer questions answered. Promotion and marketing through a nutritionist was also piloted.

Farmers are being prepared as suppliers by the teaching of postharvest techniques to maintain the quality of their beans. Progress has been made regarding production of seed that is sold to community members. Availability of good quality seed is expected to lead to increased bean production.

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

Farmers in Kamuli own and cultivate an average 1.5–3.5 acres, and half borrow or rent land for their agricultural activities. Most were growing maize and beans, but initially only a few (15 percent) were harvesting at least 50 kg of beans to sell. Project households have increased the area planted in beans by approximately 50 percent and have increased market participation in beans, with 50 percent more smallholder farm households now producing beans in sufficient quantities to earn income by selling some (currently 65 percent of farm households). Improved crop management practices and technologies have stimulated market participation by increasing the quantity available for sale. Basic value-addition activities such as proper drying, sorting, grading, and storage that minimizes insect infestation and damage, have helped improve quality, demand, and price.

The project in Uganda has sought to strengthen the collective marketing capability of farmer groups. The project assisted farmer groups in developing business plans, improving record keeping and analysis, promoting gender equity and improved group dynamics, and creating opportunities for farmers to learn

from successful farmers and seed production groups. With selected groups, local community-based production and sale of quality seed has been promoted to improve widespread smallholder farmer access to improved varieties and other yield enhancing inputs.

A multistakeholder bean value chain forum has been established to enable participants to identify key constraints and solutions for broad-based successful market participation. Bean farmers have consequently established stronger links with traders. Further, input traders have started to extend their services to farming communities and are willing to provide some advisory services. Bean farmers are now accessing information on market prices and demand of various bean varieties from different regional markets.



Baked and fried food

Participating in the CRSP project has enhanced farmers' assets and capabilities, both individually and collectively. Their social capital has been enhanced through stronger groups and group connections. Their human capital has been enhanced through technical knowledge and its application. Their political capital has been enhanced through elected leadership roles and growing awareness of how to work with government officials to gain support for their initiatives, particularly marketing. Cultural capital can be seen in the five of six groups currently headed by women. Natural capital has been enhanced through increased land cultivation and by growing and selling beans. Physical capital has been enhanced through acquiring improved bean varieties that are high yielding and tolerant to environmental stresses; some groups have even acquired oxen and an ox plough. As farmers grow more beans, group members are very interested in labor saving production technologies (threshers, larger scale storage, etc.) that will lessen their work in large scale production, storage, and bulking centers for improved marketing and increased access to microfinance services. Our efforts to support development through the bean value chain stakeholder forum have already enabled project farmers to establish contacts with organizations that provide microfinance services

Networking and Linkages with Stakeholders

NaCRRRI has been multiplying more than 200 bean lines high in iron, zinc, and protein that it received from the University of Nairobi through CIAT. ISU has received germplasm from breeders that reflects variation in drought and seed nutritive composition.

VEDCO holds biannual community review meetings where CRSP project partners and farmers participate. VEDCO organized the first bean value chain stakeholder workshop for Kamuli in Jinja in May 2012. KIST's research has led to collaboration with HarvestPlus to determine the iron, zinc, and phytic acid content of beans cultivated in Rwanda.

Two Uganda-based organizations participated in our Bean Value Chain Stakeholder Forum in Jinja in May 2012 and our Project Dissemination workshop in Kampala in July: AgriNet and Farm Radio Africa/Uganda. AgriNet monitors markets for agricultural commodities and collects and disseminates market information to farmers. AgriNet supplies market demand and price information to VEDCO, which shares this information with farmer groups.

Leveraged Funds

Drs. Westgate and Mazur have successfully leveraged more than \$92,000 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

The project in Uganda has sought to strengthen the collective marketing capability of farmer groups. The project assisted farmer groups in developing business plans, improving record keeping and analysis, promoting gender equity.

Contribution of Project to Target USAID Performance Indicators

Cumulatively, we have been mentoring 27 students for degree training, seven at the graduate level (of whom three are women) and 20 at the B.S. level (of whom 10 are women).

Sixty-seven farmers (58 women and 9 men) have participated in short-term trainings for farmers in Kamuli. Other members of farmer groups have also participated in the training sessions. During FY12, bean crop management practices and technologies were disseminated to an additional 642 farmers (70 percent women). At least 954 households have benefitted directly from CRSP project interventions, 104 more than anticipated. We have four technologies and management practices under research, four under field testing, and 12 ready for transfer. The 381 additional hectares under improved technologies or management practices exceed the anticipated 350. Farmers' marketing associations in two subcounties are benefitting directly from project activities. We are providing technical assistance directly to 82 community-based organizations in which women constitute the majority of members. There are four host country partner organizations benefitting. The public-private sector partnership between Makerere University and Nutreal Limited is being established as a result of this USAID-funded project.

Contribution to Gender Equity Goal

Among the core team of 12 research scientists and professional practitioners, 50 percent are women. We have been mentoring 13 female graduate and undergraduate students. Eighty percent

of the 30 farmers participating in field experiments are women; similarly, 87 percent of the 67 farmers who received extensive project training are women; approximately 70 percent of the 642 farmers who received training and improved bean seeds during FY12 are women. Of the 954 households that have benefitted directly from CRSP project interventions, 15 percent are female headed.

Progress Report on Activities Funded Through Supplemental Funds

Supplemental funds received for FY12 supported training, technology dissemination, and research. The project team developed new training materials and media on improved management practices and technologies used in training more than 800 farmers at 60 demonstration sites. Topics covered by print and posters included germination testing; plant spacing;

soil nutrient amendments (manure application); pest and disease management; harvesting, threshing, drying, and moisture testing; solarization and triple bagging; sorting and seed selection; and processing and preparation of food products from beans. Three different

training methods/media, all followed by field demonstrations, were used in training on planting and triple bagging: interactive training, animated video, and farmer acted video.

Research by an ISU-based graduate student in communication and sustainable agriculture explored the effectiveness of video to complement or replace existing lecture/demonstration training for small farmer groups in rural Uganda. Results showed that video could effectively complement or, in some circumstances, replace conventional training methods in knowledge improvement, attitude, and intention to adopt improved management practices and technologies.

Publications

Kisekka, Rashid and Timothy Mukalazi Mubiru. (2012) "Preventing PostHarvest losses of Beans and Maize through Solarization and Triple-Bagging." Final B.Sc. Report. Department of Food Technology and Nutrition, Makerere University.

Nassozi, Adrian and Margaret Mbaziira. (2012) "Culinary, Sensory and Nutritional Properties of Local and Improved Bean Varieties." Final B.Sc. Report. Department of Food Technology and Nutrition, Makerere University.

Mushabe Wilson, Ndahabwa Manasseh, Ndayisaba Emmy. (2012) "Culinary Properties of 16 Bean Varieties Newly Released by ISAR in Rwanda." Project report for B.S. degree. Department of Food Science and Technology. Kigali, Rwanda: Kigali Institute of Science and Technology.

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 MSU released three new bean varieties in three market classes in 2012, including a new high-yielding upright, full-season pinto bean variety, *Eldorado*; upright pink bean variety, *Rosetta*; and an early season white kidney bean variety, *Snowdon*. *Eldorado* has outstanding yield potential, erect architecture, white mold tolerance, and good canning quality. *Rosetta* possesses virus resistance, erect architecture, has good seed color, and does not exhibit the stem breakage observed in the *Sedona* pink variety. *Snowdon* is a high yielding variety that matures seven days earlier than the *Beluga* variety and produces a larger, brighter white kidney bean seed than *Beluga*. The widespread adoption of the new variety *Zorro* from MSU's breeding program has provided growers with opportunity to direct harvest the crop and reduce production costs.

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 future breeding programs.

In Ecuador a new bean variety is being released to farmers in the northern valleys. *INIAP 484 Centenario* is large-seeded and red-mottled and possesses resistance to the foliar diseases rust, anthracnose, and angular leaf spot (ALS), and to the root disease *Fusarium* wilt. *INIAP 484* was bred exclusively by the INIAP research team and released through evaluation and participatory selection with CIAL members in Carchi and Imbabura. More than 10 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 Mira and Chota.

In Rwanda, the breeding program expanded the crossing program and produced more than 50 ton of breeder and prebasis seed of bush and climbing beans that was distributed to NGO partners and seed companies for additional multiplication and distribution to small farmers. Thirteen new climbing bean varieties for high altitude zones, including five iron-biofortified varieties, were released in 2012. Farmers who evaluated the lines during participatory variety selection field days preferred them because of their high yield, resistance to major diseases and pests, and seed that met urban market preferences. Two doctoral students concluded field research in Rwanda in 2012; one screened genetic populations for drought tolerance and the other evaluated participatory cropping systems in grower fields, comparing 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 consumed in Ecuador and the most important protein source in Rwandan diets, with about 120,000 hectares cultivated annually in Ecuador and 300,000 hectares grown in Rwanda. Both bush and climbing beans constitute an important economic income for farmers and staple food for Ecuadorian families and the majority of small-scale farmers in Rwanda. Beans occupy 20 to 31 percent of cultivated land area in Rwanda and are grown by almost 95 percent of farmers in all major regions of the country. The country has the highest per capita bean consumption in the world, exceeding 40 kg. Climbing beans have been adopted by households across a range of farm sizes, gender, and economic classes. Drought tolerant climbing bean varieties have been released recently in the Eastern province and contribute to increased productivity in this drought prone area. The yields are three to four tons per hectare for climbers and 1.5 to two tons for bush beans. In highland areas where climbing beans are the most popular, the production has increased significantly due to crop benefits from mineral fertilizer residues from crop rotation with maize.

Beans are produced by resource-poor farmers in Rwanda and Ecuador and are more vulnerable to attack by diseases, insects, and abiotic stresses. The significant diseases of beans are ALS, anthracnose, ascochyta blight, and root rot. Breeding for disease resistance is a key element in the successful boost of the bean productivity in both countries. Improvement of bean genotypes for Ecuador environments has a potential spinoff for Rwanda upland farming systems.

Smallholder farmers, many of them widows supporting families, are keenly interested in rebuilding their bean genetic stocks and expanding into new markets. Building on international bean germplasm, particularly on the Ecuador experience and germplasm,

a valuable opportunity exists to develop and deploy improved varieties in Rwanda, using the latest molecular and client-oriented plant improvement techniques. An improved understanding of plant traits and genotypes with resistance to multiple stresses from abiotic and biotic sources will provide unique materials for small-scale farmers, while providing insight into plant tolerance mechanisms to enhance plant breeding methods. Results of this project should contribute to improved yield, farm profitability, and human resources.

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 United States.

Smallholder farmers, many of them widows supporting families, are keenly interested in rebuilding their bean genetic stocks and expanding into new markets

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 productivity and market quality of beans under development.
7. Initiate selection for diseases resistance under screen house inoculation conditions at Rubona.
8. Yield evaluation of advanced lines in range of seed types in Ecuador, Rwanda, and the United States to exchange the most promising materials among the three programs.
9. Initiate characterization of biofortified lines for iron and zinc for use as parents in Ecuador and Rwanda.
10. Evaluate lines and varieties for Ecuador's canning industry.
11. Continue seed increase of the most promising lines. Expand on-farm trials with advanced lines in Rwanda and Ecuador.
12. 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 in Ecuador.



Results

Foundation seed increases of the three new varieties: *Eldorado pinto*, *Rosetta pink*, and *Snowdon white kidney*, released by the MSU breeding program in 2012 were produced in the western United States. The widespread adoption of the high-yielding, upright black bean variety Zorro has provided growers in Michigan with opportunity to direct harvest the crop and reduce production costs.

- A total of 3,900 plots were harvested for yield and 1,977 single plant selections were made in the early generation nurseries as part of the MSU breeding program activities in 2012. A new great northern line is under consideration for release, based on high performance and improved seed quality. Sources of common bacterial blight (CBB) resistance were identified in advanced kidney and cranberry bean lines. A group of high-yielding bush cranberry breeding lines with CBB resistance will be sent to Uganda and Zambia for testing in 2013. Rust is becoming an increasing threat to navy, black, and small red bean producers in Michigan, and resistance to the emerging race 22:2 has been identified in new navy, black, and small red bean lines.
- Genotyping of the SEA5xCAL96 RIL population continued at MSU. A genetic map representing 86 percent of the bean genetic map was constructed. A quantitative trait loci (QTL) analysis identified 41 QTL associated with drought tolerance-related traits studied in the field in Rwanda and in Colombia.
- The bean breeding program in the Rwanda Agriculture Board (RAB) increased crosses by more than 200 to incorporate resistance to anthracnose, ALS, and BCMV into elite lines with yield potential or high levels of iron and zinc.
- Selection for micronutrient rich lines from advanced populations introduced from CIAT in 2012 has continued. The best will be tested across all agroecological zones before distributing seed for national performance trials.
- Fourteen bush bean varieties were selected from among 104 biofortified bush bean lines introduced from CIAT.
- An adaptability test trial was planted at three sites in Rwerere and Bukonya. Four varieties were tested; their performance will be compared to popular climbing beans in the region.

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 United States.

1. Continue to select parental breeding materials for crossing in Ecuador, Rwanda, and United States.
2. Expand groups of lines from Rwandan breeding for crossing with newly introduced differential lines from Ecuador, MSU, UPR, and the CIAT/PABRA-interchange.
3. Cross Rwandan sources of resistance for bean common mosaic virus (BCMV), angular leaf spot, rust, anthracnose, Fusarium wilt, 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* in screen house/greenhouse tests.
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 MAS at one central lab (Rubona) in Rwanda.

- The breeding program in Ecuador participated in the prerelease of red-mottled disease resistant variety *INIAP 484, Centenario*. Ten red mottled lines with rust, anthracnose, and ALS resistance were selected and coded as FMR (Beans with Multiple Resistance). The FMR3 line showed high levels of resistance to both pathogens. The Concepción check was susceptible to both pathogens, while Portilla was resistant to anthracnose and susceptible to ALS. Similarly FMR3 line was evaluated in field in the presence of the soil borne pathogen *Fusarium oxysporum* and was resistant to this pathogen. In the 2012a cycle, seed of FMR3 line was increased with the seed growers in CIALs La Concepción and Pablo Arenas and in the same cycle FMR3 line was prereleased as an improved bush bean variety INIAP 484 Centenario.
- Centenario was evaluated as breeding line FMR 3 for four years over six growing seasons in research plots and in participatory trials in the Mira and Salinas valleys. The overall performance of Centenario in experimental trials was 1723 kg/ha compared to 1238 kg/ha for the Portillo check.
- The breeding program in Ecuador continues to combine resistance to rust, anthracnose, ALS, and *Fusarium* wilt in a range of seed types.
- In 2011b, six F₃ populations of solid red beans, 20 populations of red mottled seed types, and seven populations of Uribe seed types were planted and evaluated. At harvest, the best 20 red mottled seed progenies were selected. In 2012a, the selected individuals were planted in two replicates and data were taken on reaction to anthracnose under greenhouse and field reaction to rust, plant vigor, pod load, and yield. Six lines showed resistance to anthracnose and will be evaluated in the next cycle.
- In 2011b at Tumbaco, five promising black bean lines were evaluated for high pod load and adaptation. The best performance was obtained with the rust resistant black lines from Honduras.
- In 2012a, nine promising F₇ solid red seeded lines plus two checks were evaluated. Based on plant vigor pod load, rust and anthracnose resistance lines (Portilla x I-402) -5-3, and -5 (Portilla x Campeon), one to nine were selected, with yields of 2,051 kg/ha and 2,144 kg/ha, respectively.
- Promising lines with type II growth habit were assessed in two growing cycles. Only (Portillo/TP6)-5F₂ was selected for resistance to rust, anthracnose, and performance.
- In 2011b, 12 promising red mottled bush bean lines and three checks were evaluated at Tumbaco. Based on vigor, pod load, and number and yield, all lines outperformed Intag (658 kg/ha) and Concepcion (447 kg/ha) checks. Similarly, FMR3 line (Centenario) was superior in performance, rust resistance, and adaptation to the Portilla control.

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 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 at two locations in Rwanda
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. Field trials and greenhouse screening will be conducted to identify root rot resistance sources in Ecuador and Rwanda.
7. Characterize germplasm for reaction to individual root pathogens at Cornell using selected promising germplasm for Rwanda, Ecuador, MSU, and TARS (UPR).



Results

- Evaluation of SEA5xCAL96 recombinant inbred line (RIL) population for drought resistance was conducted over multiple seasons in Rwanda and in Colombia. Overhead sprinklers were used to irrigate both RF and RFS plots to ensure good plant establishment and early growth as needed until the early pod filling. Thereafter, irrigation was discontinued in RF plots while the irrigated plots continued to receive supplemental irrigation twice a week. In Karama, drought caused a 14 percent yield reduction. Drought did not affect days to flowering, maturity, harvest index, pod harvest index, and 100-seed weight. The number of pods per plant under rain-fed conditions ranged from five to 17, with an average of eight while they varied from five to 17 with an average of 10 pods/plant under irrigation. Seed yield was significantly affected by drought. Under rain-fed condition, yield varied from 99 to 222g/m². Under irrigation conditions, mean yield was 161g/m².
- The same experiment was conducted at CIAT research station in Palmira, Colombia, where all lines matured earlier than in Rwanda. The average pods per plant and seed per pod were nine and three, respectively. Partitioning indices, harvest index, and pod harvest index varied from 13 to 59 percent and 38 to 70 percent. The average seed yield was 60 g/m². Genotypes that performed well under Palmira conditions include RSA142, RSA60, RSA118, RSA129, and RSA54.
- The RAB651/* Concepcion inbred backcross line (IBL) population, developed in Ecuador, was evaluated for drought resistance in Rwanda. Overhead sprinklers were used to

irrigate both RF and RFS plots to ensure good plant establishment and early growth. Thereafter, irrigation was discontinued in RF plots while the irrigated plots continued to receive supplemental irrigation twice a week. Variables, including days to flower, days to maturity, number of pods/plant, and number of seed/pod, yield, and 100-seed weight were evaluated. RAB651 and Concepcion combined very well. Under rain-fed conditions, there was no significant reduction of seed size. There were no significant differences for number of days to flower and number of day to maturity between water regimes. However, yield was significantly reduced by drought stress. There was a 21 percent yield reduction in rain-fed plots as compared to plots that were irrigated. Under rain-fed conditions, the average yield was 99g/m². In irrigated plots, yield varied from 42 to 326g/m². Number of pods per plant and the number of seeds per pod were also reduced by drought.



- *Field evaluation of bean breeding lines and germplasm for root rot resistance in New York.* A total of 27 bean lines and varieties were evaluated in the root rot field at the Vegetable Research Farm, Cornell University, Geneva, New York. Root rot severity ratings exhibited by the tested materials varied significantly, ranging from 3.2 (Medalist) to 5.8 (Pink Panther) on a scale of one (no disease symptoms, healthy) to nine (>75 percent of root and stem tissues affected and at late stages of decay). All other promising breeding lines tested exhibited significantly lower root rot severity ratings than the susceptible checks. In addition, the yield of the selected materials harvested differed significantly and correlated rather well to their root rot ratings.

- *Cover crops for managing root diseases of beans and other agronomic crops.* The third and final year evaluation of the replicated nine cover crop treatments (rye grain + Vetch, oat, sudex, forage radish, red clover, rapeseed, buckwheat, wheat, and a fallow check) was completed in 2012. The future IPM field had the healthiest roots and the highest soil quality, whereas the conventionally managed field had the poorest root and soil health levels. The overall snap yield continues to be highest in the field with the highest soil quality and healthiest roots, whereas the lowest average yield is that of the plots in the conventionally managed field.
- *Long-term tillage, rotation, and cover crop trial (soil health site).* This collaborative site of the Cornell soil health team was planted to beans in 2012. The effect of zone vs. plow tillage practices on yield and root rot severity of CLRK was conducted in replicated plots at the Vegetable Research Farm. Emergence, stand establishment, root rot severity ratings, and seed weight did not differ significantly among the two systems.
- *Evaluation of selected pea varieties for resistance to root pathogens.* Root rot diseases of peas are prevalent and damaging to peas. Disease symptoms commonly observed in New York were *Fusarium*-root rot and *Fusarium*-wilt. Symptoms of infections caused by *Thielaviopsis*, *Pythium*, and *Aschochyta* were also observed. In 2012, the reaction of 40 pea varieties was evaluated in naturally infested soil in a greenhouse test and a production field where two or more varieties were planted in the same field. Root rot severity ratings ranged from 3.0 to 8.5.
- Nine promising lines and three red mottled bush bean checks were evaluated for resistance to *F. oxysporum* at Tumbaco. All the tested lines produced higher yields than the control Portilla.
- In the 2012a cycle at Tumbaco, 14 sources of resistance to *F. oxysporum*, 12 for rust, 10 for anthracnose, eight for ALS, and six for CBB were planted and evaluated. Morphological (flower color) and agronomic (days to flowering, vigor, pod load, days to harvest and rust resistance) data were taken. Under strong natural rust pressure, all resistance materials reactions were one to four on the one to nine CIAT scale.
- In the 2012a planting cycle at Tumbaco, elite bush bean lines developed in different commercial seed types were planted and evaluated under drought stress. Morphological, agronomic, and rust resistance data were taken. Of the mottled red lines tolerant to drought stress, line (S23 /3/ YUNG // S23/DOR466) 2F₂ was selected for rust resistance and yield performance. Among black beans, line 9-10 from Honduras was selected for rust resistance, adaptation, and performance over the control Afroandino. Large-seeded yellow line (C. CHOTA x TB2)-6F₂, was selected for its resistance to rust, good growth vigor, superior performance, and pod load compared to INIAP-Chota. The large white seeded FMR line-7 (FABE ARG x TB2)-1F₂-4F₄, was better than the control INIAP Fanesquero in rust resistance, vigor, yield pod.



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 the characterization of root rot isolates collected in northern and southern regions of Ecuador.
4. Phenotypic evaluation of Rwandan germplasm for resistance to local isolates of anthracnose, ALS, and BCMV under field conditions, screen house, 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. Continue 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, anthracnose by CIAT/ISAR, and MS theses.

Results

- Rust was collected from bean fields in Michigan, and the strain was similar to that collected over the last five seasons. The new strain characterized as race 22:2 defeats many of the current resistance genes in Michigan. A similar race 20:3 was recently detected in North Dakota. Resistance has been identified in elite MSU black and navy bean germplasm and crossing has been initiated to transfer resistance. Given the persistence of this race, an extensive screening of all MSU germplasm is being conducted in the greenhouse this winter.
- Twenty-three isolates of anthracnose from Ecuador have been characterized and 17 races were identified. In regard to the effectiveness of resistance genes, the *Co-3* gene from Mexico

222 and the *Co-4²*, *Co-5²*, *Co-7* genes from G2333 were the only ones that showed full resistance to all isolates characterized.

- To characterize isolates of ALS, the program has adopted a system of inoculating detached trifoliolate leaves of the 12 differential varieties in a Petri dish with a moistened absorbent paper. Six samples of ALS collected in the villages of Tumbaco (Pichincha), Tena (Napo), Pablo Arenas, Urcuquí, and Intag (Imbabura) were characterized on the differentials and three races were identified. An additional six monosporic isolates will be characterized in the coming weeks. This work is critical to breeders deciding which resistance sources to use to control ALS in the different production areas of northern Ecuador.
- Five new races of rust were identified from five samples collected in the villages of Tumbaco (Pichincha), Carpuela, Cotacachi, and Intag (Imbabura). These results indicate the large variability of rust in the country and indicate that races are highly Andean in origin. Concern exists that many of the resistance sources have been defeated.
- Surveys to diagnose major root diseases in Rwanda and collect isolates of root pathogens for additional characterization were initiated this year. Root rot samples and diseased leaf samples of angular leaf spot, bean rust, and anthracnose will be collected from affected bean plants. Characterization of the isolates will be done in collaboration with all partners in the new NIFA project. Field trials and greenhouse screening will be conducted to identify root rot resistance sources for our breeding program. A complete survey to collect root rot samples infected with *Pythium*, *Fusarium* wilt, *Rhizotonia* and *Macrophomina*, and foliar disease samples has started and will continue until December 2012. Screening of promising resistant germplasm in Rwanda will follow the disease characterization when isolates will be ready for artificial inoculation.

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 midaltitude and high agroecological regions in Rwanda.
 - Plan genotype by environment farmer participatory assessment of advanced lines within intercrops and sole crops; initiate trials in 2011 and terminate in 2012.
 - On-farm assessment of promising lines conducted in sole crop and intercrop on-farm 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 in Ecuador and Rwanda.
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 to enhance adoption in Rwanda.
7. Initiate interchange of experience in Rwanda on participatory methods and seed production for local community use with smallholder farmer members. Train trainers on seed and farming system production, and work with progressive farmers.
8. Draft a manuscript by August 2012 for review at Rwanda workshop; circulate for comment and input.



Results

- Experimental research on cropping systems and bean varieties was conducted in northern Rwanda using a mother-baby-grandbaby trial system. The second round of climbing bean and maize GxE trials was planted in September 2011, monitored, and harvested in February 2012. Five climbing bean genotypes, a farmer bean mixture unique to each site, and a single maize genotype were planted in a randomized complete block design with 14 plots in three treatments: a sole crop, bean intercrop with maize, and a bean mixture with maize broadcast. Trials were planted and replicated four times at two research stations (mother trials) and single replicates were planted on farm in eight communities (baby trials). Data on yield, leaf area index, and multiple plant traits were collected.
- Farmer trainings on crop monitoring, rain data collection, and participatory genotype selection.
- At the start of the season, farmers chose two bean genotypes from the trials to plant in their own modified experiments on-farm to learn more about farmer experimentation and allow farmers to compare genotypes qualitatively. Approximately 80 farmers planted three plots each: a monocrop, an intercrop, and a control plot of the farmer's mixture in a monocrop.
- To compare advanced line selection by breeders and farmers, farmers in each location evaluated the cropping systems, the best genotypes for a monocrop, and the best genotypes for an intercrop with maize. Data were collected on their preferences and following voting, discussions were conducted to understand why farmers chose these genotypes.
- In February and March, visits to farmers' grandbaby trials, surveys, and semistructured interviews were conducted in

each community with approximately 60 farmers to gather basic demographic information, how farmers experimented with the genotypes and cropping systems, farmer genotype preferences, and the impact of monoculture policies on farmers' livelihoods.

- Increased grower awareness and knowledge of participatory breeding methods, plant nutrition, root, and soil health issues have further improved bean productivity. Land management through bean crop intensification and its place in land consolidation program has boosted the production of beans.
- Demonstration plots have been established in Karama to promote five new drought tolerant bush bean varieties. Different varieties were under seed increase for production of breeder seed in RAB research stations. A total of 1,096 kg of different varieties, including the five bush beans and four climbing, were produced and distributed to seed producers for production of foundation seed—a total of 6,595 kg of basic seeds of drought tolerant and drought tolerant biofortified bean varieties.
- Thirteen new climbing and snap bean varieties in 2012, including five high yielding, biofortified beans were officially released in 2012. Farmers preferred these beans because of high yield, resistance to major diseases and pests, and urban market preferences.
- Seed multiplication of two bean varieties (MAC 44 and RWR 2245) on nine ha in Rubona produced 26 tons. Production with farmer partners on 10 ha was more than 20 tons.
- Evaluation and participatory selection of bush bean germplasm with local CIALs continued in Ecuador. In the town of La Concepción, three elite lines selected in the previous cycle plus a control variety were planted in large plots (200 m²) at two locations. The CIAL de la Concepción selected FMR3 as the future variety Centenario based on its high performance, overall health, and seed quality; they produced 855 kg of seed for the next cycle.
- In the town of Pablo Arenas (Imbabura), FRM3 line (Centenario) was planted at two fertile sites by the CIAL and participatory data were taken on plant growth, vigor, and yield. In both locations, the vigor of the FMR3 line was superior to other materials. Farmers from the Pablo Arenas CIAL selected FMR3 as the future choice and produced 855 kg of seed for the next growing cycle.
- Nine lines and varieties with black, red, and yellow seed types selected by agribusiness for canning were evaluated in La Concepción, Pablo Arenas, and Tumbaco. Rust and ALS were particularly severe in Pablo Arenas; only Negro San Luis and Afroandino showed intermediate resistance to ALS. All lines were resistant to rust, except Afroandino.
- Seed of Afroandino and Rocha was also sent to be tested under precision farming systems (drip irrigation and fertilization) in Santa Elena against the interest of commercial soybean producers in the region. Information to date suggested good adaptation of black bean Afroandino and poor adaption of yellow bean Rocha.

- Seven lines and two improved varieties of climbing beans with red, red mottled, and yellow (canary) seed types were evaluated near the town of Chillanes, Bolivar province. According to preliminary results the large solid red Creole line bombolin was the best for quality, pod load, and overall health.

Nondegree Training – Extension Activities

- Two posters and a booklet that provide a description of bean varieties released were developed, printed, and presented during the official release of new improved bean varieties at a ceremony in the Musanze district. Small packs of bean seed of new bean varieties were disseminated to participants along with other technologies.
- Training of trainers was organized in partnership with Win–Win Seed Company and Agro dealer on seed and farming systems as well as how to work with progressive farmers in Rwanda.
- A “Participatory Research Methods Workshop: Client-Oriented Plant Breeding and Agronomy” workshop was held for 33 participants. Participants gained an understanding of theory and new methodologies in participatory breeding and agronomy for improved bean-based farming systems.
- A molecular breeding workshop was conducted in the biotechnology lab at Musanze, with 15 participants attending. The first day focused on molecular plant breeding theory and its application and the second day on hands-on sessions. Participants learned the use of DNA markers that are tightly linked to the target loci as a substitute to assist phenotypic screening through MAS to speed up breeding and selection for different traits.
- Drs. J. Kelly and G. Abawi met with collaborators in Ecuador at INIAP to review research activities, visit field research sites, and participate in the prerelease of the new Centenario variety.

Contribution to USAID Performance Indicators

The Target Outputs

1. The development and release of locally adapted, acceptable, and disease resistant bean cultivars for the major production regions in Rwanda, Ecuador, and Michigan.
2. Increased sustainable productivity and profitability of bean production, due to increased yield and reduced inputs.
3. Improved grower income and stability of bean production contributing to better nutrition and health of farm families.
4. Increased awareness and knowledge of participatory breeding methods, root health, and soil health issues to further improve bean productivity, long-term land management, and environmental risk.
5. Identification of germplasm sources to benefit improved, select bean traits for the U.S. market.
6. Enhanced human resource development, gender equity, and improved infrastructure capacity of participating institutions in Rwanda and Ecuador.

Networking and Linkages with Stakeholders

- The project forged closer collaboration with 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-led 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.
- A bean stakeholder meeting was organized in RAB eastern zone to initiate platforms for main actors in bean production chains.
- International NGOs, including AFRICARE, ADRA, CARITAS Rwanda, and Catholic Relief Services were among key partners this year 2012. Local NGOs, such as DERN, INGABO, CSC, IMBARAGA, and individual farmers contributed to the dissemination of new bean technologies.
- The program interacted with more than 10 different NGOs in Ecuador.

Leveraged Funds

In addition to the Dry Grain Pulse CRSP project in Rwanda, funding was secured from Harvest-Plus, Bio-Innovate, AGRA, ASARECA, and the PABRA network. Support from the government also was provided for variety selection and further seed increase. Support from Borlaug LEAP was received for the additional training of a doctoral CRSP candidate.

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- Kelly, J.D. and K.A. Cichy. 2012. Dry Bean Breeding and Production Technologies, pp. 23–54. In *Common Beans and Pulses: Production, Processing, and Nutrition*. Editors: M.A. Uebersax, M. Siddiq, K.D. Dolan. John–Wiley Publishing Co.
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- Roman-Avilés, B., J.M. Lewis and J.D. Kelly. 2012. *Fusarium* Genetic Control: A Long Term Strategy, pp. 65–108. In *Control of Fusarium Diseases*. Editors: Fernando M. Alves-Santos, Julio J. Diez, Research Signpost 37/661, Kerala, India.
- At the World Food Prize Conference in October 2012, Gerardine Mukeshimana was awarded the 2012 BIFAD Student Award for scientific excellence by a student working in a USAID CRSP project.

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

Research achievements have focused on diagnostics for the bean and cowpea markets in Angola and Mozambique. Cowpeas are now included in both countries and have different market structures than the common beans as well as different production zones. Quality issues remain in both countries for marketing, with varietal mixing in sales, stemming from the producer level, and off-season pests problems in the stored commodity. In both Mozambique and Angola, market opportunities could be improved by strengthening agricultural research into varieties and cropping cycles with market opportunities, improving storage options and technologies to avoid storage damage, and improving farmer and trader knowledge of trade through market information.

In Mozambique, there are clear differences on the consumption budget allocated for common beans and cowpeas. Urban households allocate a larger proportion of their budget to common beans than to cowpeas, whereas rural households allocate the same amount for each.

Analyses reveal that cowpea is produced by women, while common beans are produced by men and women. Price variability can be high for both crops, seasonally and between seasons, due to weather variability and policy considerations in Mozambique and neighboring countries. In Mozambique, cowpeas are generally a food security crop, with low per household sales, while common beans have been highly marketed in the more concentrated production zones, which suggests there are more developed marketing channels for common beans. Women are active traders in the common beans wholesale markets of Mozambique. In Mozambique, the common beans value chain is relatively short and does not take advantage of regional/export opportunities.

The most visible impact in Angola will continue to be the training on value chains, policy, and price analysis. For CESE/IIAM, training on value chain and investment and cost benefit analysis has contributed to research that can better address the issues farmers and traders face.

Project Justification and Objectives

Angola. The development of the common bean and cowpeas value chains depends heavily on understanding producer constraints on production and marketing as well as identifying leverage points within the value chain where cost savings and better coordination can be achieved. Prior to this project, very little was known about the marketing channels for these beans or how farmers engaged with the markets. The initial objectives included collaboration to ensure a market information system (MIS); however, due to lack of political will for MIS, the project has focused on farmer and trader surveys to help identify

constraints and opportunities to then engage stakeholders in discussions for change. In FY2012, field research on cowpeas was added to the earlier work on common beans. A key component of this project is the training of students and supervision of student theses and the utilization of value chain approaches in their thesis research.

Mozambique. In Mozambique, cowpea and common bean value chains were considered distinct, but research and analysis were needed to understand the dynamics of farmers and traders to highlight opportunities for investment and better value chain coordination. A key research question is whether or not market traders can be more efficient or whether markets can function more efficiently overall. Price analysis was needed to understand the spatial relationships among the markets and the seasonal tendencies of market volumes and prices. One of the areas of research involves identifying opportunities for growth. Training with IIAM/CESE analysts will introduce concepts related to investment and cost benefit analysis to complement earlier partial budget analysis. This training will help analysts to evaluate opportunities with a view to profitability for farmers and traders.

In Mozambique, there are clear differences on the consumption budget allocated for common beans and cowpeas. Urban households allocate a larger proportion of their budget to common beans than to cowpeas, whereas rural households allocate the same amount for each.

Objectives

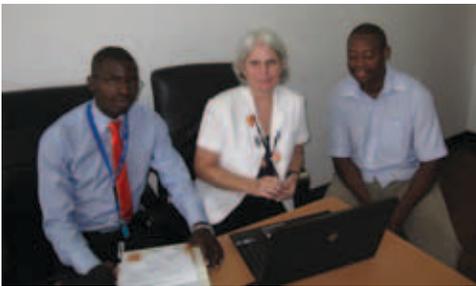
1. Angola: Identify efficiency in marketing channels of beans and cowpeas and leverage points to increase farmer profits and trader volumes.
2. Mozambique
 - a. Identify efficiency in marketing channels and leverage points to increase farmer profits and trader volumes.
 - b. Develop cell phone-based information system for beans to link farmers and traders to market prices and availability.



Research and Outreach Approaches, Results, and Achievements

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

Kiala and Donovan worked with students to develop cowpea field research on marketing channels conducted in FY2012. The research team interviewed traders, consumers, and a few producers in the rapid appraisal in Huambo province in late 2011. Later, students visited selected areas in lower elevations. Strong seasonality was highlighted by the lack of large volumes in the off-season and the generally poor quality of cowpeas available in the market due to pests. In their market visits, the UCR team found similar results, with varietal mixing in the marketed quantities, although larger volumes were found in the lowland production areas. There was no evidence of any volume trading in the highland zones or of large private sector traders participating in cowpea markets.



Women are the principal participants in selling common beans and cowpeas in the highlands. Women traders from Huambo and other cities travel to purchase from producers in the rural areas. In general, women producers are responsible for selling common beans, and often sell in small volumes to meet household expenses. The producers do not travel from their farm or their local market to sell. It is the buyers who purchase from several or many sellers to accumulate sufficient volumes to transport to larger towns and cities. They then sell at wholesale or retail levels in larger markets. These buyers are familiar with the best local markets, so they can arrive, buy, and transport within a day to avoid local storage. Although not unseen, it is less common for producers to bring together larger volumes to transport and sell in the wholesale markets because they are not as adept at arranging transport and negotiating prices to minimize loss. When it does occur, however, it is men conducting this volume trade of producers.

As a marketing channel, there are limited large-scale traders dealing in wholesale markets who purchase bulk common beans in the highlands to transport to the major markets of Luanda and Benguela. In spite of recently improved transport systems, there have been no significant changes in this pattern. Common beans are generally not seen as a key commodity by these traders.

Paved primary roads facilitate transport between key markets; however, the lack of all-weather secondary and tertiary roads limits producer and trader transport within the production zones. The poor quality of these roads results in few

transporters traveling them; further, farmers and traders face major transport constraints due to a limited number of operational trucks. The rehabilitated railway and continued investments in secondary roads will provide new opportunities for commodity transport in the zone.

Producers of common beans and cowpeas face another constraint: lack of adequate storage facilities and technology, which affects the quality of grain available in the market and the traders' potential to use temporal arbitrage. Farmers want to sell commodity as soon as possible to avoid degradation, often selling, consequently, at low harvest-time prices. Traders face the challenge of grain that deteriorates over time if stored inadequately. In the market of Kamacupa, researchers saw degraded cowpeas that had been ground into a rough flour for sales.

Field research focused on both common beans and cowpeas in the two public markets in Huambo. The traders in the main Alemanha market do not face high barriers to entry; they simply pay a daily market fee. In the Municipal market in town, however, space is limited and traders obtained their licenses and space when the market was built—and pay fees for their fixed locations. There is no longer space for new entrants in the Municipal market. Consequently, there are fewer legume vendors in the Municipal market than in the Alemanha market, and cowpeas are only sold as a side commodity by common beans sellers, with no specialization in cowpeas among traders.

There are significant numbers of common bean traders in the Alemanha markets but many fewer cowpea traders; a few common bean traders also had small quantities of cowpeas for sale. When questioned, traders indicated that cowpeas were considered by consumers as an inferior good, sought by poor households that cannot afford to purchase common beans. The quantity sold per buyer was said to be much lower for cowpeas than for common beans. It should be noted that this may be directly related to consumption habits in the highland region and could be quite different in lower elevation, drier areas. Research in late 2011 indicated that markets in zones with higher production of cowpeas had major sales of cowpeas during the main harvest season, but quality issues, especially pest infestations, limited availability and sales in the off-season.

Women are the principal participants in selling common beans and cowpeas in the highlands.

Overall, market research shows little change in the market systems since 2009, with reliance on informal trading, limited numbers of agents, and few options for the informal sector traders. Research identified four main marketing channels.

- Channel 1. Farmers may sell directly to consumers in their local market, especially when markets have specific market days.
- Channel 2. Farmers may arrange transport to local town or city markets to sell at wholesale and retail levels.

- Channel 3. Farmers sell to itinerant women traders that arrive in local markets or at farm gate; these traders arrange transport to the larger towns and cities to sell to retailers, who sell to consumers in urban markets.
- Channel 4. Small groups of farmers arrange transport to Luanda or Benguela to sell beans; this circuit is seen as highly risky because of difficulties in arranging transport, price uncertainty, and quality degradation in transport.

Missing are the large-scale buyers who have the transport and storage capacity for spatial and temporal arbitrage.

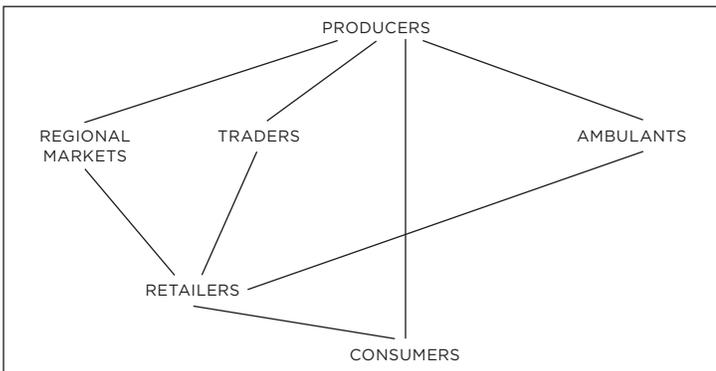


Figure 1. Market Channel Map of Local Common Beans, Angola

World Vision continues to work on collecting market prices, and Donovan worked with their system coordinator, suggesting improvements to the data system to deal with nonstandard units and to control for quality.

Objective 2: Mozambique.

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

Isabel Cachomba, CESE analyst, came to MSU from August 12–25 to work with Cynthia Donovan on the consolidated common bean and cowpea report, using price information and household survey results combined with rapid market appraisals.

To determine whether consumption in urban areas was significant, nationally representative datasets from 2009/2010 were analyzed. The analysis revealed clear gaps in our knowledge of the cowpea value chain. Consequently, Isabel Cachomba led the socioeconomists Amancio Nhantumbo and Cassamo Sumila in collecting data on the cowpea value chain,



including production gross margins in Niassa and Zambezia provinces, in November and December 2012. This field research included focus group interviews with farmers to evaluate their cowpea cropping to develop crop budgets, followed by analysis of technology options with partial budgeting analysis.

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

Analysis by Cachomba and Donovan on TIA data indicates that farmers are increasing their ownership of cell phones; however, SIMA has not moved forward on the development of the cell phone system. Funds were recently allocated by MINAG/SIMA to hire consultants and obtain equipment for this effort.

Objective 3: Institutional Capacity Building

Short-Term Training

Collection and Analysis of Market Prices training was completed in December 2011 at Faculdade de Ciências Agrárias, Chianga, Huambo, Angola.

FCA students; NGO staff members for World Vision, ADRA, and others; and staff members from the Ministry of Commerce, Agricultural Research Institute, and Agricultural Development Agency (total of 13 men and eight women) received training that examined the role of prices, price discovery, and analytical challenges with prices as times series data, including a

computer-based session on price analysis. Although it became clear that the analytical skills and computer skills needed to conduct thorough price analysis were lacking, attendees were able to develop simple graphs and estimations of real prices.

Introductory Training on Investment and Cost Benefit Analysis was completed in April 2012 at IIAM, Maputo, Mozambique.

Staff members of IIAM Center for Socio Economic Studies and two students from FCA, UJES, Angola (total of 6 men) received training based on examples and exercises on the key concepts and research issues of investment analysis and cost benefit analysis.

Intensive English language training in both classroom and living environments was partially completed by two trainees (both men) over six weeks in March and September 2012, respectively, in Capetown, South Africa. Training was completed in January/February 2013.

Contribution to Gender Equity Goal

Training continues for Ana Lidia Gungulo, a woman scientist from IIAM/CESE.

For the value chain analyses in Angola and Mozambique, the main traders are women. Women are often the household members responsible for cowpea cultivation, harvest, and selling. Value chain improvements that can meet their needs will contribute to household income. Market information will help to improve the competitiveness of women farmers and traders. Combining market knowledge with technology, especially new varieties, will enhance the role that cowpeas play in livelihoods. Women also participate with men in common bean production and marketing in Mozambique.

Networking and Linkages with Stakeholders

USAID Mission was visited in Luanda in December 2011; Mission staff members present included Scott Jackson and Gastao Lukanu. For all training activities, members of IIA are included; in the FY2012 markets and prices training, staff from various NGOS and government agencies were included.

In Mozambique, the Mission has been kept up-to-date on Pulse CRSP activities through informal interactions and brief meetings. USAID Agrifuturo continues to be engaged.

Leveraged Funds

The PIs and collaborators in this project have successfully leveraged external funding to increase smallholder income in selected crops, including common beans, in the Highlands of Angola from the World Vision ProRenda project and Gates; and for a training program for policy analysis with IIAM socioeconomists from USAID and Michigan State University.



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

This project has realized substantial progress for all its objectives, including the development of new bean lines with greater tolerance to drought and low soil fertility in Central America and Mozambique; phenotypic profiling of many bean lines for root traits of value for bean breeding; the discovery of new traits that can be used to select stress-tolerant cultivars; the discovery of genetic markers controlling these traits; the characterization of several important agroecological benefits from bean lines with superior root traits, including reduced soil erosion, enhanced water utilization, and greater biological N fixation; comprehensive analysis of community preferences for bean lines and obstacles to seed dissemination; and training of bean researchers from Mozambique.



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 genetic improvement, 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 in the developing world, especially for common bean. Phosphorus limitation is the most important nutrient constraint to bean production, followed by the acid soil complex of excess aluminum, excess manganese, and low base supply.

Nutritional stress in the bean production systems of Latin America and Africa cannot be overstated. Fertilizer use is negligible in many developing countries, especially in sub-Saharan Africa. Integrated nutrient management is needed—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.

Research has shown substantial variation in bean P efficiency that is stable across soil environments in Latin America. Analysis of the CIAT germplasm collection identified several sources with outstanding P efficiency. Studies with these genotypes identified a number of 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.

Drought is a primary yield constraint to bean production throughout Latin America and Eastern and Southern Africa. Beans vary substantially in drought tolerance, due primarily to variation in root depth and access to soil water, earliness (drought escape), and, secondarily, to seed filling capacity. Drought tolerance has been identified in several races of common bean, but it 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 nutrient-efficient bean genotypes. Bean genotypes with superior root systems may enhance the utilization of rock P, thereby improving P availability and N availability in maize/bean systems. Similarly, bean genotypes with deeper root systems may be synergistic with soil management techniques to conserve residual moisture.

We need a better understanding of socioeconomic factors determining adoption of stress-tolerant bean germplasm and the likely effects adoption may have on income and nutrition.

Drought and poor soil fertility are primary constraints to pulse production in developing countries. 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 substantially improve bean production in Africa and Latin America.

Objectives

1. Develop bean genotypes with improved tolerance to drought and low P.
2. Develop integrated crop management systems for stress tolerant bean genotypes.
3. Socioeconomics

Research and Outreach Approaches, Results, and Achievements

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

Drought and poor soil fertility are primary constraints to pulse production. Several root traits that enhance bean productivity under drought and low fertility stress have been identified. Improved bean production in Africa and Latin America through genetic improvement is possible.

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 will be collected and systematically screened for key root traits, including root hair length, root hair density, basal root whorl number (BRWN), basal root growth angle (BRGA), and adventitious rooting.

Introgression of root traits conferring greater drought tolerance and P efficiency will be carried out by developing inbred backcross (IB) populations composed of 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 (selected elite cultivars and/or advanced lines for CA/C and African target countries) and the donor parents (selected germplasm with the higher expression of key root traits), 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. Selection for some disease resistance will be conducted in the field.

Participatory plant breeding and PVS approaches will be used in field trials to evaluate the performance of IB lines under drought/low P, agronomic adaptation, and commercial seed types. We will engage both male and female farmers in these activities.

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

The selection process of IBC lines having similar background of Amadeus 77 was continued during FY12 using drought and low

P conditions on greenhouse and field trials. Selection was based on agronomic adaptation, yield, and seed color, shape, and size similar to those from the recurrent parent Amadeus 77.

Thirty IBC lines and the parental lines Amadeus 77, L88-13, L88-33 and L88-63 were evaluated under irrigation. The best lines out yielded their recurrent parent and their donor parents.

An additional field trial including 18 IBC lines and the parental checks Amadeus 77 and L88-13 were evaluated using fertilizer and without fertilizer treatment during the second raining season. Seed yields varied from 864 to 2,023 kg/ha with added fertilizer, and from 396 to 948 kg/ha without fertilizer treatments.

Another field trial including six IBC lines and the checks Amadeus 77 and Seda (landrace) was conducted during the May 2012 season using fertilizer and no fertilizer treatments in a low soil fertility plot at Zamorano. The line BRT 103-182 has greater seed yield than the two checks under no fertilization and IBC lines; however, the cultivar Seda has the second greatest seed yield and highest shoot dry weight, nodulation score, and number of adventitious roots. On various studies, the cultivar Seda has been identified as one of the best landrace under drought and low fertility conditions and is currently used as a parent in crosses with the best tolerant small red and black lines for improving the adaptation to these conditions in Central America.

A set of 18 drought and low fertility tolerant lines and two check cultivars were field evaluated at Zamorano in a low N and P soil with and without soil fertilization. Superior genotypes from this study will be used as donor parents for improving tolerance to drought and low fertility of small red and black commercial bean cultivars.

Two field trials were conducted during FY12 in a low N, P, and other soil nutrients plot at Zamorano to evaluate 20 small red and black bean improved cultivars with and without fertilization treatments during the second and first raining seasons. The significant adoption of these improved cultivars in these regions is due to their superior disease resistance and greater adaptation to a variable array of production conditions, where drought and low fertility are some of the major production problems encountered by most small farmers. Root traits measured on these field trials included number and angle of coronary roots; number, diameter, and density of adventitious roots; and diameter of primary roots. Although significant variation was observed for most root traits under the fertilized and unfertilized treatments, only some of the top yielding cultivars were superior on number and density of adventitious roots; this difference was observed mainly under no fertilization treatment. In collaboration with the UPR/Beaver DGPC Project, landrace cultivars and elite breeding lines have been used for developing improved small red and black bean cultivars with better adaptation to limiting soil fertility and rainfall conditions at Zamorano. Several advanced lines have been developed by inbred backcross and triple crosses using improved cultivars and breeding lines as donor parents.

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

1.2.1. Diversity of root phenes of common bean from Andean and Mesoamerican gene pools

Low phosphorus availability and drought are major constraints to common bean production in many developing countries. The root system is an important factor for plant productivity. Plants have evolved a wide range of adaptations to enhance phosphorus (P) and water acquisition from the soil. Variation in root traits has been reported in many crops. Information on diversity of root traits is crucial for development of genotypes adapted to a specific environment. To assess the diversity of root phenes in beans, 165 accessions from the bean core collection from CIAT were planted in the laboratory and field in 2010 in Pennsylvania. Fifteen root phenes were evaluated from one root crown: adventitious root number, length, branching and diameter, basal root number, length, branching and diameter, basal root growth angle, primary root length, branching and diameter, basal root whorl number, number of nodules, and root rot infection. Substantial phenotypic variation in root traits among genotypes was found in adventitious, basal, and primary root traits. Variation among genotypes within gene pools and genotypes within country of origin were significant for all 15 root phenes. A positive correlation between root hair length and root hair density was observed, indicating that genotypes with long root hairs also have many root hairs, traits that confer P efficiency acquisition in plants. Genotypes with root traits associated with adaptation to low P availability were found in both gene pools, and traits associated with adaptation to drought stress were mostly evident in the Mesoamerican gene pool. Andean accessions have root traits that are suitable for regions with low P availability that is associated with volcanic soils of the Andes regions, while most of the Mesoamerican accessions were reported to be tolerant to drought stress and low soil fertility. Breeding for multiple root phenes could enhance acquisition of multiple soil resources, particularly in developing countries.

1.2.2. Heritability of root hair traits in common bean

Root hairs play an important role in phosphorus uptake. Information about the mechanisms of inheritance of root hair traits in common bean is lacking. The objectives of this study were to characterize root hair traits in two bean populations and to study the mechanisms of inheritance of root hair traits in common bean. More than 150 bean genotypes from CIAT were screened in the laboratory to identify parents contrasting for root hair traits.

Results: Root hair length varied significantly among individuals within populations of both F3 and F4 generations.

Although we used only two populations, our results indicate the existence of genetic variation in root hair traits within bean populations as previously reported, and these traits can be selected for genetic improvement and development of genotypes adapted to regions with low P adaptability. We have shown that root hair traits vary genetically and these traits can be targeted in breeding programs to genetically improve bean cultivars.

1.2.3. Yield performance of F6 lines under low P stress

To develop bean varieties adapted to the agroecological conditions of Mozambique, five single crosses were performed using parents with root traits adapted to low P and drought, and generations of selfing were advanced to F6. In 2012, F6 lines developed from introgression of long and dense root hairs into lines with short root hairs were tested for yield performance. The objective of the study was to evaluate yield performance of 22 bean lines derived from crosses of two Mesoamerican parents under low P stress and under normal P fertility. Significant differences for yield were detected among lines. The trial was



repeated where P levels were considered normal. Strong positive correlation between yield performance under low P stress and root hair length was observed in 14 bean lines. Results indicate that the new lines had superior yields under low P stress, and long and dense root hairs influenced positively on yield increase under low P stress. Promising lines were selected for trials.

1.2.4. Yield performance of 27 Andean bean lines

The objective of this study was to evaluate yield performance of 27 F6 bean lines derived from a cross of Andean bean genotypes under low P conditions, one with long and dense root hairs, the other with short and sparse root hairs. Significant differences for yield were detected among lines. The average number of pods per plant varied from 24 to 14. The average yield among these lines under low P stress were superior to the local check and varied from 803 to 1975 kg/ha. These results indicate that our new lines have the potential of increasing bean productivity in regions with low P soils.

1.2.5. Yield performance of six bean genotypes with contrasting root phenotypes

Six genotypes that were contrasting in basal root growth angle, basal root whorl number, and basal root number (BRN) were planted in Chokwe to assess yield performance of six genotypes with different root architecture under drought stress. Root traits measured in the field included adventitious root number, length, and branching, BWRN, BRN, BRGA, basal root branching, primary root length and branching, number of nodules, and root rot infections. Root phenes were evaluated 45 days after planting. Significant differences in root phenes among genotypes were detected for adventitious root number, length and branching, BRGA, BRWN, BRN and basal root length, primary root branching, and number of nodules.

Significant differences among genotypes were detected for yield, indicating that the performance of the six genotypes were different. Results indicate that genotypes with deeper BRGA also had relatively higher yields. We also found a positive correlation between BRGA and yield.

1.2.6. Evaluation of common bean lines tolerant to drought stress

To identify bean genotypes adapted to drought, 21 bean genotypes introduced from CIAT and local germplasm, including genotypes known to be drought tolerant, were used and evaluated; data included number of pods per plant and yield performance.

Although we did not have strong drought stress due to the abnormal rainfall off-season in Southern Mozambique, we found significant differences among genotypes in and number of pods per plant in 2011—from 2,233 to 1,017 kg/ha. In addition, the number of pods per plant varied from 17 to 44.8. Tio Canela and BAT 477 are drought tolerance genotypes and previous study showed that these genotypes have deeper basal roots. The local check Bonus was among the genotypes that had relatively good yield, while another local variety had a yield of 1636 kg/ha. Overall, genotypes with deeper roots were among the best in yield performance, confirming the importance of root architecture for water acquisition.

Results from the 2012 season did not show significant differences among genotypes in number of pods per plant and yield performance at the 1 percent level of significance in both stressed and nonstressed treatments. Genotypes selected as source of drought tolerance: Bat 477, Tio Canela, and SEQ 1003.

1.2.7. Planned crosses

We have started and planned crosses for introgression of root traits into local genotypes to improve the existing varieties. Doctor and Kakhi are local varieties with good grain type but relatively low yield. Seed of other parents was increased and planned crosses for drought and low P adaptability will be performed next season. In this season we increased seed of about 290 LPA (low P Andinos lines) from crosses to improve root traits.

1.2.8. Seed multiplication

Seed increase and germplasm maintenance are conducted every season. Several bean lines collected in Mozambique and introduced from CIAT were increased in 2012 in Chokwe and Sussundenga. We also increased seed of 290 lines from Andes developed for low P environments. Seed of local varieties was also increased.

1.2.9. Phenotyping bean diversity panels

Several collections have been phenotyped to quantify variation for root architectural traits, identify sources of beneficial traits, and understand the genetic control of root traits. A 300-entry BeanCAP diversity panel representing North American common bean diversity was phenotyped for root traits for the second time in 2012. After the third phenotyping, data will be compiled and a genetic analysis performed, with the goal of identifying QTL related to root traits and establish coefficients of relatedness to promote an understanding of how root traits are inherited.

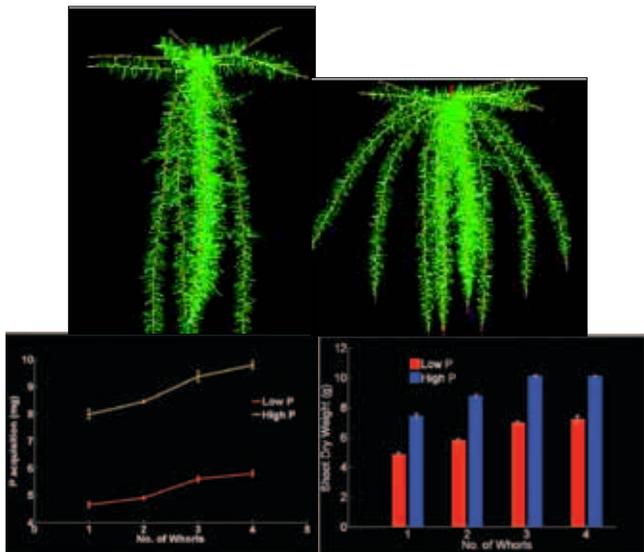
Among a reference collection assembled by CIAT representing global genetic diversity, we found substantial variation in adventitious root number and length, basal root number and length, and basal root whorl number. Most Mesoamerican genotypes had two basal root whorls with seven to eight basal roots, and these Mesoamerican genotypes had deeper roots that confer tolerance to drought stress. Andean genotypes had two to three basal root whorls, with several basal roots and shallow basal roots compared to genotypes from Mesoamerican origin. Our results indicate that sources of tolerance to drought and low P stress can be found in both Andean and Mesoamerican gene pools. Bean breeders could use this information to develop varieties adapted to specific edaphic stress.

The 50-line IBC population developed at Zamorano was phenotyped in the field for eight architectural traits. This information was used to select genotypes with contrasting root systems for intensive physiology studies evaluating the utility of root class dimorphism and multilines for combined water and phosphorus limitations.

1.2.10. Utility of Basal Root Whorl Number for water and P acquisition
Greater BRWN was associated with better growth under low P in a trial in Mozambique and a drought trial in Pennsylvania, indicating that BRWN could be a valuable trait for individual and combined stresses. The red oxisol typical of smallholder production zones in Africa had six ppm P in the low P plots and 19 ppm P in the medium P plots. Soil cores were used to determine root length by depth and three whorl genotypes had more shallow (0-15cm) than deep genotypes and more roots overall. Plant tissue was analyzed for P content and three-whorl genotypes accumulated more P than two-whorl genotypes. Shoot biomass collected at R5 showed no differences between whorl groups under medium P, but under low P three-whorl genotypes had significantly more biomass accumulation than two-whorl genotypes. We conclude that greater BRWN increases shallow rooting, P acquisition, and shoot biomass under low P.

To determine the utility of BRWN for water acquisition from drying soil, we evaluated genotypes with one, two, and three whorls. We concluded that greater BRWN is related to increased biomass accumulation under drought.

A trial at Sussundenga sought to evaluate the utility of BRWN for combined water and phosphorus stress. Stratified soil samples were collected (separated into 10 cm segments) to determine soil P availability by depth. Leaf discs were collected to determine plant P status. An association between greater BRN and biomass at flowering as well as greater BRN and yield was found. Fitting the data to a linear mixed effect model indicates that each additional basal root adds 0.5g biomass at R5. We concluded that increased basal root number is associated with greater performance, and that greater BRWN is a useful trait for combined P and water limitation



Functional-structural plant modeling showing effects of genetic variation in BRWN (top left one whorl vs. top right two-whorls) on P acquisition and biomass accumulation.

A QTL map for BRWN and BRN has been generated using composite interval mapping to perform a quantitative trait loci (QTL) analysis for BRWN and BRN using two populations of recombinant inbred lines (RILs). Phenotypic data on the number of basal root whorls and number of basal roots was measured on seedlings three days after imbibition. We found a total of 23 QTL associated with BRWN and BRN in the two populations. In the DOR364 x G19833 RIL population, we found three QTL in the first dataset with one QTL controlling 14.6 percent of the variation. For the fourth dataset, we found seven QTL with one QTL controlling 23.8 percent of the variation in BRWN. For BRN, we detected three QTL in the 2005 dataset with one QTL controlling 13.7 percent of the variation. In the fourth dataset, we found seven QTL on five linkage groups. One of the QTL on linkage group B7 controlled 25.9 percent of all the variation for BRN in that population. Variability in BRWN in the G2333 x G19839 RIL population was controlled by only one locus on linkage group B3. For basal root number in the DOR364 x G19833 RIL population, we detected four QTL on B3, B6, and B7 in the first trial, and two QTL on B2 in the second trial. No QTL was found in the third trial. For the fourth dataset we found one QTL in linkage group B3 controlling 19.3 percent of the variation in BRWN. This proportion of variation explained by relatively few loci suggests that the potential for genetic manipulation of these traits via these loci is very good.

Work demonstrating the utility of BRWN constitutes a major breakthrough with the potential for widespread and immediate impact.

1.2.11. Root class dimorphism and multilines

The combined drought and low phosphorus situation is complex since shallow soil generally has greater P availability and deep soil has more available water. This motivated the search for root phenes that can explore both deep and shallow soil zones, what

we termed *dimorphic root systems*. To evaluate the utility of dimorphic root class pairings, we phenotyped genotypes from three different sets to three genotypes with superior root traits. We grouped the genotypes into phenotypes, such as strong adventitious roots with deep basals, strong adventitious roots with a strong tap, or shallow basals with a strong taproot. The best performing lines under control and phosphorus stress both have shallow BRGA and many adventitious roots.

We found a consistent benefit of bean architectural multilines compared to the average yield of the component lines under stressful conditions in on-farm trials in Honduras. We repeated this study under combined P and water stress and again found slight benefit of multilines under P stress. This type of trait differs from other single trait studies in that the benefit of multilines would become more obvious in large plot and field scale evaluation and when evaluated at a greater number of locations.

1.2.12. Root phene synergism for P acquisition

Shallow basal root growth angle (BRGA) increases phosphorus acquisition by enhancing topsoil foraging, since in most soils phosphorus is concentrated in the topsoil. Root hair length and density (RHL/D) increase phosphorus acquisition by expanding the soil volume subject to phosphorus depletion through diffusion. We hypothesized that shallow BRGA and large RHL/D are synergistic for phosphorus acquisition, meaning their combined effect is greater than the sum of their individual effects. To evaluate this hypothesis, phosphorus acquisition in the field in Mozambique was compared among RILs of common bean having four distinct root phenotypes: long root hairs and shallow basal roots; long root hairs and deep basal roots; short root hairs and shallow basal roots; and short root hairs and deep basal roots. Results revealed substantial synergism between BRGA and RHL/D. Compared with short-haired, deep-rooted phenotypes, long root hairs increased shoot biomass under phosphorus stress by 89.3 percent while shallow roots increased shoot biomass by 57.7 percent. Genotypes with both long root hairs and shallow roots had 298 percent greater biomass accumulation than short-haired, deep-rooted phenotypes. The utility of shallow basal roots and long root hairs for phosphorus acquisition in combination is therefore twice as large as their additive effects.

1.2.13 Utility of root cortical aerenchyma (RCA) for nutrient acquisition

The formation of root cortical aerenchyma (RCA) reduces root respiration and nutrient content by converting living tissue to air volume. It was hypothesized that RCA increases soil resource acquisition by reducing the metabolic and phosphorus cost of soil exploration. To test the quantitative logic of this hypothesis, SimRoot, a functional-structural plant model with emphasis on root architecture and nutrient acquisition, was employed. Sensitivity analyses for the effects of RCA on the initial 40 days (d) of growth for common bean were conducted in soils with varying degrees of phosphorus availability. With reference to future climates, the benefit of having RCA in high CO₂ environments was simulated. The model shows that RCA may increase the growth of plants faced with suboptimal phosphorus availability up to 14 percent for bean after 40 d of growth. Maximum increases were obtained at low phosphorus availability

(three mM). Remobilization of phosphorus from dying cells had a larger effect on plant growth than reduced root respiration. The benefit of both these functions was additive and increased over time. Larger benefits may be expected for mature plants. Sensitivity analysis for light-use efficiency showed that the benefit of having RCA is relatively stable, suggesting that elevated CO₂ in future climates will not significantly affect the benefits of having RCA. The results support the hypothesis that RCA is an adaptive trait for phosphorus acquisition by remobilizing phosphorus from the root cortex and reducing the metabolic costs of soil exploration.

1.2.14. Bean polycultures have greater nutrient acquisition than bean monocultures

Since ancient times in the Americas, maize, bean, and squash have been grown together in a polyculture known as the “three sisters.” This polyculture and its maize and bean intercropping variant have been shown to have greater yield over monocultures on a land equivalent basis (LER > 1). We showed how below-ground niche complementarity may contribute to this yield advantage. During two seasons, we grew monocultures and polycultures of maize, bean, and squash in field plots differing in nitrogen and phosphorus availability. The polycultures of maize, bean, and squash or only maize and bean had greater yield and biomass production on a land equivalent basis than the monocultures. The differences in root crown architectures, vertical root distribution, and uptake of shallow and deep placed tracers among the components of the three sisters suggest that these species have different, possibly complementary, nutrient foraging strategies. Maize acquired the greatest fraction of nutrients from the topsoil; common bean explored the vertical soil profile more equally, while squash’s root placement depended mostly on phosphorus application. These differences in root placement may have reduced interspecies root competition and increased total soil exploration, with consequent positive effects on plant growth and yield.

1.2.15. Physiological evaluation of common bean genotypes for root traits associated to phosphorus acquisition efficiency in low P soils

During the 2010/11 growing season, we received common bean genotypes from Honduras and CIAT headquarters for evaluation in Mozambique. These genotypes were planted for seed increase so that the seeds can be sufficient to establish an experiment with two P treatment levels with four replications and for destructive phenotyping in the field at Sussundenga Research Station. During this growing season these genotypes were planted and soon we will be conducting destructive phenotyping in the field, where root traits associated to P acquisition efficiency will be evaluated. In addition, an experiment with the objective of testing the performance of genotypes contrasting for a number of root traits, grown under multiple stresses, including low P availability, drought, and the combination of the two stresses will also be evaluated.



Physiological evaluation of common bean genotypes for root traits associated with phosphorus acquisition efficiency in low P fields at Sussundenga Research Station.

1.2.16. Field Evaluation of Mesoamerican and Andean genotypes for performance under low P availability

Five sets of experiments involving 191 genotypes were established in the field in Sussundenga Research Station. The experiments included yield evaluation under low P availability of 49 Mesoamerican and 24 Andean materials received from CIAT in Colombia; evaluation of 46 Mesoamerican materials received from Honduras, 32 Andean materials from BILFA collection, and 40 lines from a biofortification project. Because of limited amounts of seeds available, the biofortified materials were only increased under optimal P availability (fertilized plots) without assessment of their yields. All the other materials were planted under low P availability. Yield data were recorded at harvest.

Data analysis of the 49 Mesoamerican materials had field yields under low P availability varying from 0.16 (Meso 40) to 4.87 ton/ha (Meso 85). Most genotypes had yields of 1.5 ton/ha while five genotypes had less than one ton/ha. Fourteen genotypes out of 49 had yields of more than two ton/ha and only two had 3.72 and 4.87 ton/ha respectively. Genotypes with more than two ton/ha under low P we consider to be P efficient.



Technology dissemination (P efficient varieties of bean) through the establishment of on-farm demonstration plots in Namiepe, Gurue.

Yield assessment of 46 Mesoamerican genotypes (small reds, blacks, and whites) received from Honduras showed yield variation from 0.37 to 6.03 ton/ha, with average of 1.70 ton/ha under low P.

Most BILFA materials are noted to be P-efficient materials when grown in low P soils. We evaluated a total of 32 materials in the field, where grain yield was recorded. The performance of the materials was not superior to other materials.

Twenty-four common bean materials from the Andean gene pool received from CIAT Colombia were evaluated under low phosphorus availability. Grain yield varied from 0.29 ton/ha to 2.47 ton/ha, with average yield of 0.87 ton/ha.

Other Activities

Utility of root hairs for K acquisition in low K soils

Root hairs have been repeatedly shown to have a positive impact on phosphorus acquisition. Potassium is nearly as immobile as phosphorus and we theorize that root hairs would have a similar positive impact in potassium uptake in low K soils.

Cowpea phenotyping and root trait evaluation

Shovelomics was performed on a 200 entry cowpea collection representing worldwide diversity. Substantial variation was observed and genotypes with contrasting root architectures were selected for future studies to establish the utility of root traits for limited water and phosphorus (P) conditions.

Crosscutting Activities

During the implementation of the CRSP project we were able to:

- Establish a low P site at Sussundenga Research Station, where more than 200 genotypes of common bean (both Andean and Mesoamerican) were tested for tolerance to low phosphorus availability, and drought. Genotypes were screened to identify and quantify root traits associated to phosphorus acquisition efficiency and drought.
- Seeds of 40 outstanding common bean genotypes (with 25 to 200 percent greater grain yields under low P availability compared to local checks) were distributed to about 200 selected farmers for field evaluation and technology dissemination.
- A 3,000 liter container attached to a tractor trailer was acquired for back-up irrigation of the field trials at Sussundenga Research Station.
- Two motor-pumps were acquired for direct irrigation to the fields and to pump water from a source to the container and then to the fields.
- A cold room facility was rehabilitated in Chokwe to better preserve bean materials.
- 200 farmers benefited from the bean seeds in Angonia.

Objective 2: Develop integrated crop management systems for stress tolerant bean genotypes

The effects of root traits enhancing P efficiency on biological N fixation

Low P availability is a primary constraint to the production of common bean in Africa and Latin America. Genetic variation for root traits that enhance topsoil foraging is being used to generate new Common bean genotypes with greater P acquisition efficiency. Results suggest that the introduction of P-efficient genotypes may greatly contribute to increased productivity and sustainability of low-input systems since a considerable amount

of N is fixed by the symbiotic nitrogen fixation process with P-efficient lines and its cost is lower than the use of inorganic fertilizer by poor farmers.

Objective 3: Socioeconomics

The socioeconomic research undertaken for this project involved a major survey in four provinces of Mozambique: Manica, Niassa, Zambezia, and Tete. Objectives of the study include identifying and understanding barriers to the widespread adoption of new bean technologies, determining household constraints towards achieving full income and nutrition potential, and, lastly, studying the intrahousehold effects of the introduction of a new bean technology.

Study sites in bean-growing regions in northern Mozambique

The socioeconomic work was undertaken in three districts in the northern (bean-growing) region of Mozambique: Tete, Zambezia, and Niassa.

A collaborative IIAM-Penn State baseline questionnaire was administered to Mozambican households in 2008-09. The survey included questions related to income sources; bean production, consumption, and sales; household decision-making structure; intra-household bean preferences in terms of varieties and traits; constraints to achieving income and nutrition potential relative to legumes; social and economic networks in place and how they can be utilized to enhance the probability of adoption of introduced improved cultivars; and disease-food security interactions, with a particular focus on malaria.

The baseline data set is very rich in content, allowing for comparison to future surveys to be conducted as new cultivars are developed and diffused. IIAM distributed thoroughly-tested cultivars for local assessment in early 2012, and a follow-up survey is now underway to assess specific constraints that households receiving the seed report face.

Summary of selected findings

1. Bean sales are a very important source of income for the vast majority of farms in Mozambique's bean-growing regions.



2. Income from bean sales averaged 4649.46 meticaís in Niassa for households selling beans, and most Niassa households fail to consider bean profitability as a problem. In Zambezia, beans sales generated an average 2246.67 meticaís annually; in Tete, bean sales averaged only 569.28 meticaís per year. The majority of farmers in Zambezia and Tete report profitability as a problem.
3. Villages in Tete are very seriously food stressed. About 80 percent of respondent households reported that not having enough food to eat is a very serious issue for them.
4. Hiring out household labor to other farms appears to be an important coping mechanism to combat food and income difficulties during the hunger season.
5. Households reported strong interest (>70 percent) in producing soybeans, if guaranteed a buyer. This was also the case for black beans: 80 percent of households in the three provinces reported a willingness to produce black beans, if a buyer was guaranteed.
6. Shorter cooking time is a preferred trait. Nearly 90 percent of respondents were interested in beans with the following characteristics: ability to improve soil, drought resistance, lower requirements for fertilizer, less labor, fewer plant and insect diseases, and an ability to intercrop with maize.
7. Introducing new bean varieties that they could sell themselves to consumers in distant markets was less important, but having new bean varieties of interest to traders coming to the farm or homestead was of great interest.
8. The major constraint to the production of more and better common beans is the lack of access to improved seed in Mozambique.
9. A majority of respondents wanted security—both social and market—before adoption.
10. There is substantial variation in social and economic networks in the villages. “Participators” are those villagers who tend to be quite active in the village and are likely to participate in seed demonstrations, seed trials, etc. The “Well-connected” are a group with ties to external networks and likely to access information and input and output markets.

These results emphasize the need for greatly improving access to new seed to improve food security and relieve hunger. The serious lack of seed companies in Mozambique is a major constraint to improved seed access.

Networking and Linkages with Stakeholders

During the present FY, several advanced breeding lines tolerant to drought and low fertility developed by the project were distributed for testing in various countries of the Central American/Caribbean region thru the Bean Research Network (VIDAC and ECAR Trials). A similar group of lines was tested by farmer groups involved in the Participatory Plant Breeding Program for the Mesoamerican Region (PPB-MA) funded by the Norwegian Development Fund. From these groups of project lines, two cultivars has already been released in Honduras and



are used by farmers; three cultivars were released in El Salvador and one cultivar will be released by the end of 2012 in Nicaragua. These locally released cultivars are being disseminated thru the Bean Technology Dissemination Project from the DGPC/ USAID and the PPB-MA Program in Central America. In addition, promising lines from the DGPC has 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 thru the Red SICTA/IICA.

Leveraged Funds

Dr. Lynch and his collaborators have successfully leveraged more than \$4.6 million in external funding for research, in part due to the assistance received from the Dry Grain Pulses CRSP.

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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 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 follows:

- In California, elite novel dry grain green blackeyes were evaluated in two on-station trials for grain quality, yield, and disease and insect resistance; data were collected to support the best one for release.
- In Senegal, an estimated 1,500 kg of the line ISRA-2065, with thrips and aphid resistance, was produced in the 2012 off-season. Following the advanced multilocation yield trials and on-farm tests conducted in five seasons (2008–2012), three new lines will be proposed for release as varieties in Senegal based on grain quality, yield, and disease and insect resistance. About 600 new breeding lines that combine high yield, grain quality, and abiotic and biotic stress resistance traits were tested in preliminary trials in Senegal. Another 1500 lines were advanced to F6 stage in 2012.

Under the seed production and delivery systems objective, the following was achieved:

- In Burkina Faso, breeder seed of 11 improved varieties was grown at Saria and Pobe-Mengao. Foundation Seed of seven varieties was produced at Saria, Pobe-Mengao Kamboinse, and by individual farmers’ organizations and agrodealers. More than 40 MT of foundation seed of seven varieties will be sold to Certified Seed producers in 2013. Another 90 lead farmers were trained as Certified Seed producers in 2012. In Senegal, two ha each of Melakh, Yacine, and Pakau Foundation Seed was produced at Bambe to supply the EWA NGO seed producer network (RESOPP). Certified Seed production and training focused on farmer organizations. Some seed was also produced at Dahra by a private group and the former Louga station manager.
- A student from Mozambique started PhD degree training in cowpea pathology genetics and breeding at UC Riverside.
- Multilocation trials of diverse cowpea lines were completed in Angola and IIA established contact with extension institute “IDA” to coordinate seed production and distribution.

Project Justification and Objectives

The primary project focus is to:

1. increase productivity of African and U.S. cowpea producers through improved varieties that possess 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
4. provide training and capacity building in modern cowpea breeding to African researchers

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 even though potential yields are five-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. Sustainable increases in cowpea productivity in Africa and the United States 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. Landrace grain types are often preferred locally, but if over produced, prices offered to farmers can be low. 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 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 in Africa and the United States. There is also considerable demand for large rough-brown seed, especially in urban centers in Nigeria, but the rough-brown “Ife Brown” is susceptible to pests and diseases. Other new cowpea products based on a sweeter and milder taste could help broaden cowpea consumption in the United States, Africa, and elsewhere.

Increasing Seed Supply of Improved Varieties. Cowpea breeding by the CRSP, African NARS, and IITA (Senegal, Burkina Faso, Nigeria, and other countries) has led to improved cowpea varieties that are near release. However, only about five percent of the



Hamado, one of our best certify seed producer in his 3 ha field at Poa near Saria.



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 (e.g., women farmer organizations), for-profit seed cooperatives (NGO-established, now largely self-sustaining), and for-profit individuals or groups, but limited scope reflects insufficient quantities of Breeder and Foundation Seed. We are supporting 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)
4. Capacity Building for Host Country NARS

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.

We are completing final testing and release protocols of lines developed under the previous Bean/Cowpea CRSP and other germplasm in the development pipeline, and initiating new short- and long-term breeding strategies to develop high-yielding improved 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. The best performing INERA lines will be planned for release in 2013 following Breeder Seed production during FY12. In Senegal, the indicated ISRA lines were planted in 20 demonstration trials during FY11, with a second year of on-farm demonstration trials in FY12. This should complete the performance data required for the formal release. The lines are high yielding with resistance to the prevailing diseases and insects (aphids), large seed size, and plot size sufficiently large to allow mechanical planting.

In Burkina Faso, the six varietal candidate lines developed at INERA were planned for on-farm trials by 30 farmers at six sites in the Central and Northern zones. The six new varieties were compared to one local check at each site.

In Angola, cowpea field evaluations were conducted in the main ecological zones to identify candidate varieties among local landraces, and Bean/Cowpea CRSP (in Ghana, Senegal and/or Burkina Faso) and IITA varieties. The trials included 26 CRSP core entries, plus a similar number of local types. The Angolan materials were SNP-genotyped to enable association mapping comparisons for major mapped cowpea traits.

In California, 12 new advanced dry green breeding lines were screened in replicated trials at two locations in 2011; from these, the seven best yielding/grain quality types were field tested in replicated plots at Riverside and Kearney.

In California, for continued development and testing of new elite blackeye lines, nine advanced lines selected from 2010 and 2011 trials were tested in replicated plots at Riverside and Kearney under insect protected conditions.

In California, for development of lygus, nematode, and aphid resistant varieties, lygus resistant breeding lines have been developed that are in different stages of testing. A subset of these lines were selected based on their performance in lygus screening trials conducted in 2010 and 2011 and evaluated for grain yield and grain damage under lygus protected and unprotected conditions at Kearney and unprotected at Riverside in replicated yield trials again in 2012. At Kearney, we also conducted large unprotected strip trial yield tests of the three 2011 highest performing lygus tolerant lines. We also initiated a new round of crosses in 2010 for breeding varieties with increased resistance to lygus and that have high quality grain, because while current lygus resistant lines combine very high yield potential and resistance to lygus bug, improvement in grain quality is needed to meet market expectations. F4 lines developed from these crosses and screened in an unprotected nursery at Kearney under strong selection for resistance to lygus and for desirable grain quality were advanced in 2012.

We are also breeding an improved version of CB46 with greater resistance to root-knot nematodes derived from IITA breeding line IT84S-2049. In FY2011 inbred BC7F2 lines were developed, evaluated for resistance to nematodes in laboratory growth pouch assays, and resistant lines increased in the greenhouse. The best lines were field tested at South Coast field station in 2012. For aphid resistance, breeding lines were developed that show strong resistance to this pest in aphid resistance screening

trials. The most resistant lines were crossed with CB46 and CB50 and advanced to F2 in 2011 as part of the process of transferring aphid resistance to adapted varieties. In FY12, these lines were advanced to F3 for screening.

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. Obvious defects in local and improved varieties are being improved by breeding in resistance to diseases and pests and other traits.

In Senegal, from the new crosses made by Dr. Cisse at ISRA, progeny selection and advancement were made to develop varieties with medium to late maturity to cope with the changing cropping season length in the northern zones and with the growing interest in cowpea in the south and eastern areas. These materials included thrips resistance and good grain size and color qualities.

In Burkina Faso, from the crosses made at INERA, progeny selection and advancement is being made to develop varieties with increased seed size of the improved varieties with important insect, disease, Striga, and nematode resistance traits.



During FY12, Senegal and Burkina Faso recurrent backcross populations were advanced and inbred to the BC2F5 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 was used to aid in the selection for multiple traits.

The California advanced inbred BC and segregating populations were either backcrossed or tested for yield performance during the FY12 main growing season. The SNP-marker genotyping described above for Senegal and Burkina Faso backcross progenies was applied similarly to check for resistance traits to root-knot nematode, *Fusarium* wilt, and aphid.

Longer-Term Breeding Strategy

The longer-term strategy is to pyramid resistance and grain quality factors in varieties desired by farmers using crosses between elite parents having complementary parental lines.

Stream One includes biparental crosses between highly drought tolerant lines. During the 2008–2010 project phase, the F1s were made at UCR, then advanced to the F2 generation and subjected to screening for drought tolerance. Drought-tolerant F2 individuals were identified and advanced to the F3 for each population. The F3 lines were evaluated for drought tolerance and the best performing 100 selected and the family bulked for further evaluation. The F4 and F5 families were planted in replicated field trials in Senegal and Burkina Faso in the off-season and main 2012 season for initial performance evaluation. Individuals from the most drought-tolerant lines will be used for crossing to the improved lines developed under the backcrossing program.

Stream Two includes a set of popular local cowpea varieties chosen by breeders in Senegal and Burkina Faso during 2010 for targeted genetic improvement through MAS or MARS. These were hybridized to sources of known thrips resistance and heat/drought tolerance. In 2009, 352 F3 families were screened for performance under postflowering drought conditions and the seed bulked. The 100 top performing bulks were re-evaluated in California in late 2010 and four single-plant selections made in the best 25 performing families. These 100 F5 selections were shipped to Burkina Faso and Senegal, where they were grown out in field nursery plots and phenotyped for thrips tolerance and grain production under drought/heat conditions in 2011. Some additional crosses made in Senegal were advanced by single seed selection to the F6 generation in 2011, with selection based on grain quality and SNP markers for drought, thrips, and *Macrophomina* resistance.

In California, Pigeon pea selections made in earlier years at UCR from GA-1 were tested in 2011 and 2012. Using four-fold replicated field station plantings, data from a yield trial conducted at Kearney in 2011 with 10 selections was used to identify the best six selections for yield and grain quality tests at Kearney and UC-Riverside in 2012.

Final Testing and Release of Varieties

In Burkina Faso (INERA). Results of two years of testing indicated that among the five tested white-grain lines, KVx 442-3-25 is the most preferred because of its high yield and large seeds. We will produce Breeder Seed of this line during the coming off-season. In 2012, 8,000 kg of Certified Seed of KVx 442-3-25 were produced. Note that a total of 879 kg of seeds of KVx421-2J, IT98K-205-8, and Melakh were also obtained.

In Senegal (ISRA). The breeding line ISRA-2065 was developed under the previous Bean/Cowpea CRSP from a cross between the high-yielding CRSP cultivar “Mouride” and aphid and thrips resistant local landrace accession “58-77,” with the objective of developing a cultivar with the yield and stability of Mouride but with resistance to aphids and thrips. ISRA-2065 is being targeted for use in the wetter part of this cowpea production zone where flower thrips are especially damaging, since it has

stronger resistance to thrips than Melakh; ISRA-2065 was released in 2011 as “Pakau”; 1500 kg of Foundation Seed was produced in 2012 off-season.

In Angola, cowpea field evaluations were conducted at three locations targeting the main ecological zones with the aim of identifying candidate varieties among local landraces and Bean/Cowpea CRSP and IITA varieties. In FY12 the highest ranked lines were tested at seven sites. In the trial planted in Mazozo, the highest performing varieties were 2870-5, 124/2469-1 local varieties, and UCR 288, IT90K-284-2 improved varieties.

In California. Replicated small plot tests of new dry-green blackeye advanced lines were conducted to determine varietal candidates. Yields of these seven breeding lines were significantly less than elite blackeye variety CB46 in the Kearney trial.

In California, a breeding nursery with several hundred F7 generation blackeye breeding lines was conducted in 2010 and seed of 15 new lines bulked for replicated tests in 2011. The best nine performing lines were retested at Riverside and Kearney (data not yet finalized) in 2012. Several lines had yields comparable to CB46 and CB50, and one had significantly higher yield than these current varieties.

In California, for development of lygus, nematode, and aphid resistant varieties, a range of lygus resistant breeding lines have been developed that appear very promising. Breeding line 07KN-74 showed the best performance in protected and unprotected conditions. However, line 07KN-74 had excellent yield in both protected and unprotected plots.

We also initiated a new round of crosses in 2010 for breeding varieties with increased resistance to lygus that have high quality grain, to meet market expectations. These were advanced to F3 in 2012.

We have developed a breeding line that is an improved version of CB46 with greater resistance to root-knot nematodes derived from IITA breeding line IT84S-2049. Line CB46-57Rk2 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.

Short-Term Breeding Strategy

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 80 lines were re-evaluated at Pobé Mengao in the 2012 main season. Farmers preferred variety K VX442-3-25 and it was released under the name “Komcalle” in July 2012.

In Senegal, two advanced yield trials were conducted at the Bambey, ISRA field station in 2010 and 2011. The first trial included 98 lines from the cross Nd. AW x Yacine and the two parents. The second trial with four-fold replication included 26 lines from Mélékh x UCR 232; CB 27 x Mélékh; Mélékh x Monteiro derived lines, and ND. AW x Yacine. The control entries were Mouride, Mélékh, Yacine, and Pakau. The trials

were used for yield and agronomic characterization of each line, and harvest data were collected. Another 20 lines with medium maturity were selected from the first trial based on 2008 performance and included in four replicated yield trials in farmer fields in 2009 and 2010 in the Mekhe and Louga areas; 20 lines were also selected based on grain size (100 grain-weight > 25g) from the second 2008 trial and tested under the same conditions. In 2012 the five best lines selected from tests conducted from 2008-2011 were introduced in large plots for the last on-farm trials.

Crosses for developing new breeding lines

In Burkina Faso, progenies of 25 new crosses were advanced to the F5 stage during 2010. The F6 bulk of these crosses were harvested in 2011 for planting in 2012 at Pobé Mengao. Single plant selection was made to increase seed size of the improved varieties for Burkina Faso.

In Senegal, for introgressing Striga resistance, Yacine was crossed with line IT90K-76. Additional crosses were also made and included Pakau, Yacine, and Melakh, each crossed with the Striga resistant lines for Macrophomina resistance. The 58-57 x Suvita and TN88-63 x 58-57 crosses were also made. These new materials were advanced in 2012 to the F6 stage.

In Burkina Faso, 27 families of the crosses using the set of parents IT84S-2246, IT93K-503-1 and Mouride were evaluated in a replicated trial at Pobé during the main 2012 season. In Senegal the F3 lines from these families were evaluated for drought tolerance and the best performing 100 selected and the family bulked for further evaluation.



Stream Two includes two four-way cross populations developed from diverse yet elite African and California cultivars with a host of desirable traits. Population 1 was made by biparental crosses between CB27 with IT82E-18 and IT84S-2049 with IT84S-288. Population 2 was made by crossing IT84S-2246 with IT93K-503-1 and IT00K-1263 with SuVita2. Then 300 four-way double crosses were made to generate 300 four-way individuals, which were selfed one generation in the greenhouse and were grown at the Coachella Valley station for within-family selection for high pod production in December 2011.

In Burkina Faso, two phenotyping trials were conducted at Saria and Kamboinse with the lines from a cross of Suvita2 and IT98K-499-35 and 248 lines of the cross 2011-002.

Marker-assisted backcrossing (MABC) is a breeding strategy that can markedly increase the progress and precision of backcross breeding outcomes. The focus is on MABC of backcross progenies to identify individuals carrying a majority of molecular markers associated with the genetic background of the recurrent parent, with the addition of the trait markers from the donor parent. 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.

In California. Pigeonpea GA-1, a selection made in earlier years at UCR from materials supplied from the University of Georgia, is being used as a source of further selection based on yield and grain quality. A small-plot, four-fold replicated trial at Kearney with entries that included 10 selections from the 2010 pigeonpea nursery at Kearney and single-plant selections derived from a plot of GA-1 grown in 2010 was harvested in November 2011. Based on yield differences, seed of the six most promising lines was used for larger-scale 2012 trials at both Kearney and Riverside in late May and early July.

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 Certified Seed production. Insufficient resources limit growing, harvesting, and storing Breeder Seed increases, in turn limiting Foundation Seed and Certified Seed for farmers.

The approach is to increase amounts of Breeder and Foundation Seed available to Certified Seed producers, help identify new Certified Seed producers, and strengthen and expand proven activities in Senegal and Burkina Faso through leveraged funding from NGOs. Working with the national extension services in Senegal (ANCAR), Burkina Faso (DVA), and Angola (SENSE) to reach the farmers' organizations in different communities is being coupled with strengthening the small private seed producers, some of them already working on cowpea.

A strategy adopted by the GCP/ICRISAT "Legumes for Livelihoods" project in Niger, Nigeria, Mali, Tanzania, and Mozambique for cowpea is to improve farmers' access to seed and enhance widespread adoption of improved cowpea varieties through the development and promotion of community seed production and promotion of local markets for seed. Several progressive farmers will be selected per village and given guidance in seed production and supplied with quality Foundation Seed for multiplication. They will become the source of improved seed for the entire village Seed Network and other programs to derive synergy in promoting local seed enterprises.

In Burkina Faso, the primary effort is to produce Foundation Seed and Certified Seed of six newly released varieties and seven existing varieties. Up to 40 tons of Certified Seed was produced in seven provinces by trained farmers in 2012. Breeder Seed will be produced in the off-season. Foundation Seed production will be made to ensure an adequate capacity on each of the four INERA stations to generate about six tons of Foundation Seed, which will address the estimated five percent shortage of Foundation Seed. A target of 90 Certified Seed producers will be trained in seed production, harvest, and postharvest handling.

In Senegal, availability of Foundation Seed has been identified as a bottleneck for adequate supply of seed to farmers. To overcome this, Dr. Cisse will produce two ha of Melakh, two ha of Yacine, and two ha of Pakau to complement the Foundation Seed production by the ISRA seed unit at Bambey. This effort will help to identify the demand level for Foundation Seed and provide seed for establishing new Certified Seed growers in cowpea production areas where there is currently no formal Certified Seed production effort. At each location, Foundation Seed will be provided and farmers will be trained in seed production, harvest, and post-harvest handling. Organizations who contact ISRA for Certified Seed will be directed to the new Certified Seed producers, to establish a supply and demand relationship that should become self-sustaining.

In Angola, we will continue to link with government and NGOs to determine opportunities for advancing the cowpea seed system. The Legume program of IIA with the support of CRSP can coordinate the breeder seed through a strong partnership with National Extension Service and National Seed Service to train future seed producers. 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 in Saria more than 40 tons of Foundation Seed was produced in 2012 using part of the Breeder Seed produced in 2011. Part of the money obtained by selling the Foundation Seed in 2011 was used for supporting 2012 seed production activities in attempts to establish a self-sustaining plant seed production and delivery system.

In Senegal. Initially, three ha each of Melakh and Yacine and one ha of Pakau Foundation Seed was produced at the ISRA Bambey station, and at least 100 kg of each variety was made available to the network of farmers cooperatives (RESOPP) now set up by the NGO EWA. RESOPP planted about 61 ha of Melakh and Yacine Foundation and Certified seeds in 2012. In the Thilmakha area, Foundation Seeds were distributed to two farmers for production of two ha of Melakh and two ha of Yacine Certified Seeds during the 2011 season. In 2012 they cultivated the same acreage with seeds obtained the year before. Certified Seed production was also conducted in collaboration with a farmers' union (UGPM) in Mekhe with 10 ha of Melakh and Yacine each in 2010. These acreages were raised to 25 ha in 2011. In 2012, three ha of Pakau were added to their operation. At Touba Toul, a locality near Bambey, 20 ha of Melakh and Yacine Certified Seeds were produced by a farmers' organization that

purchases inputs in bulk. Training of farmers during the 2012 season for seed production consisted of field selection, removal of off-types and diseased plants, and both harvest and postharvest handling. Triple bags obtained from the PICS project are being provided to farmers for storage of the 2012 seed.

Objective 3. Technology Dissemination (Seed of Improved Cowpea Varieties in West Africa).

INERA, Burkina Faso. In Burkina Faso during FY12, Breeder Seed of 11 improved cowpea varieties were produced at Saria and Pobe. Theory and practical training were conducted at two locations to train farmers, of which at least one-third were women. The trained farmers also were guided in producing Certified Seed of the INERA improved cowpea varieties. Breeder and Foundation Seed of the seven varieties were also produced during the 2012 growing season.

ISRA Senegal. In Senegal, the availability of Foundation Seed is a bottleneck for adequate supply of seed to farmers. Additional Foundation Seed will be produced of three varieties of Pakau to supply new Certified Seed to growers. During the 2012 rainy season, six ha of Melakh, Pakau and Yacine foundation seeds were produced. The project worked with the National Extension Service and 80 farmer organizations at five locations where 100 to 200 farmers will be trained in seed production, harvest, and postharvest handling. At least 100 ha of Melakh and Yacine will be grown initially for Certified Seed, with the goal of scaling up in future years.

INERA, Burkina Faso. In Burkina Faso during FY12, Breeder Seed of 11 improved cowpea varieties was produced on eight ha, yielding 250 to 900 kg of Breeder Seed of each variety. Theory and practical training was conducted at Saria and Pobé-Mengao during April to June 2012 to train 90 farmers, of which 25 were women. The 90 farmers were guided in producing Certified Seed of the INERA improved cowpea varieties. This activity generated approximately 35 T of Certified Seed on 50 ha. Foundation Seed of seven varieties was also produced during the 2012 growing season (July–October) by individual seed producer farmers, Farmers Organizations, Agro dealers, and NGOs. An estimated 40 T of Foundation Seed will be produced when all harvest is counted.

ISRA Senegal. To address the Foundation Seed bottleneck for adequate supply of seed to farmers, additional Foundation Seed was produced of three varieties (Melakh, Yacine, Pakau) to supply new Certified Seed to growers. Two T each of Melakh and Yacine and 1500 kg of Pakau Foundation Seeds were produced during the off-season under irrigation. It is estimated that 50–60 T of Certified Seeds will be produced after completion of the 2012 harvest. Seed is being packaged in four kg bags. During the 2012 growing season, five to six T of Foundation Seed was produced at the ISRA Bambey Research Station. Support was provided to the NGO FONGS, RESOPP, and Millennium objectives project to expand cowpea seed production.

Objective 4. Capacity Building for Host Country NARS

Training current cowpea breeders in the development and application of DNA-based markers for MAS in the cowpea

breeding programs has been embedded in the research effort under objective 1. Cowpea breeders were trained in marker application utilizing their own breeding populations generated by the high x high crosses and recurrent backcrossing for existing variety improvement made within the programs. The focus has been on the Senegal and Burkina Faso programs and to some extent on Angola. This training utilizes the KBiosciences (LGC Genomics) KASP SNP genotyping platform populated with 1,057 informative markers derived from the Illumina SNP-genotyping platform we developed for cowpea. The genotyping results are being co-analyzed by the United States and HC team. Additional training was made through joint interpretation of data sets and progeny selections at a hands-on MAS and MARS experience in a two-day training workshop conducted in conjunction with the Pulse CRSP Global meeting held in February 2012 in Kigali, Rwanda. We also invited the Pulse CRSP common bean breeders and trained at the Kigali workshop. Training was also conducted at meetings in Addis-Ababa, Ethiopia, in May 2012, linked with CGIAR GCP-organized meetings. The genotyping allows all target traits described under Objective 1 to be advanced by indirect selection. Markers linked to traits, including drought tolerance and Macrophomina and thrips resistance are being emphasized.

Networking and Linkages with Stakeholders

We worked closely with national and international cowpea breeders and other scientists at IITA, IAR in Nigeria, Eduardo Mondlane University in Maputo, Mozambique, the University of Virginia, and Purdue University. We worked closely with the California Dry Bean Advisory Board and its Blackeye Council on research priorities of the industry. We worked with Inland Empire Foods, an important legume processor based in Riverside, on developing Akara (or “Bean Tots”) for inclusion into the California school program and with another major U.S. manufacturer on utilization of several products. We have provided Pulse CRSP PIs with cowpea seed. Under the CGIAR-GCP funded project Tropical Legumes 1, we are leading the cowpea improvement objective and interact 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 continued on-farm tests of improved varieties and we are helping them to produce Certified Seed. Linkages have also been maintained with five farmer organizations: “Song Koadba” at Donsin near Ouagadougou; “Six S” at Pobe Mengao; Producteurs de Semences de Diouroum; Producteurs de Semences at Pobe Mengao; and Producteurs Semenciers Song Woaga at Saria. In addition, collaboration was continued with a Seed Producer Association named Venegre and two seed entrepreneurs (Famille Kabre and Agrisem). Linkages also have been made with two Agro Dealers.

In Senegal, collaboration was established with the extension service 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), an individual (Moustapha Diop)

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 the Faculty of Agrarian Sciences, Institute for Agrarian Development, AFRICARE, an American NGO, and Small Farmers Association/Community Based Organizations (CBOs).

Dr. David Kiala of the Faculty of Agrarian Sciences has carried out evaluation of germplasm across agroecological zones, capturing farmers' preferences and gender considerations for selecting cowpea varieties. José Pedro of the National Center for Phyto-genetic 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 U.S. \$3.0 million in external funding to achieve objectives related to this project.

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Contribution to Gender Equity Goal

Among the target beneficiaries of the project work, the activities in Burkina Faso and Senegal resulted in producer/community-based organizations, comprised of women and men, receiving technical assistance. In addition, Host Country partner organizations/institutions in Burkina Faso and Senegal benefitted from the seed systems technology. More specifically, women's organizations received technical assistance in Senegal and Burkina Faso.

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 a nonoption for many farmers, and transgenic cowpeas, if they become available, will only control one of the six major pest species. There is an urgent need to develop a comprehensive, cost-effective IPM strategy for the insect pests that attack cowpeas using a diversity of control strategies, comprised of three major steps, on which we have made significant progress in FY12:

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. This information is extremely important 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. Most notably we have a neem-virus combined spray that is as effective as or better than traditional pesticides in increasing cowpea yields in the field.

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

This past year, the program has exploded to include more than 24 partner groups. The animation technologies and deployment strategies have been used for cowpea-related activities in West Africa across multiple groups. The SAWBO program and infrastructure is now addressing many other education-related issues.

Project Justification and Objectives

Arguably, the greatest biotic constraints on cowpea production are insect pests. There is currently a dire need for deployment of pest control strategies to improve the livelihoods of those who produce and consume cowpeas. 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. Biocontrol agents offer immediate, tangible, and cost-effective pest control solutions to farmers; 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 and their institutions have had major successes with the use of biological control agents for pests of other crops and have tested other practical control methods 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.

The best place to release biocontrol agents is where the insects are endemic and can support the biocontrol agent populations. 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. Characterization of Pests of Cowpeas (*Maruca vitrata*) and Molecular Markers
2. Characterization of the Other Pests of Cowpeas (other than *Maruca vitrata*) and Molecular Markers
 - a. Insect Pests on Cultivated Cowpeas.
 - b. Survey Wild Alternative host plants (in and off season)

In the long-term, pesticides are likely to become a less viable option for control of pests on cowpea.

3. Development of Infrastructure for Release of Information to Extension Services, NGOs, and to Cowpea Farmers as well as Short-term and Long-Term Training
4. Dissemination Project Capacity Building of Host Countries
5. Build capacity at host country institutions for the rearing and mass release of biocontrol agents that are currently ready for release.
6. Collections of biological control agents for sequencing and development and of IPM-omics tools
7. Bring new biocontrol agents into the pipeline for development and deployment
8. Other Institutional Capacity Building training activities in rearing and release of biological control agents

Objective 1: 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.



Light trapping was done over the past 12 months at the existing locations in Niger and Burkina Faso. Adults were monitored and collected from the light traps on a daily basis 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. Because *M. vitrata* is an important pest of cowpea in Southern to mid-Burkina Faso, control efforts for this pest should be concentrated in the south and midpart of the country.

We have a large collection of *M. vitrata* from throughout Burkina Faso and Niger being used in molecular analysis of the populations, which has allowed us to 1. build institutional infrastructures to monitor *M. vitrata* using light traps, 2. develop multiple standard and novel molecular approaches for studying *M. vitrata* population dynamics, 3. use these genomics tools for insect management decisions for the next phase of our project, and 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 the combined light trapping and genomics data of *M. vitrata* populations to determine how to most cost-effectively deploy insect control strategies for this pest of cowpeas. We now have a better understanding of when and where biological control agents should be released to optimize the 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.
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 around the world and (c) determine which genes vary locally and regionally (in West Africa). We can now characterize *M. vitrata* populations from distinct locations in West Africa to determine their movement patterns.
3. We have used 454 sequencing technology to determine single nucleotide polymorphisms (SNPs) across hundreds of *M. vitrata* nuclear genes to determine the exact locations in these gene that will and will not typically vary from insects found around the world and which components of the genes vary locally, regionally, or across the plane, and to characterize *M. vitrata* populations locally, regionally, and across continents and to gather critical data on their movement patterns in West Africa.
4. We have used the above molecular tools to determine that *M. vitrata* is actually two separate species of insects and determine important information on the migratory patterns of this pest in West Africa.
5. Based on the above molecular strategies, we have also developed diagnostic PCR-based assays for other researchers to further test details of *M. vitrata* populations. These approaches will allow African host country institutions to sequence genotypes with basic molecular biology equipment to easily characterize *M. vitrata* populations.
6. Our increased insights into the movement patterns of *M. vitrata* have been important for the development of modeling strategies for minimizing resistance in the insect populations if or when the transgenic cowpea is released in West Africa.
7. All of the molecular tools we have developed, along with their applications for insect control, were applied in FY2012 to the other pest insects that attack cowpea, this year particularly to the sequencing and use of polymorphic markers of two of the biocontrol agents (*Apanteles taragamae* and *Ceranisus femoratus*) that have also been released in field trials. This same

molecular marker information will be used to demonstrate that successful biocontrol agents came from our release populations and will ultimately determine if there are specific molecular markers associated with successful biocontrol agents that have been released.

8. We have collected insects from all the other pest species and performed the first steps of sequencing these populations to perform the same type of studies as with *M. vitrata*.

Objective 2: Characterization of the Other Pests of Cowpeas (other than *Maruca vitrata*) and Molecular Markers.

2a Insect Pests on Cultivated Cowpeas

This activity has provided the basis for a better understanding of the problems of pest insects of cowpeas within the host countries. It has also allowed for cross training in pest insect biology across the three host countries. This work will also lay the basis for the development of an IPM plan for *Bt* cowpea and provide the basis for other IPM-based pest control strategies for *Maruca* and other pest insects of cowpea. We will also test the impact of viral and neem sprays on cultivated cowpea crops to determine if these approaches 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, the coreid pod sucking bugs, the groundnut aphid, and, thrips. During FY11 and FY12 viral sprays (against *M. vitrata*) and neem sprays (against all the pests) were applied to cowpea crops.

Summary of findings

1. We have collected the necessary samples of insects and begun the process of 454 sequencing of mixed samples within each species to discover polymorphisms used for SNP analysis.
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 were confirmed. Detailed experiments in Benin indicated an average of 67 percent yield increase in the first cropping season as compared to the untreated plot. Even better, a combination of aqueous formulation of neem oil combined with the virus doubled the cowpea grain yield.
4. Neem sprays were effective in decreasing pest populations, increasing yield, and, coupled with host plant resistant strains, were most effective in reducing the pest populations.

Implications for pest control strategy

1. We have defined which regions we should focus the deployment of specific biocontrol agents to control specific pest species for long-term suppression of pest populations.
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.

Details of Efforts over FY12

We have performed the above experiments over the past five field seasons. 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, and we recorded all the details of which pests attacked which plants and at what time interval; we have analyzed the datasets and are able to ascertain which pest insects represent the greatest problems (and at what time interval) in northern Nigeria, Niger, and Burkina Faso.

We have performed field experiments where we have tested pest tolerant varieties of cowpeas on their own and in combination with neem sprays, and tested viral sprays to control *M. vitrata*. Both strategies were successful in reducing pest numbers and increasing yield.

Other important trends have emerged in insect control efforts. In Niger, earlier flowering varieties did not sustain the same levels of insect attack as did the medium and late flowering varieties. In host plant resistance, this phenomenon is termed avoidance; the plants simply mature before the pest populations reach their peak numbers and thus simply avoid the time intervals of pest attack. These experiments have also helped us determine where to deploy certain biological control agents for given pest insects and the regions where there is little need for such control measures.

We have made large-scale collections of insects for our genomics experiments to better understand the movement of pest populations. The materials collected in this part of the project will be critical for the development of genomics tools to understand the nature of these pest populations and where and when to release biological control agents.

2b Survey Wild Alternative host plants (in and off season)

This activity will provide the basis for a better understanding of the problems of pest insects of cowpeas within the host countries, both during the growing season and when the cowpea is not in season to help us determine where the pest populations are occurring when the cowpea is not being grown.

A standardized scouting plan will be established within the first six months of the project. Scouting of pests of cowpea on alternative host plants will occur both during and outside of the cowpea-growing season. No fewer than one scouting trip will occur per country, per six-month budget period.

Surveys of wild alternative hosts around and near cowpea fields were assessed in each country during the growing season. Farmers' fields were surveyed for the numbers of insects on cowpeas in relationship to any nearby wild alternative hosts. Observed insects were collected to generate the necessary polymorphisms used to study the insect populations and the movement patterns.

Implications for pest control strategy

Our results support the hypothesis that pod sucking bugs, thrips, and aphids occur in the dry season in areas where cowpeas are grown during the wet season. If these results are

supported by molecular data, that the pest populations are endemic, then biocontrol agent releases locally should support local pest populations over the long-term.

Details of Efforts over FY12

We have performed a series of scouting trips in Niger, Benin, and Burkina Faso. The results have already provided an important basis for indicating the best locations where biological control agents for *M. vitrata* need to be released to achieve the greatest potential impact on *M. vitrata* populations that affect cowpea crops in northern Niger and Burkina Faso. For example, in Burkina Faso our work has shown that *M. vitrata* is endemic in the southern most region of the country (which is farther north of where it had previously been thought to have been endemic). At least 14 plants species belonging to the Fabaceae family are alternate host for *M. vitrata* in Western Burkina Faso. Most of these host plants maintain the population of *M. vitrata* during the cowpea off-season. Our scouting data (coupled with our molecular data) strongly suggests that *M. vitrata* moves almost directly north from these endemic areas during the growing season and impacts cowpea crops in the central areas of Burkina Faso. Based on our findings, biological control agents useful in controlling *M. vitrata* should be deployed in Southern Burkina Faso and in the northern parts of the countries in Burkina Faso's southern border. The parasitoids useful in control of *M. vitrata* are the *Apanteles taragamae* and *Nemorilla maculosa*.

For both pod-sucking bug species, there are at least six local wild alternatives that support these populations during the dry season. Cowpea aphids are hosted by peanuts and vegetables during the dry season.

We extended scouting and molecular approaches to other pests of cowpeas to determine where the biocontrol agents would be 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 3: Development of Infrastructure for Release of Information to Extension Services, NGOs, and to Cowpea Farmers as well as Short-term and Long-Term Training.

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 that will help us deploy these approaches on a larger scale, and 3. educational tools and resources for

training host country scientists, extension educators, and farmers in the most effective pest control strategies. 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 highly cost-effective strategies for rearing *M. vitrata* and the production of biocontrol agents for release.
4. We have partnered with other organizations to deliver pest control strategies to farmers.
5. We launched the online information sharing system for extension materials (Sustainable Development Virtual Knowledge Interface, <http://susdeviki.illinois.edu>).
6. We developed a series of animations (in local languages) 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. The biocontrol video has been completed and is soon to be released. 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) which will also be producing videos for other development and socially related projects and programs (with other funding sources). Our videos have been shared with Drs. Robert Mazur and Cynthia Donovan. Contact has been made with staff of the Ministry of Communication in Benin to promote this technology within their current strategy of introducing ICT innovations to the rural areas. SAWBO has developed links with more than 30 partner groups who are helping to create new videos, do voice overlays in local languages, and perform local deployment. Between known field-deployment, views, and downloads, we estimate more than 100,000 people have seen these videos.
7. We have studied the potential for spreading these animations via cell phone networks in Burkina Faso and Niger. The results suggest that the videos can spread through Bluetooth® from cell phone to cell phone.
8. The SAWBO program now has more than 20 other videos on malaria, dengue, prevention of postharvest losses, tuberculosis, West Nile virus prevention, removal of cyanide from cassava flour, and a host of other important topics.

Farmer Field School (FFS)

Farmer field schools have been used as an effective method of deploying information into rural communities, along with developing the skills sets for farmers to adopt new technologies for crop production. We have performed a minimum of two farmer field schools in each of the host countries in FY12, with a 50/50 mix of men and women. Each farmer field school has a minimum of 20 individuals and represent multimonth half-day a week training sessions. The objective of these FFSs is to

educate farmers about the pests of cowpeas so they can play an active role in assessing, disseminating, and releasing improved methods for pest control (and overall production) in cowpeas. Farmers are trained to identify the major pests of cowpea and understand their basic biology and the impact on their crops. Animations on cell phones were also distributed to villages.

As part of the farmer field schools, farmers set up test plots with different technologies for cowpea production (host plant resistant lines, neem sprays, and viral sprays), assessed insect attacks along with the impact of other production technologies, and made decisions on the outcomes of these experiments.

Technologies deployed 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. how to minimize the use of traditional pesticide sprays in areas 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 the greatest concern in various regions of each country and give farmers the ability to identify early on in the season which pest problems they have so they can take logical measures to minimize the pest populations.

Our long-term goal has been to release biological control agents into those areas where we have held FFSs. The FFSs will continue to monitor the pest populations and also the presence of the biological control agents. This way we will engage farmers to assist us in playing a role in determining if the biological control agents have a practical (or at least perceived) impact on these 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:

1. We have held one-day sessions where other farmers, production groups, and people from other villages can interact with the FFSs to see the impacts of the various pest control strategies (and other technological improvements).
2. Improved seed varieties have been given out to other farmer organizations for them to assess, multiply, and use.
3. We are currently producing printed and electronic media that can be used by future Peace Corps volunteers for deployment of technologies to assist in cowpea production.

As part of the FFSs we have also been focused on determining the needs and roles of women in various aspects of cowpea through production of a project to identify targeted issues regarding gender roles and outcomes.

Technician Training

Research assistant exchanges occurred between IITA-Benin, INERA, and INRAN to demonstrate and implement various methodologies for rearing of *M. vitrata* and its parasitoids under laboratory conditions.

Objective 4: Dissemination Project Capacity Building of Host Countries.

Accomplishments

1. Low-cost/highly efficient system for mass rearing techniques for *M. vitrata* (and its parasitoids) using cowpea sprouts adapted for HC conditions and currently in use in Benin, Burkina Faso and Niger.
 - a. Approximately 30 times less expensive and much easier than the previous rearing technology
 - b. Parasitoids and viruses can be produced on a much larger scale for a fraction of the cost.
 - c. The technology can be easily transferred to new programs and organizations.
 - d. Videos have been developed for online training of technicians on techniques for use within our project.
2. In-field rearing and slow-release delivery systems developed for the parasitoids *A. taragamae*, *T. eldanae*, and *G. fulviventris* are currently being experimentally field-tested in Benin
3. A detailed field experiment in Benin confirmed the good results (a grain increase of 67.2 percent in a plot sprayed with aviMNPV and a 106.8 percent yield increase in a plot using a combination of anaqueous formulation of neem oil and MaviMNPV).

Objective 5: Build capacity at host country institutions for the rearing and mass release of biocontrol agents that are currently ready for release.

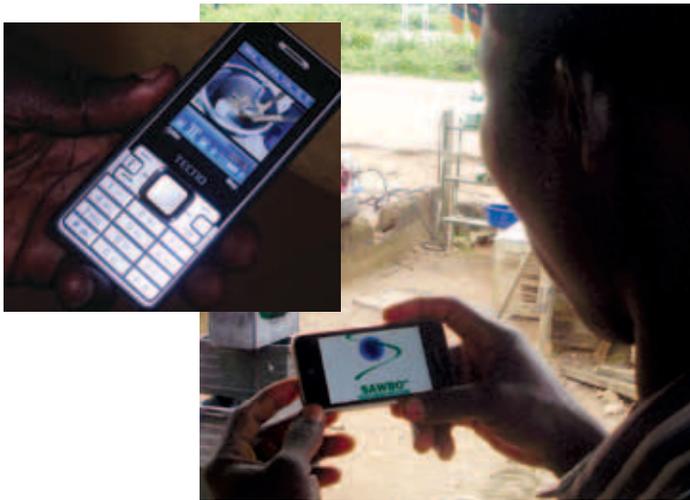
We are developing rearing and delivery systems for biological control agents (including training of staff, extension agents, and farmers where necessary) against major cowpea pest infestation that can easily be implemented by Host Country collaborators. In particular, we are:

1. Refining and validating the recently developed mass rearing technique for *M. vitrata* using germinating cowpea sprouts. The rearing methodology will be used to mass rear the parasitoid *A. taragamae* in HC laboratories for field inoculations. We will also be developing an 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.
2. Establishing nursery plots of the host plant *Tephrosia candida* in HCs for in-field mass rearing of the thrips parasitoid.

Results

The germinating cowpea sprout method validated in FY11 is currently in use for rearing *M. vitrata* at IITA in Benin and Nigeria, and in our collaborators' lab in Burkina Faso and Niger. This cheap and efficient rearing method is also routinely used to rear the *M. vitrata* parasitoids.

Female wasps took longer to develop compared to males regardless of feeding substrates. Significant differences were observed in the wasp longevity between Tawa and artificial diet, and females lived longer than males on all feeding substrates. The parasitoid sex ratio varied significantly between feeding substrates with a higher proportion of female wasps on the improved varieties TVX2336 and local variety Kpodji-guèguè. Rearing *M. vitrata* larvae on germinating cowpea grains is 4.38 times cheaper than on artificial diet.



Cowpea sprouts from Tawa are being used to produce the *MaviMNPV* virus. Results indicate that, for virus production, the soaking time of 48 h remains the best, while the infestation of the sprouts with neonate *M. vitrata* larvae is best delayed until appearance of the first green parts of the germinating seed.

Following up on the successful field deployment of the combination of neem oil and *MaviMNPV* reported in FY11, more detailed studies have assessed the nature of these interactions. Lab studies have investigated the effect of the compounds, alone or in combination, on key insect pests of cowpea, *Maruca vitrata* Fabricius, *Aphis craccivora* Koch, and *Megalurothrips sjostedti* Trybom. The numbers of insects killed were recorded each day for five or 14 days and the larval, pupal development, and adult emergence assessed. Combination of *MaviMNPV* resulted in a significantly higher larval mortality than treatment with either virus or botanical insecticide alone at the corresponding concentrations. Co-infected insects died sooner than those infected with only one control agent. Combinations between *MaviMNPV* and botanical oils produced additive or synergistic effects. No evidence of antagonistic effects was noted.

Neem oil has been reformulated as an emulsifiable mixture of neem and essential oil from lemon grass, which is planted by women's groups and sold to the self-help enterprise with the

neem seeds, providing additional household income. This mixture is being sold commercially by the Bio-Phyto communal enterprise under the brand name "Top-Bio." We are assessing the viability of the *MaviMNPV* virus kept in the Top-Bio for several months to determine if the two components can be premixed and sold in the same container. Concurrently, Top-Bio has been dispatched with the virus to our collaborators in Burkina Faso, Niger, and Nigeria for proper multilocal field testing.

Objective 6: Collections of biological control agents for sequencing and development and of IPM-omics tools.

Priority natural enemies for sequencing:

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

Dr. Manu Tamò is 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.

Results

1. *Apanteles taragamae* samples have been processed for RNA extraction and sent to UIUC for sequencing. Polymorphisms have been identified and used in molecular studies.
2. Samples of *C. femoratus* have been collected from Benin, Ghana, and Cameroon, and have been processed for RNA extraction and sent to UIUC for sequencing. Polymorphisms have been identified and used in molecular studies.

Objective 7: Bring new biocontrol agents into the pipeline for development and deployment.

In partnership with HC collaborators, we will be developing rearing and delivery systems for the following priority natural enemies:

Against *M. vitrata*

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

Against *C. tomentosicollis*

1. The parasitoid, *Gryon fulviventre*

Results

A detailed study was undertaken to optimize the mass production of *Trichogrammatoidea eldanae* Viggiani, a major egg parasitoid of *M. vitrata*, on eggs of the factitious host *Corcyra cephalonica* Stainton in the laboratory and the release system of this parasitoid in the field.

Four feeding substrates were compared for *C. cephalonica* larval rearing: whole millet grains, a mixture of 50 percent whole millet grains and 50 percent millet flour, a mixture of 50 percent whole millet grains and 50 percent broken millet grains, and flour of milled cowpea grains.

It appears that mixing whole millet grains with either broken grains or flour gives better results in terms of egg parasitism, adult wasp emergence, and, more important for trichogrammatid egg parasitoids, the proportion of female wasps.

Releases of *T. eldanae* were performed using a novel rearing-cum-release device in the laboratory, in the greenhouse, and on an experimental plot in the field. The results show that this device is suitable for rearing *C. cephalonica* under field conditions and releasing *T. eldanae* adult parasitoids. Under field conditions, sentinel eggs of both *C. cephalonica* and *M. vitrata* were discovered by the parasitoids emerging from the device and parasitized up to a distance of 50 meters from the release site.

Following the robust establishment of a quarantine colony rearing the parasitoid *Nemorilla maculosa*, a series of prerelease studies, focusing on host range were initiated.



Colony rearing of the parasitoid *Nemorilla maculosa* on *M. vitrata* larvae feeding on sprouting cowpea seeds

We are currently assessing host specificity for the following organisms: *Helicoverpa armigera*, *Spodoptera littoralis*, *Plutella xylostella*, *Sesamia calamistis*, and *Coryra cephalonica*.

Chemical ecology studies using a modified 4-arm olfactometer are being used to assess the preference of the parasitoid for odor sources of different origin. We are studying the potential of *N. maculosa* to discriminate between different hosts being offered individually or simultaneously and will be assessing the host-finding behavior of the parasitoid in the presence of kairomones derived from different host plants, such as cowpea.

An experiment has been initiated to assess multiple interactions between two soil-beneficial microorganisms alone and in combination, different watering regimes simulating drought stress at different phenological stages of the cowpea plant, the cowpea plant and the legume pod borer *M. vitrata*.



Rainout shelter experiment assessing multiple interaction between soil beneficial micro-organisms, drought stress, the cowpea plant, and the pest *M. vitrata*

Objective 8: Other Institutional Capacity Building training activities in rearing and release of biological control agents

- July 2012. Training of 48 students, including 16 females from 16 farmer's field schools, on the process of FFS establishment at CERRA Maradi.
- July/August 2012. Training of 240 students on IPM of cowpeas. Topics included pest identification, use of neem extract, and virus and chemical extract at 16 FFSs.

Networking and Linkages with Stakeholders

Dr. Pittendrigh has visited both the USAID missions in Mali and Nigeria. Dr. Tamò is continuing to work with collaborators in Ghana to ultimately request funds from a USAID mission office in regards to a biological control program of insect pests of cowpeas in Ghana (an IITA activity).

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.

In Niger, our program has partnered with NGOs for the development of joint farmer field schools. Additionally, numerous farmer organizations have been engaged to help distribute pest control technologies, including seeds of cowpeas that are from insect tolerant lines of cowpeas.

Leveraging of CRSP Resources

Dr. Pittendrigh and his co-principal investigators have successfully leveraged more than \$650,000 in external funding 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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Dannon, E., Tamò, M., Huis, A., Dicke, M. (2012). Effect of *Maruca vitrata* (Lepidoptera: Crambidae) host plants on life-history parameters of the parasitoid *Apanteles taragamae* (Hymenoptera: Braconidae). *Insect Science* 19: 518-528.

Ganyo, K. K., Tounou, A.K. Agboton, C. Dannon, E. A. Pittendrigh, B. R. Tamò M., (2012). Interaction between the aphid parasitoid *Lysiphlebus testaceipes* (Hymenoptera: Aphidiidae) and its hyperparasitoid *Syrphophagus africanus* (Hymenoptera: Encyrtidae). *International Journal of Tropical Insect Science* 32:45-55.

Onstad, D.W., J. Kang, N. M. Ba, M. Tamò, L. Jackai, C. Dabire and B. R. Pittendrigh. (2012). Modeling evolution of resistance by *Maruca vitrata* (Lepidoptera: Crambidae) to transgenic insecticidal cowpea in Africa. *Environmental Entomology*. 41(5): 1045-1276.

Contribution to Gender Equity Goal

We have made every effort to maintain gender equity in graduate student, farmer, and technician training; we have also made significant efforts to bring female professionals onto the project. We addressed the access women have to materials for neem and viral sprays. For host plant resistance seed lines, we have made sure seeds are given to women's organizations to ensure gender equity.

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

UPR-1

Principle Investigators

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Consuelo Estevez, University of Puerto Rico, Puerto Rico

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António Chicapa Dovala, IIA, Angola



Abstract of Research Achievements and Impacts

Significant progress was made during the past year toward research and training objectives. The small red, disease resistance bean cultivars were released in Costa Rica and Honduras. The small red cultivars were released in El Salvador, using PPB approaches. Web blight and common bacterial blight resistant germplasm lines PR0401-259 and PR0650-31 were released; and multiple-stress-tolerant black bean germplasm TARS-MST1 and SB-DT1 were released.

Populations were developed from crosses between commercial seed types used in Angola and sources of resistance to BCMV, CBB, and ALS resistance. 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. Black and white bean breeding lines that combine resistance to bruchids, BGYMV, BCMV, and BCMNV have been developed. White bean lines have been selected that combine resistance to BGYMV, BCMV, BCMNV, and all races of rust when tested at Beltsville, MD. Greenhouse trials conducted in Honduras identified lines with higher nodulation scores and greater root and shoot dry weights under low N conditions. Inbred-backcross populations were developed to study the expression of nodulation and N₂ fixation traits. Selected lines were evaluated in the field using inoculation and N fertilizer treatments. The most promising F₄ lines derived from crosses between diverse parents having good nodulation selected in FY11 were recombined to form the second cycle of recurrent selection for enhanced biological nitrogen fixation. During FY12, populations were developed and advanced to the F₄ generation. Lines from these populations will be screened for higher nodulation in 2013. Significant *Rhizobium* strain x bean line interaction for seed yield was observed in field trials planted at Isabela, Puerto Rico. Lines were identified that nodulated and yielded well when inoculated with either strain CIAT899 or UMR1597. USDA and UPR scientists collaborated in the identification of the dominant gene, *Xap-1*, which confers resistance to CBB. Preliminary results suggest that an additional recessive gene is needed to achieve high levels of CBB resistance. The response of common bean to ashy stem blight was evaluated in the field and greenhouse. An inoculation technique using Band-Aids proved to be successful in the identification of Andean bean lines with greater levels of resistance to ashy stem blight. A CAPS marker using ENM-FWe/RVe primers and the *RsaI* restriction enzyme 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 from Haiti, the Dominican Republic, and Puerto Rico 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 field trials planted at Isabela, Puerto Rico, and Mazozo, Angola.

Genotypic evaluation using SNP markers was also completed. Elemental composition of seed from the Puerto Rico trials was measured 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 were tested in Honduras, the United States, Angola, and Puerto Rico, and two lines with superior performance are being considered for release as improved germplasm.

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 Laura Lara (Zamorano student) at the USDA-ARS-TARS. Workshops describing research techniques dealing with biological nitrogen fixation and *Rhizobium* inoculant production were conducted in Honduras in November 2011 and in March 2012, and in Mozambique in April 2012.

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 common 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 15 years, however, only a limited number of black bean cultivars have been released in the region. This limited release of germplasm is the result of 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. As a consequence, 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. During the past five years, the Pulse CRSP project has developed a sizeable number of black bean breeding lines that have been distributed to bean research network members in Nicaragua, Guatemala, and Haiti. The bean research network supported by the Pulse CRSP is a key element in the success of the cultivar development program in Central America. Although this Dry Grain Pulse CRSP project emphasizes the development and 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 breeding lines that have resistance to BGYM, BCMNV, and other diseases of economic importance.

The Pulse CRSP breeding programs are in the position to continue 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 in an advanced stage of development.

Improved bean breeding lines developed by the Pulse CRSP in Central America and the Caribbean have proven useful in Angola. Some small red and black bean cultivars and breeding lines developed in Central America and the Caribbean have resistance to diseases and tolerance to abiotic stresses that are important constraints to bean production in Africa. Central American bean breeding lines with resistance to BCMNV, common bacterial blight, and web blight may be of particular value to northeastern Angola or Tanzania where small red beans are produced. The lowland bean breeding team has also developed Andean (red mottled, yellow, and light red kidney) bean breeding lines with resistance to BCMNV and rust that may be useful in Eastern Africa.



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 disease resistance with enhanced resistance to pests and greater tolerance to abiotic stress. 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 each generation in environments that were 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 Central America and the Caribbean.

Basic seed stocks of bean varieties developed and released by the project were multiplied and small lots of seed distributed to farmers in Central America and the Caribbean for testing. Performance 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.

During the past five years, the project initiated collaborative research with Mr. Antonio Chicapa Dovala, head of the Legume Program of the Instituto de Investigação Agronómica in Angola. 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. During the past few years, breeding lines derived from crosses between local landraces and sources of disease resistance have been field tested in Angola.

Development of breeding populations

Small red, black, white, and Andean bean breeding populations were developed and evaluated during the past year. The overall goal is to incorporate disease resistance and abiotic stress tolerance into black, small red, white, red mottled, and yellow bean breeding lines. Parents used in the crosses include 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 improved 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 and F_3 to F_5 families were evaluated and selected for highly heritable traits. One group of crosses identified as the *Seda Connection* was made by crossing the landrace Seda with elite high yielding, disease-resistant cultivars and breeding lines. The cultivar Seda is an early maturity, well adapted Honduran small red landrace with tolerance to drought and low fertility; Seda's performance has been outstanding. This landrace nodulates well and possesses root traits associated to tolerance to drought and low fertility. Populations derived from crosses including local landrace cultivars were developed using participatory plant breeding approaches in collaboration with farmer groups and researchers from El Salvador, Honduras, and Nicaragua.

The performance of breeding lines derived from crosses between landrace and improved cultivars to improve the most common Honduran small red bean landraces and the widely grown Salvadoran landrace Rojo de Seda was validated in farmer fields in several bean production areas in Honduras. At least one cultivar will be ready for release in Honduras in 2013, one in Nicaragua.

During the current period of funding, the project has made a major effort to develop superior black bean cultivars for Guatemala and Haiti. This effort has led to the release of several black bean cultivars in both countries. Promising black bean cultivars are being tested in field trials in Nicaragua and El Salvador. Promising breeding lines derived from crosses were evaluated in farmer fields in Costa Rica, resulting in the programmed release of a black bean cultivar in the North Huetar region.

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. BC1S4 families were selected for superior agronomic performance, desirable seed traits, and SCAR markers for BGYMV and BCMV resistance. These selected BC1S4 lines will be screened for resistance to BCMNV at the University of Puerto Rico during the upcoming year. The black bean lines under development should 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 were largely susceptible to ALS—important diseases. Several of the Angolan lines showed good BNF potential, while most were not well adapted to high temperatures in Puerto Rico. Based on testing of improved varieties and breeding lines in Angola over the past five years, lines were selected that are adapted to Angola and have resistance to BCMV, BCMNV, CBB, and ALS. Populations were developed from crosses between the commercial seed types used in Angola and sources of disease resistance. Marker assisted selection (MAS) and field and greenhouse evaluations were used to select breeding lines with CBB and BCMV resistance. Evaluation of more than 400 F₃ lines, 130 F_{4,5} lines, and 46 F_{6,7} lines in Huambo, Angola, was begun this last year.

The yellow bean is a preferred seed type in Haiti and Angola. Azufrado bean breeding lines from Mexico (yellow bean) were obtained and crossed with red mottled bean breeding lines that have multiple disease resistance. MAS and greenhouse evaluations were conducted in Puerto Rico to identify lines that have genes for BGYMV and BCMV resistance. Lines were also selected for common bacterial blight resistance. The performance of a group of the yellow bean breeding lines with BGYMV and BCMV resistance was tested in Haiti, Puerto Rico, and Angola.

The common bean weevil is a major seed storage pest. The University of Puerto Rico received black and light red kidney breeding lines resistant to the bean weevil. A bioassay was developed to screen the BC₃F_{4,5} bean lines for resistance to the

bean weevil. Seed of both Andean and Middle American bean cultivars were severely damaged by the bean weevil. Three light red kidney lines from the OSU populations had useful levels of resistance. The date of first emergence of adults of the resistant lines was approximately three weeks later than the susceptible checks. Most of the seed of the resistant lines was undamaged at 90 days after infestation. Seed of resistant lines had greater than or 65 percent seed without holes.

The common bean weevil resistant red kidney line AO-1012-29-3 was crossed with black and white bean breeding lines having BGYMV, BCMNV, and BCMV resistance. During the past year, individual plants with commercially acceptable seed types and superior agronomic traits were selected in F₃ and F₄ nurseries planted at Isabela, Puerto Rico. Seed of each F₅ line was infested with *Acanthoscelides obtectus* Say in laboratory trials. Lines that expressed resistance to the bruchid were re-infested. Residual seed of the F₅ lines was used to screen the lines in a greenhouse at the UPR Campus for reaction to BCMNV. DNA was extracted from the plants in the greenhouse and screened using markers for resistance to BGYMV and BCMV. Six black, one white, and one dark red line were identified that combine bruchid and virus resistance. Seed of these lines were increased in 2012 for preliminary performance trials in 2013. During the past year, AO-1012-29-3 was also crossed with red mottled bean breeding lines having BGYMV, BCMNV, and BCMV resistance.

Regional performance trials

Advanced bean breeding lines were derived from crosses between disease resistant and abiotic stress tolerant parental lines, that have commercially acceptable seed types. More than 70 small red and black bean breeding lines were distributed to collaborators from the NBP from Central America and the Caribbean. More than 200 on-farm cultivar validation trials were conducted in Central America and the Caribbean. Results identified several promising breeding lines for additional testing.

The project is developing small red and black breeding lines that recombine resistance to BGYMV and BCMV, tolerance to drought, and low fertility with resistance to ALS. During the past year, a regional trial including ALS resistant lines was evaluated in Costa Rica and Honduras. In this trial, some small black and red lines show foliar and pod resistance to ALS similar to the best resistant check, the Andean accession G05686.

The UPR developed red mottled bean lines that combine resistance to BGYMV, BCMNV, BCMV, and CBB. Seed of the most promising lines was increased in Puerto Rico during the past year. The red mottled lines PR0633-10 and PR0737-1 were among the highest yielding lines in the trials. And have the *bgm-1* gene for resistance to BGYMV and the *bc3* gene for BCMV and BCMNV resistance. PR0633-10 also has the SW12 QTL associated with resistance to BGYMV and the SAP6 QTL associated with common bacterial blight resistance. PR0633-10 and PR0737-1 produced mean seed yields as great as or greater than check varieties PC-50 and JB-178 in trials. PR0633-10 and PR0737-1 are candidates for release as cultivars in Haiti and should serve as useful sources of resistance to BGYMV, BCMV, BCMNV and common bacterial blight.

Elite black bean breeding lines from the UPR were evaluated in Haiti and the Dominican Republic during the past year. Two of the lines have been selected for adaptation to low N soils. Five white bean breeding lines have resistance to BGYMV, BCMV, BCMNV, and rust.

Lines developed for Central America and the Caribbean were evaluated in Angola during the main production seasons from 2008 to 2012. Results helped identify economically important diseases in Angola's bean production regions.



On-farm validation of promising breeding lines

The PASEBAF trial, a Red SICTA activity including CIAT and Zamorano lines tolerant to drought and low fertility, was funded by IICA/COSUDE from 2007 to 2010. These lines were tested in Central America over the past two years with support from the Pulse CRSP. The small red cultivars *Campechano JR* and *Don Kike* released in Honduras in 2012 were entries included in the initial PASEBAF trial.

There is increased interest in the lowlands of Guatemala in the production of small red beans for export to El Salvador and the United States. Cultivars with higher yield potential and greater disease resistance are needed for this purpose. Ten of the most promising small red bean cultivars and breeding lines from Central America were sent in 2009 to a Pulse CRSP collaborator for testing in the most important lowland bean production regions. During the past year, packages of seed 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.

Release of cultivars and seed multiplication

In February 2010, the Instituto Dominicano de Investigaciones Agropecuarias y Forestales (IDIAF) formally released DPC-40. During the summers of 2011 and 2012, the National Seed Service

in Haiti produced 10 MT of DPC-40, which was distributed in Haiti in collaboration with the Feed the Future supported Bean Technology Dissemination project. During the past year, more than 200 kg of basic seed stocks of BGYMV, BCMNV, and BCMV resistant black bean lines XRAV40-4 and MEN2201-64 ML were produced in Honduras and shipped to Haiti for further multiplication.

The white bean breeding line PR0634-13 was released by the UPR as *Beniquez*. This cultivar has resistance to BCMV, BCMNV, and BGYMV and will provide protection against the possible emergence of BCMNV in Puerto Rico.

During FY12 the disease resistant small red bean cultivar *Tayni* was released in Costa Rica. The small red cultivars *Campechano JR*, *Don Kike*, and *Chepe* were released in Honduras, and cultivars *San Antonio FPI*, *La Presa JF*, and *Guazapa 1* in El Salvador.

The small red cultivar *Paisano PF* was released in 2011 in Honduras. Seed production and dissemination of the cultivars released in 2011–12 were conducted in FY12 in collaboration with farmer CIALs and NGO technical personnel.

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

Inadequate soil nitrogen is a frequent yield constraint for common beans in the Tropics. The use of nitrogen fertilizers increases production costs and, in some intensive bean production systems, can contribute to groundwater contamination. Researchers have pointed out the need to develop integrated soil nutrient management practices for beans that 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. 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 to take advantage of nitrogen and other nutrients.

Recurrent selection has proven 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 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. The root rot resistant black bean line PR0443-151 from Puerto Rico and the CIAT bean breeding line VAX 3 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 serve as a source of root rot

resistance. Breeding lines were evaluated in the F_3 and F_4 generations in replicated field trials that received low levels (20 kg/ha) of N fertilizer. The bean lines were inoculated with recommended bean *Rhizobium* strains to create conditions favorable for biological nitrogen fixation. The breeding lines were evaluated for root rot resistance. The most promising lines from each cycle 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. The plants were grown in a soil:sand substrate low in organic matter and N. Significant variation among bean lines for nodulation using a one to nine scale (one=none or very few, small nodules; nine=maximum number of large nodules), root, shoot and total dry weight (DW), and root/shoot ratio was observed. The cultivars and lines with higher nodulation scores also had greater root, shoot, and total DW.

Twenty-five accessions with the higher nodulation and total plant DW from the first trial were inoculated with a mixture of *Rhizobium* strains and grown in a soil:sand (1:2) substrate low in organic matter and N. 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. Significant differences were observed among bean lines for all variables. The lines with higher nodulation had almost twice nodule DW and 50 percent greater plant DW, than those with less nodulation.

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 CIAT 899 and CIAT 632 and to identify potential parents for a recurrent selection program for high nodulation and N_2 fixation. Since the common bean is also nodulated by strains of *R. leguminosarum*, additional experiments are being conducted including the strain UPR 2010 of *R. leguminosarum*.

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. Complementary controlled studies will include an evaluation of differences in nodulation speed and nodule occupancy using mutant strains. These IB lines have Amadeus 77 genetic background. The similarity of the genetic background of the IB lines will facilitate the study of nodulation expression and N_2 fixation traits, and their contribution to plant growth and seed yield as well as the response to inoculation under highly variable environmental conditions.

Populations from recurrent selection for increasing BNF in common beans were developed from crosses between 24 lines selected from the screening of F_4 families from 25 bean populations from Cycle 1 in Honduras under greenhouse conditions using a soil:sand (1:1) substrate low in total N and organic matter. The F_4 lines were inoculated with a mixture of three *Rhizobium* strains at four and eight DAP. Plant samples were taken at 40 DAP (flowering stage); nodulation and shoot dry wt. were determined. Nodulation (number and size) was scored visually using a one to nine scale as defined previously. Cycle 2 populations were advanced to the F_3 generation in FY12. During 2013, these populations will be advanced to the F_4 generations and at least 20 families per population will be tested for higher nodulation under greenhouse conditions.

During the 2012 summer season, improved small red and black lines from the VIDAC, ECAR, and ERSAT regional trials were tested under inoculation with a mixture of the three *Rhizobium* strains mentioned previously. The lines were grown without soil added fertilizer and under drought stress conditions. Nodulation and shoot dry weight were recorded at flowering and seed yield at maturity. In general, nodulation, plant growth, and seed yield were lower due to the low fertility and drought conditions; however, some small red and black breeding lines and the landrace cultivar Seda performed well under the limited conditions of these trials.

A set of 32 (17 Mesoamerican and 15 Andean) bean genotypes are being tested with single and mixtures of inoculants to develop a set of differential genotypes for evaluating the response of *Rhizobium* strains and bean germplasm in diverse soil and climatic conditions.

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 the *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 Fortuna Substation, there were significant differences among lines and strains in the nodule position at twenty days after planting. Soil rhizobia plant infection counts were considered high at 10^4 rhizobia per gram of soil. Among the genotypes evaluated, PR9745-232 (Andean) nodulated with CIAT 899 in the upper one cm of the tap root and had greater number of nodules when compared to strain UMR 1597. In contrast, lines PR0401-259 and 10IS-2421 had greater nodule number 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 and 20 nodules that compared to the best performing cultivars. Root and shoot dry weights were not different between strains and lines. DPC-40 had greater shoot dry weight when inoculated with strain CIAT 899. Pinto line 10IS-2417 produced greater shoot dry weight with UMR 1597. PR0401-259, PR0137-1, PR0137-2, 10IS-2435, *Verano*, and DPC-40 had the best plant vigor scores in the trial. The highest yielding lines were *Beniquez*, DPC-40, *Verano*, and PR0401-259. The site at the Isabela Substation was previously planted with common beans and the soil rhizobia population was 102 viable cells per gram of soil. Nodulation was evaluated at 21 days after planting using the CIAT scale one to nine. The pinto line 10IS-2417 formed nodules in the crown with both strains CIAT 899 and UMR 1597. The most number of nodules were produced in lines PR0401-259 and 10IS-2423. Seed yields were excellent. The small red line 10IS-2423 produced a mean yield > 2,000 kg/ha. The red mottled line PR0737-1 had the best overall performance for an Andean bean line. With the exception of DPC-40 and *Badillo* (opposite response), most of the lines produced similar seed yields when inoculated with strains CIAT 899 and UMR 1597. The pink bean line PR0401-259 nodulated well with CIAT 899 and UMR 1597.



Competition with the established rhizobial population is a factor that can limit the success of an inoculant. At the Fortuna Substation, the rhizobial counts were considered high and strain

x bean line interaction was not significant for seed yield. The Isabela Substation had a lower native rhizobium population and better response to inoculation. In subsequent experiments the effectiveness of inoculants produced with single strains and/or a combination of different *Rhizobium tropici* and *Rhizobium etli* strains was evaluated. Inoculants with a single or a combination of *Rhizobium* strains increased nodulation. Strain 1597 alone had a positive effect in dry matter and yield. Strains CIAT 899 in combination with all the tested strains had a synergistic effect on nodulation. The noninoculated control had the lowest number of nodules. The use of both combinations of *Rhizobium* strains resulted in the highest grain yields along with the NPK treatment. The noninoculated control had the lowest grain yield. In the trial in Damien, Haiti, the response of inoculation using the same strains and combinations resulted in no differences in nodulation and seed yield among the NPK, Nitrogen.

Rhizobial strains suitable as inoculants should be highly competitive, fast in nodulation, tolerant to environmental stresses, and capable of forming nodules that fix nitrogen. In a field experiment at the Fortuna Substation, five strains were compared to reference strain CIAT 899, an un-inoculated control, and a commercial inoculant (Nodulator). Three additional treatments included fertilization with Nitrogen (N); Nitrogen Phosphorus and Potassium (NPK); and Potassium (K). Although there were no significant differences among treatments for seed yield, the treatments that produced the highest yields were inoculated with *Rhizobium* strains UMR 1512, CIAT 899, and UMR 1597.

As result of the advances in the identification of superior *Rhizobium* strains and implementation of the inoculant production technology, extensive testing and dissemination activities are being conducted in Honduras, Guatemala, Nicaragua, and Haiti. More than 2,000 farmers received small bags to inoculate their bean fields with a mixture of the best *Rhizobium* strains in Central America.

Objective 3: Develop 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. New recombinant inbred line populations are being developed from crosses between BAT 477 and susceptible bean lines to identify new molecular markers for the charcoal rot resistance genes using bulk segregant analysis (BSA).

Marker-assisted selection is currently used by only a few programs in Latin America and the Caribbean. The molecular marker lab at Zamorano will assist other bean research programs in Central America in the use of this technology by providing informal training and assistance in screening elite bean breeding lines and in applying any new molecular markers developed by this project.

The RAPD markers previously reported to be linked to genes for charcoal rot were screened with a set of seven susceptible genotypes and eight resistant genotypes. Consultation with another group working with *Macrophomina phaseolina* in common bean confirmed that B386₉₀₀ and B459₁₆₀₀ do not have utility for charcoal rot.

Because the putative RAPD markers were proven ineffective, recombinant inbred lines (RILs) from crosses between BAT 477 and susceptible bean lines were pursued for the development of novel markers. These lines were planted over a three-year period in Isabela, Puerto Rico, in a replicated field trial inoculated with the pathogen. The disease reactions of the RILs will be used to initiate the search for molecular markers for resistance to ashy stem blight.

A detached leaf technique for *Macrophomina phaseolina* evaluation has been implemented for screening the BAT 477 x DOR 364 RIL population. In addition, the Andean Diversity Panel (ADP) has been screened using a novel Band-Aid inoculation method and markers will be evaluated based on the SNP genotyping of that population of ~350 lines. Once SNPs have been identified, indel markers can be screened for the development of breeder-friendly markers.

USDA and UPR scientists collaborated in the identification of the dominant gene, *Xap-1*, which confers resistance to common bacterial blight of beans. This represents 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 and found to be associated with the *bc3* gene in known genotypes of common bean through a CAPs assay. Results suggest that this marker has potential for use in marker-assisted selection

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 was 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.

Tepary bean, 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 outside 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, initiated breeding of tepary for increased seed size and improved architecture has begun.

Seventeen lima bean accessions from the UPR collection were screened for adaptation in Honduras. When planted in Honduras in June, four landraces flowered less than 60 days after planting, suggesting that these varieties could be planted in the Central America and the Caribbean throughout the year. The 12 most promising accessions continue to be evaluated.

The diversity of Angolan cowpea germplasm was evaluated through phenotypic characterization in field trials in Puerto Rico in 2010 and in Angola in 2012. Lines were evaluated for general adaptation, phenology, growth habit, yield components, seed characteristics, and elemental composition of the seed. Angolan bean landrace varieties were identified that produced greater than 1,000 kg/ha in Puerto Rico during both growing seasons. Cowpeas in Angola currently produce an average yield from 200 to 300 kg/ha. Cowpea leaves, which are commonly consumed in Angola had greater percent protein than cowpea seeds. Angolan germplasm lines with higher levels of Fe and Zn in the seed were also identified.

Nineteen cowpea lines from the 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 was distributed for testing during the postrera season of 2011 to several organizations from Honduras and to INTA in Nicaragua. During 2012, ERCAUPI trials have been provided to collaborators from Honduras, Nicaragua, and El Salvador. Seven red seeded cowpea accessions from the USDA PI collection were received from UPR for testing in Honduras to determine if these red seeded type cowpea lines would have better consumer preference in the Central American region where people mostly consume small red bean. During 2012, seed of the best red seeded cowpeas accessions were increased for distribution to collaborators from Honduras, Nicaragua, and El Salvador.

Superior lines of tepary beans were evaluated in trials in Puerto Rico and Nebraska in 2007 and 2008, and adapted, large seeded lines were selected for population development. Lines were developed from crosses between this elite germplasm and previously identified line with superior agronomic characteristics. Lines from the resulting populations were selected with increased seed size and seed quality, improved architecture characteristics, bacterial blight resistance, drought and heat tolerance, bruchid resistance, and yield. Several of these lines are being considered for release and have been distributed to programs in Burkina Faso, Angola, Rwanda, Mozambique, Honduras, Haiti, and the United States. Future efforts will include efforts to transfer BCMV and BGYMV resistance from common bean to tepary bean.

Networking and Linkages with Stakeholders

The UPR bean breeding program collaborates with Dr. Graciela Godoy-Lutz, Instituto Dominicano de Investigaciones Agropecuarias y Forestales plant pathologist, in a locally-funded project entitled *Evaluación, multiplicación y adopción de líneas avanzadas de habichuela con resistencia a limitantes bióticas desarrolladas en el proyecto Bean/Cowpea CRSP* that was approved by the Consejo Nacional de Investigaciones Agropecuarios y Forestales. The project provides an opportunity to continue to test the most promising lines from the Pulse CRSP breeding programs in the Dominican Republic. This collaboration has resulted in the development and release of disease resistant black and red mottled bean lines, such as PR0737-1 and PR0633-10, of potential benefit to farmers in Haiti and the Dominican Republic.

The UPR Pulse CRSP breeding program collaborated with the MSU bean breeding program in the evaluation of a RIL population derived from the cross *Zorro x Puebla 152*. The lines were planted in a low N field at the Isabela Substation and were inoculated with a mixture of *R. tropici* strain CIAT 899 and *R. elli* strain UMR 1597 provided by Dr. Consuelo Estevez. Differences among lines were observed for nodulation, vigor, leaf SPAD scores, and seed yield. The small red check line 10IS-2423 yielded as well as the most productive RILs in the trial (18). This line was among the highest yielding lines in other trials in Puerto Rico conducted in low N soils.



Leveraged Funds

Scientists involved in this project have effectively used Dry Grain Pulses CRSP support and institutional linkages to leverage more than \$3.7 million in external funding in 2012 to achieve objectives related to this project.

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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

Field demonstration trials were conducted on research station sites across a range of agroecological zones to test the effectiveness of locally and U.S. produced inoculant for common beans. In Uganda, trials conducted at NaCRRRI research stations yielded less than the equivalent of 1,000 kg/ha—far less than yield potential of the varieties tested. Averaged across varieties, there was no consistent response to inoculant or phosphorous fertilizer. In Tanzania, trials indicate large G x E interaction for most yield and BNF traits; response to inoculants and phosphorous fertility was highly variable. In Rwanda, trials showed a large yield advantage for climbing beans and confirmed inoculation alone or in combination with inorganic P did not always provide a consistent yield increase. Inoculant from Becker Underwood, however, increased seed yield about 10 percent.

Extensive phenotypic information was collected on 100 RIL (Recombinant Inbred Lines) derived from parental lines with unique rooting characteristics with potential for improving BNF. Data are being used for QTL analysis with a pre-existing linkage map of SSR and SNP markers. A panel of 284 Andean bean genotypes was SNP genotyped with 533 SNP markers. These diversity data show clustering of many lines collected from Africa, which is useful in identifying the best Andean-derived parental lines for improving BNF. Phenotyping studies were conducted to determine if consistent relationships existed between ureide concentration in individual plant tissues and total plant N or biomass accumulation. Ureide concentrations measured postflowering varied dramatically among lines and plant tissues. Pods accumulated the highest concentrations of ureides, followed by stems, roots, petioles, and leaves. Concentrations across tissues, however, were not consistent within an individual line. Analyses are in progress to relate these results to genetic variation in total N accumulation.

Training materials were developed to include benefits of the Nitrogen fixing bacteria in the soil, safety precautions to be observed during storage of inoculum, and procedures to be followed when inoculating seeds. Extensionists and farmer trainers were trained on the benefits of BNF and inoculant application techniques. Eighteen demonstration gardens were established with farmers groups in Uganda. On average, farmers harvested more beans from inoculated and fertilized plots. In most cases, yields were greater with a combination of FYM + BNF than with either treatment alone, which indicates improved soil fertility management can help farmers achieve better bean yields in the lowland areas of Kamuli District. A Knowledge, Attitudes, and Practices survey revealed highly positive attitudes among smallholder farmers about the technology, but they had little knowledge of benefits and uses. Three graduate students completed their thesis research. All undergraduate internships were completed.

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 about 38 percent of utilizable protein and 12–16 percent of daily caloric requirements. Improved bean production in Uganda, Rwanda, and Tanzania offers a unique opportunity to address the deteriorating food security situation there and in sub-Saharan Africa.

Loss of soil fertility is recognized as the most important constraint to food security in sub-Saharan Africa. Low levels of nitrogen and phosphorous are the primary fertility constraints. Because soils are becoming increasingly degraded, an affordable means of improving soil fertility and productivity of nitrogen-accumulating crops is critical. Properly nodulated legumes can leave up to 350 kg nitrogen per hectare in the soil, depending on effectiveness of the nitrogen fixation process, type of legume, length of time the legume is grown, soil nutrient levels, and nitrogen already available. Because inoculum is much cheaper than inorganic fertilizer, use of inoculants can provide an affordable and sustainable way to improve production of nitrogen fixing legumes.

Numerous studies have shown the potential of improving legume productivity by enhancing nodulation through proper use of a biological inoculant. Yet field trials in sub-Saharan Africa have provided mixed results. Likely causes for variable response include poor quality control of inoculant formulation, failure to compete with local rhizobia, inhibition by indigenous microbial flora, or failure of the inoculant species to survive in low pH and/or droughty soils. 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. These stacked inoculants have been shown to decrease chemical fertilizer use in crop rotations, increase legume yields, suppress root diseases, and improve rhizosphere conditions for root growth.

To optimize BNF, it is essential to identify germplasm with the greatest capacity for this trait. Although common bean has the potential for BNF, it is reported to have the lowest percent N₂ 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. Superior BNF lines have been used as parents in crosses to generate populations for genetic studies and to examine selection and breeding for improved BNF. Few breeding lines with improved BNF, however, have been developed. The optimal selection environment for BNF is under low soil N since application of nitrogen fertilizer reduces N fixation capacity. 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 smallholder farmers in sub-Saharan Africa whose health and well-being depend heavily on legume production.



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 biologically stacked inoculants on local and improved germplasm.
 - b. Quantify genotype by environment 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. 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. Conduct on-farm demonstrations comparing inoculant strategies.

- c. Strengthen farmers' collective capabilities to purchase inoculants and incorporate them into a profitable and sustainable system for smallholder farmers.

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 biologically stacked inoculants on local and improved germplasm.
 1. Test common bean varieties along with nonnodulating controls and high/low-N treatments at all HC trial locations (NaCCRI, ISAR, SUA).
 2. Quantify yield advantage of inoculation for second cropping season (NaCCRI, SUA, ISAR).
- 1b. Quantify genotype by environment interactions and constraints to enhancing BNF of inoculated plants.
 1. Complete analysis of plant/soil/weather data (ISU, Makerere, NaCCRI, SUA, ISAR).
 - a. Initiate modeling studies of seasonal soil moisture profiles and bean yield.
 2. Confirm phenotype and yield response to inoculant x genotype x environment (ISU, Makerere, MSU, WSU, NaCCRI, SUA, ISAR).
 - a. Incorporate new inoculants from Becker Underwood and local companies in field trials.
 - b. Quantify plant N, biomass, nodule classes, ureide levels prior to pod fill.
 - c. Quantify yield, yield components, NUE, NHI.
 - d. Quantify nodule classes and occupancy.
3. Confirm indigenous rhizobia levels and relate to local environmental conditions (ISU, WSU, MSU, Makerere).
4. Confirm soil rhizobia soil populations and strain diversity at field sites.
 - a. Collect nodules, soil samples at field sites, store for analysis.
5. Initiate root/nodulation study in greenhouse on selected lines (ISU, MSU, WSU).
6. Complete initial studies on strain x host interactions for BNF (WSU).
7. Identify most effective genotype-inoculant combinations for each eco-zone tested in HC (Makerere, NaCCRI, SUA, ISAR).
8. Calculate economic return for inoculation treatments for season 1 and 2 field trials (NaCCRI, SUA, ISAR).

Uganda

The project had four demonstration sites established in Uganda on which objective one was to be conducted. The sites included

- the Namulonge site at National Crops Resources Research Institute (NaCRRI) in Wakiso district (low altitude area)
- the Mbarara stock farm site at Mbarara Zonal Agricultural Research Development Institute (MbaZARDI) in Mbarara district (medium altitude zone)
- the Kabale site at Kachwekano Zonal Agricultural Research Development Institute (KaZARDI) in Kabale district (high altitude zone)
- the Nakabango site in Jinja district along the Jinja–Kamuli road (low altitude zone) and was established a season later

There were three common bean varieties considered and these had a high market preference, one, Kanyebwa, a landrace. The site in high altitude was selected for climbing bean and three other varieties; all these sites were planted and treated identically.

Land preparation, demonstration layout and treatments. The 200m x 40m field was mechanized to the fine seed bed. The block was demarcated and subdivided into smaller plots for NaCRRI and Nakabango and for MbaZARDI and KaZARDI on station. There were three major bio-inoculants: the Mak-Bio-fixer (RM) from Makerere University, Bio-N-fix (RN) from Nairobi University, and BioStacked® (RU) from the Becker Underwood United States of America. Phosphorus was applied to all treatment at two rates 0 kg P ha⁻¹ and 40 kg P ha⁻¹.

Data collection. Soil samples were collected at the beginning of the season to evaluate fields with low nutrients, especially nitrogen. The type of data collection used was a destructive method at both flowering and harvesting stages of growth within a season. Data collected flowering included leaf area index, nodule numbers per plant, plant biomass, plant height, and, at harvest: number of pods per plant, number of seeds per pod, 100 seed weight, and total yield.

Results

Low altitude zone. Namulonge site

In general, seed yields on the small research plots at the NaCRRI station were less than the equivalent of 1,000 Kg/ha—far less than yield potential of the varieties tested. Averaged across varieties, there was no consistent response to inoculant or phosphorous fertilizer. The combination of Nairobi University inoculant and Phosphorus application provided the greatest positive response, but the yield of unfertilized plots was quite low and the increase relative to the noninoculated plots was not significant.

There was no significant ($P \leq 0.05$) or consistent impact of inoculum used or Phosphorus fertilizer application on 100 seed weight of the three varieties. Seed size was fairly consistent across treatments except for K131, which responded positively to fertilizer P with Nairobi University inoculant and negatively to phosphorus application with Makerere University inoculant. There was no apparent advantage of the Biostacked inoculant on this yield parameter.

The application of inoculant had a significant impact on the stability of seed weight, which varied about 30 percent between years in the noninoculated controls. The seed weight harvested

from plots treated with Biostacked and Nairobi University inoculants varied less than 10 percent across years.

All varieties produced/supported a small number of nodules that ranged from about 7 to 10 per plant. The same varieties grown in the greenhouse typically support 50 to 100 nodules). Application of inoculant had very little effect on nodule numbers measured on these field grown plants.

Medium altitude zone: Mbarara site

Seed yields at the Mbarara site were consistently greater than those at the lowland site. The local variety, Kanyebwa, generally achieved the highest yields, reaching nearly 2.8 ton/ha in response to inoculant and P fertilizer. Differences in the seed yield among varieties and inoculants and P levels were significant in many cases. Most notable were the K132 response to inoculant, and Kanyebwa response to P application. The positive response to inoculant and P inputs at the higher yield levels is consistent with results of inoculation studies in the N2Africa program.

High altitude zone: Kabale site

Climbing bean varieties were evaluated at the high altitude site in SW Uganda. In general, these varieties achieved the greatest seed yields of all three locations tested. Yield response to P fertilizer was positive in most but not all cases. The response of NABE12c was particularly variable. Likewise, the response to inoculant was inconsistent, with no particular trend evident across varieties or P levels.

Nodulation score for NABE10c responded positively to P fertilizer, except when the Makerere University inoculant was used. Otherwise, there did not appear to be a benefit of inoculation on nodulation among climbing bean varieties tested. Averaged across varieties tested, there was a significant ($P \leq 0.05$) impact of Biostacked and Makerere Univ. inoculum on 100 seed weight. Although there was no effect of Nairobi-sourced inoculum on seed weight, there were significant increase in 100 seed weight in response to 40 kg P ha⁻¹ for noninoculated and Nairobi University inoculated plots.

Variation across ecological zones for yield and yield components reflects both the genetic potential of varieties examined and the local weather conditions during the two growing seasons. In 2011 for examples, Namulonge had the highest rainfall amounts in May while for Mbarara and Kabale, it was October and September, respectively. For Mbarara and Kabale, the onset of the rains for the first season began in January as opposed to February in Namulonge. The dry spell for all the districts was generally June to August, although Namulonge experienced a substantial amount of rain in August. While rainfall in March and August are generally beneficial for bean yield, excessive rainfall and high temperatures during vegetative growth are most often detrimental. We have collected available weather data at all test sites and are using these data to assess yield limiting conditions and potential impacts on plant response to inoculants.

Tanzania

Materials and Methods

Experiment 1. Field trials were established at Selian in Arusha

(mid altitude 1000–2000 masl) and SUA in Morogoro (low altitude 450–600 masl) . In both locations soil samples were collected and analyzed. The treatments included P application (P+ and P-) and Rhizobia application . Five bean genotypes were used. Phosphorous application was the main plot, rhizobia inoculation as subplots, and genotypes as sub-sub plots.

Experiment 2. Field trials were established at three sites: Selian in Arusha, SUA in Morogoro, and Uyole in Mbeya (High altitude 2000–2500 masl). In this experiment, 16 bean genotypes were used and two nonnodulating bean genotypes.

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, seed yield, and nodule weight. For this experiment, data for leaf and seed N were not collected because of inadequate funds to perform such analysis.

Results

There was variation among genotypes and among rhizobia inoculant types. Overall, varieties Bilfa-4 and Jesca were the best performers. Bilfa-4 was selected from the Africa Low Soil Fertility Bean Nursery and has proven to do well both at low soil fertility and in well fertilized soils. Jesca is released from the Selian Agricultural Research Institute. About the Rhizobia, the results show that at Arusha Nitrosua was a better inoculant than others while at SUA, Biofix from Makerere and Nitrosua performed well compared to inoculant from Nairobi University.

There was no consistent impact of phosphorus on seed yield and other yield parameters. As expected, data on nodule counts and nodule dry weight have relatively high CV. Apart from the inoculated rhizobia there could be some native rhizobia in the field that were not distributed uniformly. Related measurements of indigenous soil rhizobia are aimed at detecting major trends in local populations and likely would not suffice to document spatial variation encountered in this experiment.

Data for Experiment 2 in 2012 are still being analyzed, but preliminary results show clear differences among genotypes and a large G x E interaction for most yield/BNF traits. Related



measurements indicated that some of the bean genotypes developed at SUA have quite good nodulation.

Soil samples from the two locations where trials were conducted have been analyzed and the results are available. Sample of soil has been sent to WSU for mineral analysis. Five years of weather data at the locations where trials were collected are available.

Soil samples from three regions in Tanzania (Morogoro, Mbeya, and Arusha) were collected and sent to WSU for determining the presence of indigenous rhizobia levels.

Rwanda

The results of the Rwanda field study confirmed that climbing beans generally out-yield bush beans. They also confirm that inoculation alone or in combination with inorganic P did not always provide consistent positive effects on seed yield. However, the U.S. inoculant whether in combination with phosphate fertilizer or not did increase seed yield about 10 percent ($P < 0.01$). The significant effects on grain yield and yield parameters reflect the greater yield potential of climbing beans and their positive response to intensive management.

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

1. Characterize soil rhizobia soil populations and strain diversity at field sites.
 - a. Establish nodule rhizobia occupancy established on selected lines (WSU).
2. Confirm season 1 BNF phenotyping of selected populations (Bean CAP and South American Core).
 - a. biomass, plant N, ureide levels prior to pod fill.
3. Conduct SNP analysis (SNP chip) on bean CAP and SA Core collection for association mapping with phenotype data (WSU, MSU).
4. Complete initial list of candidate genes associated with BNF for SNP associations (WSU, MSU).
5. Advance selected RILs to F3 (MSU,WSU)

Bat477 and Dor364 are two Mesoamerican bean genotypes known to have different root system architectures and contrasting responses to abiotic stress. Bat477 is tolerant to low P soils and Dor364 is drought tolerant. A RIL population of BAT477 x Dor364 consisting of 100 lines was evaluated for BNF capacity in a greenhouse screen. Three replications of each line were inoculated with rhizobia and grown in eight inch plastic pots in a nitrogen free perlite/vermiculite mix until flowering. Low levels of P and sufficient levels of all other nutrients were given in a nutrient solution. At flowering, root weight, shoot weight, and shoot N, P, and S were measured. The means, ranges, and correlations for these traits are shown in Table 1.

A panel of 284 Andean bean genotypes was SNP genotyped with 533 SNP markers. A portion of the Andean diversity panel (275 lines) was grown in Michigan in 2012 and evaluated for biological nitrogen fixation capacity. Nodulation was estimated at flowering by digging up three plants per genotype per replication. Some lines were identified with large numbers of nodules, but overall the CV for this trait was high (37 percent). Nodule score was weakly correlated to days to flower and plant growth habit. Nodule score was negatively correlated to aboveground biomass at flowering. The genotypes with the highest nodulation scores were mostly African in origin.

Variable	Mean	Std Dev	Minimum	Maximum	Fold Difference
root wt. (g)	1.45	0.52	0.29	3.84	13
shoot wt. (g)	3.61	1.28	0.45	8.02	17
% N	2.39	0.36	0.74	3.26	4.4
% S	0.25	0.06	0.16	0.73	4.6
% P	0.32	0.07	0.18	0.66	3.7

Table 1. Traits related to BNF in a greenhouse screen of 100 RILs of BAT 477 x Dor.

Development of Andean RIL populations with parental lines contrasting in BNF capacity continued with advancement to the F3 generation. Crosses were also made to improve BNF in kidney beans.

Analysis of tissue Ureide levels in selected common bean lines

Ureides are the principal forms of nitrogen (N) transported in common beans, which originate predominantly from N₂ fixation. As such, the level in tissues is an indirect indicator of the extent of N₂ fixation by the nodules. The variation among plant tissues, genetics, and environmental effects, however, makes it difficult to relate tissue level to N₂ fixation directly.

In this study, we extracted ureides from plant tissues (petioles, stem, leaves, and pods) and measured relative levels spectrophotometrically against an allantoin standard. The objective was to determine which plant tissue, if any, provided a consistent relationship between ureide concentrations and total plant N and biomass accumulation.

Average values for individual lines varied nearly 10-fold, from 0.25 to 2.3 μ Moles/g DW. Ureide concentrations of individual leaf petioles varied between 0.1 and 2.8 μ Mol/g dry weight in the petioles. Petioles from cultivar 30-72c had the highest level of ureide concentration while petioles from cultivars 29-308, 30-17c, and 30-41b had the lowest ureide concentration levels.

Ureide concentrations in individual leaf samples varied between 0.1 and 0.8 μ Mol/g dry weight. Leaves typically exhibited the lowest levels compared to values observed in stems pods or petioles. Leaves from cultivar 29-101 had the highest level of ureide concentration, while leaves from cultivar 29-111 had the lowest ureide concentration level. The ureide concentrations varied between 0.1 and 3 μ Mol/g dry weight in stems. Stems had the second highest levels of ureide concentrations. Stems from cultivar 29-111 had the highest level of ureide concentration

while stems from cultivars 30-41b and 29-308 had the lowest ureide concentration levels. The ureide concentrations varied between 1 and 90 μ Mol/g of dry weight in pods. Pods had the highest levels of ureide concentrations of all tissues measured. Pods of cultivar 30-20b had the highest level of ureide concentration while pods from cultivars 29-111 had the lowest ureide concentration level.

The results clearly show that ureide levels varied dramatically among lines and plant tissues. While pods accumulated the highest concentrations of ureides, pod development and pod load were not uniform among all lines when samples were taken. Ureide concentrations typically correlated with tissue (petioles, leaves, stems and pods) biomass. Analyses are in progress to relate ureide and biomass values with total N accumulation.

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

1. Conduct field days in each HC to sensitize farmers and present research results (VEDCO, NaCCRI, SUA, ISAR).

3b. Conduct on-farm demonstrations comparing inoculant strategies.

1. Conduct on-farm trials initiated with selected farmer cooperators in all HC (VEDCO, NaCCRI, SUA, ISAR).

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

1. Create training materials to disseminate through PELUM farmer network.

2. Conduct information dissemination meetings on BNF with PELUM-associated farmer groups in Rwanda, Tanzania, Uganda, and Kenya (VEDCO).

3. Incorporate research results into extension training programs, farmer advocacy meetings, and the PELUM network website (VEDCO).

4. Determine the potential for engaging international funding agencies to expand current technology transfer efforts (ISU, Makerere, VEDCO, NaCCRI, SUA, ISAR).

5. Conduct advocacy meetings with farmer groups and agribusiness interests (VEDCO).

VEDCO Farmer Outreach and Training

Create awareness among extensionists at host country institutions on benefits of BNF and inoculant use as seen in soybeans. Four Community Based Trainers (1 female, 3 male) and two VEDCO Project Extension Officers were trained on the benefits of BNF and inoculants by research staff at NaCCRI and Makerere University. The training involved understanding basic terms and definitions like BNF and the type of bacteria that fix nitrogen in the soil as well as the importance of Nitrogen to crops as one of the macronutrients required by plants. The training also focused at the advantages

and disadvantages of BNF versus other inputs (fertilizers and Farm Yard Manure). Extensionists were trained on handling of the inoculum during storage and the inoculation process for common beans. After the training, extensionists visited one of the field demonstration sites where they participated in a practical session of identifying active and inactive nodules, differentiating between nodules and nematode egg sacs on plant roots, and observing the field performance of inoculated plants from Makerere University.

Initiate training materials on BNF and seed inoculation for extensionists, community-based trainers, farmers. Training materials about BNF and seed inoculation were sourced from Makerere University through its Department of Agricultural Production. The content in the training materials includes benefits of nitrogen-fixing bacteria in the soil, safety precautions to be observed during storage of inoculum, and procedures to be followed when inoculating seeds.

Evaluate Current Farmer Knowledge, Practices and Attitudes (KAPS) about BNF and Inoculation. The KAPS data collection tool was developed, reviewed, and approved with an input from all regional PIs and the lead PI from ISU. Upon approval of the tool, research assistants were mobilized and trained on how to collect information from the targeted respondents. Farmer surveys were preceded by pretesting of the tool and about 40 percent (400) of the total households in VEDCO operational areas where beans are grown as a food crop.

Establishment of farmer field demonstration gardens with BNF as part of the treatments. Eighteen half-acre demonstration gardens were established with farmer groups in Kamuli District, Uganda, during the second growing season of 2012. Four treatments were planned for each site: seed inoculation (BNF), addition of farm-yard manure (FYM), both seed inoculant and farm-yard manure (FYM + BNF), and an untreated control. The bean variety was local. The goals were to introduce farmers to an alternative soil management technique to improve bean yields, to compare the performance of BNF with that of locally available manure, and to observe how the Makerere inoculum performed under difficult soil conditions. Of the 18 sites, 12 included untreated areas to serve as a control comparison.

Averaged across all locations, farmers harvested more beans from the inoculated and fertilized plots. Yields were almost always greater with a combination of FYM + BNF than with either treatment alone. These results indicate improved soil fertility management can help farmers achieve better bean yields in the lowland areas of Kamuli District, where bean yields are typically 300 to 600 kg/ha—well below genetic potential.

Networking and Linkages with Stakeholders

Dr. Tenywa and two of his students contributed to the construction of baseline data collection tools used by VEDCO to evaluate the knowledge gaps of the targeted farming communities, collaborated with VEDCO in the design and laying out of demonstration plots in Kamuli district, and coordinated the analysis of soil samples and the processing of plant samples.

Contribution of Project to Target USAID Performance Indicators

Graduate and undergraduate training is central to this project. Supporting advanced education for HC students with world-class scientists and training field technicians will contribute directly to HC capacity building.

Training of farmers and farmer groups on technologies to improve bean productivity will contribute to income and food security of smallholder farmers.

Improved on-farm productivity will enhance marketing opportunities for farmer associations.

Advancing inoculant technology for legumes will promote agricultural enterprises associated with inoculant production and sales.

New knowledge on bean germplasm x inoculant x environment interactions to inform ongoing variety development programs in the United States and host countries about specific improvements in BNF is needed to realize enhanced yield, nutritional value, and marketability of dry beans and other pulses.

Seven graduate students and at least five undergraduate students trained in agricultural research and extension.

Contribution to Gender Equity Goal

Four of seven graduate students and three of five undergraduate students trained through this program are women. These individuals are being mentored to continue their academic careers and assume leadership roles in agricultural fields.

Most (60 to 65 percent) of the smallholder farmers and farmer groups served by VEDCO are women. Improving productivity of common beans intentionally targets women farmers since they are generally in charge of growing this crop and managing the proceeds from it.

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

MSU-3

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Abstract of Research Achievements and Impacts

Four hundred and forty one HIV-infected children and adolescents from two to 15 years of age are receiving one of three food supplements in two locations in Tanzania. The supplements contain 100 percent of the U.S. Daily Recommended Intake for vitamins and minerals, maize, and one of three protein rich foods: common dry beans, cowpeas, or small fish (sardines). The supplements are precooked and are prepared by suspending the supplement in water and boiling for five minutes. Each child or adolescent is provided with 130 g of dry supplement per day. The study will determine if bean or cowpea is more efficacious in promoting growth and immune function than the supplement containing fish. This a single-blinded, community-based, longitudinal study; the investigator compiling and evaluating data is blind to subject treatment and therefore treatment differences cannot be evaluated at this time.

Treatment with highly active antiretroviral (HAARV) drugs is expensive (U.S. \$8 to \$10 per day) and is crippling national economies. A previous study showed that feeding the bean–maize supplement improved the immune system (based on CD4 cell counts) and the children were not placed on HAARV treatment because their CD4 cell counts improved when the supplement was consumed. Food supplement cost is approximately U.S. \$0.75 per day and represents a reduction in treatment costs of \$7 to \$9 per day. The potential savings are great.

HIV-infection damages the mucosal lining of the intestine and promotes development of inflammation in the intestinal mucosa with resulting poor absorption of nutrients. Studies with rodents demonstrated that repair of damaged intestinal mucosa is more effective when a bean-based diet is fed. These results confirm previous results, and we believe the bean-based supplement enhances immune response and growth in HIV-infected children in the absence of HAARV by promoting greater integrity of the intestinal mucosa.

Project Justification and Objectives

The overall goal of the research is to determine if eating beans will improve the immune status of children that 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*; 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, and there are approximately 33 million people in the world infected with HIV. Approximately 2 million children younger than 15 years have HIV and 90 percent of the children living with—and dying from—HIV live in sub-Saharan Africa. Furthermore, 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 common 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 uninfected counterparts.

We previously showed that providing HIV+ children with a bean–maize supplement containing minerals and vitamins could reverse malnutrition if present and improve the immune system (increased CD4 counts), even without the children receiving highly active antiretroviral drugs. This is an extremely important finding, since 50 percent of HIV+ 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 that have access to HAARV medicine. Consuming a bean-based supplement could improve the lives of millions of HIV-infected people, which would at the same time benefit the entire bean value-added chain from farmers to consumers.



Objectives

1. Determine if HIV-infected, HAARV naïve, 2- to 15-year-old children and adolescents eating a bean–maize or cowpea–maize supplement will maintain a higher CD4 percent than HIV-infected, HAARV naïve, 2- 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. (Note: this completes the data gathering begun in FY10.)
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, 2- 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, 2- to 15-year-old children and adolescents eating a fish–maize supplement.

Approaches and Methods

1. Purchase ingredients, cook and package food supplements, and transport and distribute food supplements to 400 subjects.
2. Every three months collect and analyze blood samples from approximately 400 subjects; 1,660 blood samples will be analyzed for CD4, CD8, CD3, and total lymphocyte counts.
3. Train two M.S. students to assist in research.
4. Provide field practical training in community nutrition and health for ten undergraduates.

Results and Outputs Achieved

At the beginning of FY12, 73 children were receiving food supplements. As the study progressed and expanded, a number of challenges required modification to our planned methodology. The first and greatest challenge related to analysis of the blood samples. The original plan was to transport the blood samples from the communities to SUA for analysis. However, it quickly became apparent that the membranes of red blood cells from HIV-infected children are considerably more fragile than red-blood cells (RBC) from noninfected children. Transport of the blood samples collected at the Centers for Treatment and Counseling (CTC) to Sokoine University (a 100 to 550 km trip over bumpy roads) caused hemolysis of some of the RBC that interfered with the determination of CD4, CD8, CD3, and total lymphocyte counts. RBC hemolysis was not encountered during method development and testing, presumably because the samples were transported only 5 km from a local CTC. After much discussion, it was decided that the various CTCs would determine the CD4 cell counts in collaboration with SUA researchers. All of the CTCs are part of the Tanzanian HIV surveillance program and they routinely determine CD4 cell counts as a component of the clinical management of HIV-infected individuals. One positive outcome is that the CTCs now bear most of the cost for determining the CD4 cell counts as part of their routine surveillance program and this Pulse CRSP project only provides miscellaneous supplies and reagents that had often previously limited the CTC's capability to perform their analyses. Thus, both the CTCs and the CRSP project benefit from this arrangement. A second positive aspect is that the benefits of the supplemental feeding will be immediately obvious to the frontline group handling HIV-infected individuals. We expect that local support from the CTCs will greatly assist us in our efforts to influence national and international policy related to care of HIV-infected individuals.

Sharing the responsibility for determining CD4 cell counts with the CTCs makes the project a more community-based study and a type of participatory research than originally envisioned. On the negative side, the CTCs are not capable of determining CD8, CD3, and total lymphocyte counts. While CD4 cells are the primary immune cell destroyed by the virus, having CD8, CD3, and total lymphocyte counts would have provided a more complete picture of immune response than what will now be available.

Our goal was to have 540 subjects complete the study in the absence of HAARV. The reality is that the availability of HAARV drugs in Tanzania is rapidly increasing, and HIV-infected individuals are given HAARV drugs per physician recommendations based on clinical symptoms and CD4 cell count. It would be unethical to ask subjects to refrain from taking HAARV drugs if their physician so recommends. An external reviewer of the project recommended a cohort of subjects that were receiving HAARV drugs be included in the study. Initially, we could not take this recommendation; however, as more and more of the HAARV-naïve children and adolescents were placed on HAARV, we were forced to accept the reviewer's recommendation. M. Bennink was a co-PI of a study conducted in Botswana that showed that HIV-infected children and adolescents receiving HAARV therapy had a significant improvement in immune response and growth compared to a control group ingesting a supplement that did not contain beans. Therefore, we still expect the group consuming the bean–maize supplement (and perhaps the group consuming the cowpea–maize supplement) will have a greater immunological and growth response than the control group consuming a fish–maize supplement.

Initiation of HAARV drug therapy during the course of the study will add another level of complexity to data analysis. For anthropometric measurements, we will utilize statistical tools to adjust for initiation of HAARV drug therapy. We have gathered data from a group of subjects who are not receiving nutritional support; this group will be our negative control group and the data from the negative control group will be utilized to statistically adjust for initiation of HAARV drug therapy. We will still test the hypothesis that HIV-infected children fed a bean–maize or a cowpea–maize supplement will have greater immune and growth improvement in HAARV-naïve patients and reduce the need for HAARV therapy compared to the group consuming a fish–maize supplement.

We currently have 441 subjects in the study. Approximately 300 of the subjects reside in the northern rural areas of Rombo and Kilema, approximately 550 km from Morogoro. The other 141 subjects come from rural areas around Turiani, about 100 km from Morogoro. We had planned to have about 200 subjects from Morogoro Rural District.

Approximately two metric tons of precooked and dried supplement is packed into 13,250 packages every month for distribution. Data continues to be collected and it is best to not break the diet code until more data is collected.



ingredients, including the maize flour, beans, cooking oil, onions, tomatoes, salt, and other spices. The amount of time spent in cooking was determined and valued in monetary form based on the government payment rates for casual laborers per day (TShs 6000.00). The value of the fuel (charcoal, firewood) used to prepare the ugali with beans was determined. Transport costs were taken into account whenever the subject paid money for transport when buying food and nonfood materials. The total cost for preparing the equivalent of 130 g of dry ugali plus beans was computed since 130 g is the weight of precooked supplement provided to each subject each day. (see Table 1).

S/N	TYPE OF PRODUCT	COST (USD) ¹ PER DAY	COST RANGE PER DAY
1	Ugali with beans	\$0.60	0.57 – 0.64
2	Ugali with sardines	\$0.63	0.62 – 0.70
3	Ugali with cowpeas	\$0.60	0.56 – 0.65
4	Extruded/fortified bean-based supplement	\$0.72	\$0.70 – \$0.77
5	Extruded/fortified cowpea-based supplement	\$0.72	\$0.69 – \$0.77
6	Extruded/fortified Sardine-based supplement	\$0.77	\$0.71 – \$0.80
7	Antiretroviral therapies ² 1. Drugs 2. Syrup	\$2.62 \$1.98	\$2.45 – \$3.25 \$1.81 – \$2.77
8	Antiretroviral therapies ³ 1. Drugs 2. Syrup	\$10.48 \$7.92	\$9.80 – \$12.98 \$7.23 – \$10.40

Table 1. Cost comparison for the various foods/supplementary foods and the antiretroviral therapies.

- ¹ USD = approximately 1,550 Tanzanian Shillings per \$1 USD
² Cost to patient; patient pays 25 percent and government pays 75 percent of the costs through Essential Drugs Program
³ Total (unsubsidized) cost of drugs

Three M.S. students in human nutrition received research training while assisting in work related to objective one. Two B.S. graduates from the Department of Food Science and Technology received practical training related to safe preparation of cereal foods. Fourteen undergraduates in the nutrition major at SUA received practical field training for a five-week period.

Objective 2: Determine the relative costs of three dietary treatments compared to HAARV drug treatment (Note: this completes the data gathering begun in FY10).

Approaches and Methods

1. Determine the costs associated with cooking beans, cowpeas, and sardines in a pot and for preparing ugali (a corn-based local food).
2. Determine costs associated with preparation of the extruded (precooked) bean–maize and cowpea–maize supplements and the thin porridge from the supplements.
3. Determine costs associated with preparation of the fish–maize supplement and the thin porridge from the supplement.
4. Determine costs associated with HAARV drug treatment.

Results and Outputs Achieved

1. *The costs associated with cooking beans in a pot and preparing stiff porridge (ugali).* Data were collected from a sample of 100 randomly selected women as follows: Rombo (30), Kilosa (15), Kimamba (11), Turiani (35), and Mtibwa (9). The subjects reported the amount of money used in buying the food

2. *The costs associated with cooking sardines in a pot and preparing stiff porridge (ugali).* Data were collected from a sample of 91 randomly selected women as follows: Rombo (21), Kilosa (19), Kimamba (8), Turiani (33), and Mtibwa (10). The subjects reported the amount of money spent in buying food ingredients, including the maize flour, sardines, cooking oil, onions, tomatoes, salt, and other spices. The amount of time spent in cooking was determined and valued in monetary form based on the government payment rates for casual laborers per day. The value of the fuel used to prepare the ugali with sardines was determined. Transport costs were taken into account whenever the subject paid money for transport when buying the food and nonfood materials. The total cost for preparing the equivalent of 130 g of dry ugali plus sardines was computed (see Table 1).
3. *The costs associated with cooking cowpeas in a pot and preparing stiff porridge (Ugali).* Data were collected from a sample of 86 randomly selected women as follows: Rombo (20), Kilosa (22), Kimamba (10), Turiani (25), and Mtibwa (9). The subjects reported the amount of money spent in buying food ingredients, including the maize flour, cowpeas, cooking oil, onions, tomatoes, salt, and other spices. The amount of time spent in cooking was determined and valued in monetary form based on the government payment rates for casual

laborers per day. The value of the fuel used to prepare the ugali with cowpeas was determined. Transport costs were taken into account whenever the subject paid money for transport when buying the food and nonfood materials. The total cost for preparing the equivalent of 130 g of dry ugali plus cowpeas was computed (see Table 1).

4. *Cost involved in the preparation of the extruded, fortified maize-beans/cowpeas/sardines composite supplementary foods.* Data were collected from six, 1,000 kg batches of food materials that were bought at different seasons to reflect market price fluctuations. For each batch of 1,000 kg, the following information was collected. Cost of food materials: maize, beans/cowpeas/sardines, sugar, salt, bicarbonate, vegetable oil and minerals/vitamins pre-mix. Overhead costs collected were hulling, milling, extruding, electricity, labor charge, transportation of raw materials, and delivery of products and packaging materials. For each batch of 1,000 kg food materials processed, the number of 130 g sachets yielded was determined. The total cost for each 1,000 kg batch (cost of food ingredients plus labor and overheads) was thereafter divided by the total number of 130 g sachets obtained from the batch. The average cost of each 130 g sachet was then established.
5. *Cost involved in antiretroviral drugs or syrup (HAART).* Data were collected from various pharmacies and from the Tanzania Pharmaceutical Industry (TPI), which manufactures the antiretroviral therapies. A total of 15 retail pharmacies were visited: Morogoro (4), Dar es Salaam (6), and Moshi (5). Retail prices for a 30-day dose were obtained from each pharmacy. Visits were also paid to the Medical Store Department (MSD) (sole distributor of essential medicines in Tanzania) in Morogoro, Moshi, and Dar es Salaam, where wholesale prices were recorded. A visit was also paid to the factory (TPI) that manufactures antiretroviral therapies in Arusha. Industrial-gate prices were recorded. From the average retail price of a 30-day dose, the cost for one-day dose of antiretroviral drugs or syrup was then established.

The food costs presented in Table 1 are based on the amount of energy required for a five-year-old child. The costs would be doubled for a 15-year-old. Costs are for foods prepared over an open fire and for foods prepared via extrusion. The data in Table 1 are based on comparable dry weights and include the cost of vitamins and minerals. The costs associated with preparing ugali and cowpeas or ugali and beans (equal amounts of protein) are similar. Preparing a similar amount of protein with ugali and sardines instead of cowpeas or beans cost about five to seven percent more. Preparing precooked flour increased the cost of the food supplements 20 to 22 percent compared to preparing similar foods in a pot over a fire.

Direct comparisons for managing HIV-infected children through diet versus drug treatment cannot be made since the two treatments don't achieve the same results. However, a daily savings of U.S. \$7.25 to \$10.00 is achieved if the child can be managed with the food supplements in this study instead of with drugs. A child managed with one of the food supplements will achieve better growth and development than a child that simply receives HAARV. Moreover, drug treatment causes deleterious

side effects and drug effectiveness often declines due to the development of drug resistance. If governments desire more normal physical and mental development in their children treated with HAARV, they should provide a nutritional supplement that would increase the treatment differential to at least \$8.00 per day. How long and how effectively an HIV-infected child can be managed with diet alone remains to be determined.



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.

Approaches and Methods

1. Analyze approximately 800 dried blood samples shipped from Tanzania for HIV load, selected proinflammatory cytokines, and R16s (a marker of bacterial translocation).
2. Develop a rodent model to study bacterial translocation from the intestine into tissues and blood. A bean-based diet is expected to reduce bacterial translocation and release of pro-inflammatory cytokines. The effectiveness of cowpeas will also be tested in this model.
3. Correlate changes in proinflammatory cytokines and R16s in the human feeding study with the changes observed in the rodent model to help establish a mechanism of how feeding beans (or cowpeas) can improve the immune status of HIV-infected children.

Results and Outputs Achieved

(Activities related to objective 3 are secondary to the activities for objectives 1 and 2.)

1. Blood samples were collected from the subjects and dried blood spot samples were prepared and stored at Sokoine

University. The analytes we are interested in are stable for years when the blood spots are properly prepared and stored. These samples, which are biohazardous due to the presence of HIV, require special clearance for international shipping. Once we have approval from the Center for Disease Control to ship these samples from Tanzania to the United States, the samples will be analyzed.

2. The intestinal mucosal barrier, comprised of goblet, cell-derived mucin and reinforced by tight junctions, normally limits passage of bacteria and other luminal antigens through the epithelium and into the lamina propria and deeper tissues. Since the intestinal barrier is not perfect, immune cells destroy bacteria and luminal antigens that get into the lamina propria and deeper tissues. The human immuno virus destroys CD4 and other immune cells and seriously compromises the ability of the immune system to prevent bacteria from gaining entrance into the circulatory systems. Bacteria and bacterial products that are not inactivated by immune cells cause inflammation in the intestinal wall resulting in poor absorption of nutrients and greater permeability of the intestinal mucosa. From previous research regarding diet and colon cancer, we have observed that eating beans improves the colonic mucosal barrier following mucosal damage.

It would be unethical to obtain intestinal biopsies from our subjects, so we performed two experiments with rats to further document that eating beans promotes greater mucosal integrity and reduces inflammation. Rats are not susceptible to HIV, so a chemical toxin was injected to impair mucosal integrity and to promote inflammation in the intestinal wall. Control rats were injected with saline rather than the toxin. Three months following toxin injection, mucosal integrity in the small intestine was evaluated. Immido black, a dye that does not permeate healthy mucosa was infused into the lumen of the intestine. Histology showed that mucosal damage by the toxin had long-term effects on mucosal integrity, regardless if rats were fed a control diet or a diet containing beans. Rats injected with the toxin and fed a control diet had the greatest mucosal permeability to the dye. Rats injected with the toxin and fed beans had reduced mucosal permeability compared to rats injected with the toxin and fed the control diet. Rats injected with saline had minimal permeability of the dye past the epithelial layer; however, rats fed beans had slightly less dye permeability than rats fed the control diet. Histology showed the presence of immune cells in the lamina propria paralleled dye permeability. These results are qualitative, but the study did show that eating beans caused better recovery of the intestinal mucosa following mucosal damage than eating a control diet that did not contain beans.

The second rat study followed the same protocol as study one, except mucosal integrity and inflammation were assessed by measuring the expression of key genes involved in innate defense and immunity. Expression of these genes in the bean-fed, saline-injected rats was slightly lower (trend, but not statistically significant) than in saline-injected rats fed the control diet. *Pla2g2a* and *RatNP-3* exhibit antimicrobial activity, and together with other proteins play an important role in mucosal epithelial defense. *Pla2g2a* is a multifunctional protein induced in a variety of inflammatory conditions. Decreased

expression of the genes *Thr4* and *Dmbt1* further suggest that eating beans reduces microbially induced inflammation. A lower expression of these genes in bean-fed rats suggests that translocation of bacteria and other antigens from the intestinal lumen is reduced by eating beans. Taken together, these two studies strongly suggest that eating beans improves mucosal barrier function and reduces inflammation in the intestinal mucosa and lamina propria.

Networking and Linkages with Stakeholders

During FY 2012, collaboration was made with several institutions dealing with food processing and management of HIV/AIDS, including:

1. *Tuboreshe Chakula*. USAID-funded project dealing with rural food fortification and social marketing of micronutrients. Mosha helped them initiate their project.
2. *Mwanzo Bora*. USAID-funded project dealing with management of HIV/AIDS cases in rural communities. Mosha provided nutritional training for their personnel.
3. *World Bank* country office. Focused on rural fortification of foods. Laswai and Mosha served as technical experts to the government and NGOs in their efforts to launch staple food fortification initiatives in Tanzania.
4. *TechnoServe*—USAID-funded NGO that hopes to develop large-scale food extrusion and fortification. Mosha provided food extrusion and fortification training to their personnel.

Publications

Rondini EA, Bennink MR. (2012). Microarray Analyses of Genes Differentially Expressed by Diet (Black Beans and Soy Flour) during Azoxymethane-Induced Colon Carcinogenesis in Rats. *J Nutr Metab* .2012;2012:351796. Epub 2012 Feb 8.

Contribution to Gender Equity Goal

Four women received advanced degree training (one Ph.D. and three M.S.). One M.S. student graduated in November 2011 and is employed by an NGO in Tanzania. The other two M.S. students graduated in November 2012 and will begin employment in Tanzania immediately. All four trainees will contribute to the pool of professionals in nutrition and food science in their respective countries (three in Tanzania and one in Jamaica).



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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Abstract of Research Achievements and Impacts

In FY12, the project team focused on completing the ex post impact assessment study on cowpea improvement research in Senegal. The survey results indicated that total adoption rate of improved varieties as reported by farmers in the three major cowpea growing regions was 44 percent. Adoption rate of the three CRSP varieties specifically identified by their names (Melakh, Mouride, and Yacine) was about 22 percent. About 46 percent of the sampled farmers were growing at least one of the CRSP varieties. Comparison of the reported adoption rates of the CRSP varieties with those found from the 2004 study reveals that the adoption of CRSP varieties has increased rapidly since 2004—from about 4 percent in the early 2000s to 22 percent in 2010, a reflection of the efforts in the past four to five years by the government, FAO, NGOs, and farmers organizations (and recently by the CRSP) in the multiplication and dissemination of seeds of Melakh and Yacine in Senegal's major cowpea growing regions. The results of the economic analysis indicate that past investments by the CRSP and ISRA have produced an internal rate of return of 18.6 percent and a net present value of \$18.6 million. The program was further found to be profitable under every sensitivity analysis scenario.

A meta-analysis of ex post impact assessments of research on dry grain pulses was initiated in FY12 to assemble a pool of studies that report the economic benefits from pulse crop research and then use that data to conduct a meta-benefit-costs analysis to identify generalized patterns from case observations and estimate an overall rate of return to research investments in pulse crop research. After reviewing and filtering for eligible studies, the final pool for inclusion in the meta-cost-benefit analysis includes 17 studies. Yearly data on benefits and costs are being systematically collected from these studies, with the intention to conduct the meta-analysis based on these 17 studies.

To implement an integrated impact evaluation strategy as part of the CRSP project design, the team collaborated with several project teams to conduct baseline assessment, design impact evaluations, and conduct in-depth case studies to better understand sustainable models of dissemination of agricultural technologies. Research studies initiated in FY12 towards this objective include:

1. Baseline assessment of the economic effects of pest problems on cowpea growing areas in Burkina Faso
2. Impact evaluation to test the effectiveness and impacts of methods of extension to disseminate materials for IPM of cowpea pests
3. Benefit/Cost (B/C) analysis of the bean-based nutrition intervention in Tanzania
4. Case study of the bean seed multiplication and distribution system in Central America.

By September 2012, two surveys in Burkina Faso and three surveys in Nicaragua were completed. More data collection efforts are under way in Burkina Faso and Tanzania.

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 essential to document outputs, outcomes, and impacts of public investments in research for development (R4D) activities. Anecdotal data and qualitative information are important in communicating impact to policymakers and the public but must be augmented with empirical data and sound and rigorous analysis.

Impact assessments are widely recognized to perform two functions—accountability and learning. Greater accountability (and strategic validation) is seen as a prerequisite for continued financial support. Better learning is crucial for improving the effectiveness of development projects and ensuring that the lessons from experience are heeded. 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 in Phase II and III. 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. 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.
 - a. Benefits of genetic improvement of cowpea in Senegal and West Africa
 - b. Meta-analysis study
2. Investigate opportunities to integrate baseline data collection and impact evaluation strategies as part of the CRSP project design.
 - a. Baseline assessment of the economic effects of pest problems on cowpea growing areas in Burkina Faso.
 - b. Impact evaluation to test the effectiveness and impacts of methods of extension to disseminate materials for IPM of cowpea pests.
 - c. Benefit/Cost (B/C) analysis of the bean-based nutrition intervention in Tanzania.
 - d. Case study of the bean seed multiplication and distribution system in Central America
3. Build institutional capacity and develop human resources in the area of impact assessment research.

Impact assessments are widely recognized to perform two functions—accountability and learning

Research and Outreach Approaches, Results, and Achievements

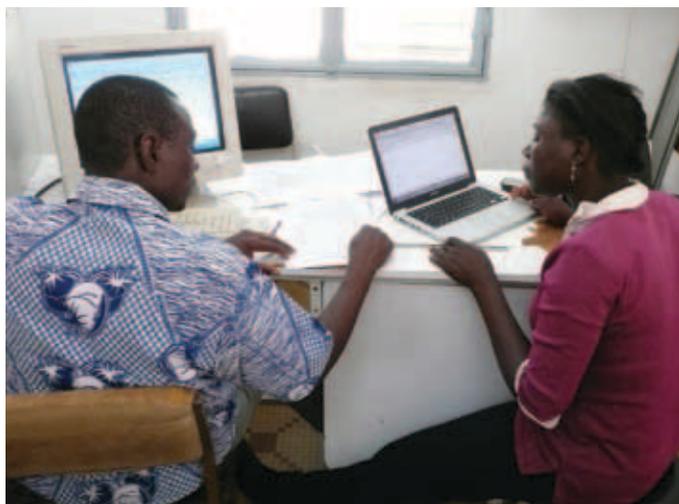
Objective 1: Conduct ex post impact assessment of Bean/Cowpea and Dry Grain Pulses CRSP Investments in Research, Institutional Capacity Building, and Technology Dissemination in Africa, Latin America, and the United States.

In FY12, the project team focused on completing the West Africa impact assessment study on cowpea improvement research and initiated the meta-analysis of ex post impact assessments of research on dry grain pulses in general and of CRSP research in particular.

Objective 1a. Benefits of genetic improvement of cowpea in Senegal and West Africa

The general objective of this study was to perform an ex post economic impact assessment of B/C CRSP investments in cowpea varietal development in Senegal, which led to the development and dissemination of three determinant, short-cycle varieties: Melakh, Mouride, and Yacine.

To assess the adoption and economic impact of these improved varieties, a survey was conducted in the Thiès, Louga, and Diourbel regions of Senegal. Two groups of farmers were surveyed, sufficient to provide seven households per local enumeration area. The sample was weighted to account for proportional representation.



The total adoption rate of improved varieties as reported by farmers in the three regions was found to be 44 percent. Adoption rate of the three CRSP varieties was half of this reported area under IV (improved varieties) adoption. About 46 percent of the sampled farmers were growing at least one CRSP variety. In terms of area, the adoption of improved varieties was found to be highest in Louga (51 percent), followed by Thiès (42 percent) and Diourbel (39 percent).

Almost all of the other improved varieties reported by farmers were in Thiès region (36 percent). Personal communication with the cowpea breeder in Senegal suggests that if these are indeed improved varieties, then they are likely CRSP varieties; however,

the economic analysis conducted to estimate the rates of return excluded other improved varieties reported in Thiès.

Comparison of the reported adoption rates of the CRSP varieties with those found from a 2004 survey of farmers from the same regions reveals that the adoption of CRSP varieties (and IV in general) has increased rapidly since 2004—from about 4 percent in the early 2000s to 22 percent in 2010, a reflection of the focused efforts by the government, FAO, NGOs, and farmers' organizations (and more recently the CRSP) in multiplication and dissemination of breeder seeds and certified seeds of Melakh and Yacine in the last four to five years.

The yield of traditional varieties ranged from 130 kg/ha in Thiès to 175 kg/ha in Louga. Reported yields of CRSP varieties are consistently higher than traditional yields across all the regions. However, the yields of three CRSP varieties varied significantly between regions, with the lowest reported yield difference between TV (traditional varieties) and IV in Diourbel, and the highest in Louga.

Adoption rates from this survey and the 2004 study were used to project adoption curves for each variety within each region. Economic benefits generated from the adoption of higher yielding Melakh, Mouride, and Yacine were estimated and compared with the value of research investment in cowpea breeding by the B/C CRSP and the Senegalese Agricultural Research Institute (ISRA). The results indicate that past investments by the CRSP and ISRA have produced an internal rate of return of about 18.6 percent and a net present value of \$18.6 million. The program was further found to be profitable under every sensitivity analysis scenario.

Objective 1b. Meta-analysis study

Toward this study, a thorough literature review was conducted to identify the research impact studies (CRSP and non-CRSP) related to beans, cowpeas, and other dry grain pulses. The goal was to assemble a pool of studies that report economic benefits from pulse crop research and then use that data to conduct a meta-benefit-costs analysis to identify generalized patterns from case observations and estimate an overall rate of return to research investments in pulse crop research.

Sixty-nine studies were identified and information entered in a database. A quick review of the abstract/summary and methodology section led to the elimination of many studies that were either duplicates or not empirically based aggregate level ex post impact assessment studies. The final pool of studies for inclusion in the meta-cost-benefit analysis numbered 17.

Currently, yearly data on benefits and costs are being systematically collected from the published reports or requested from authors if these data are not included in published outputs.

Objective 2: Investigate opportunities to integrate baseline data collection and impact evaluation strategies as part of the CRSP project design.

2a. Baseline assessment of the economic effects of pest problems on cowpea growing areas in Burkina Faso

This is a joint activity with the UIUC-INERA PII-UIUC-1 project team. To address the field insect pest problems like

legume pod borer, bruchids, and pod sucking bugs for which conventional breeding has not been effective, the PII-UIUC-1 project has developed alternative strategies for control of these insect pests to reduce the levels of pesticides used on cowpea crops. One of the strategies developed is to implement a comprehensive biocontrol program to generate long-term impacts on cowpea growers in the region: 1. health and environmental benefits from the reduction in the use (and misuse) of pesticides and 2. economic benefits resulting from increased productivity (due to reduction in crop losses) and increased profitability (due to reduction in input costs) to cowpea growers in the region. The realization of these impacts critically depends on the following realities: 1. the movement and spread of biocontrol agents in relation to where the pest population is present and 2. the pest control strategies practiced by farmers to control the pests in the absence of biocontrol agents.



To estimate the long-term benefits of this research requires three types of data: 1. a better understanding of the spatial distribution of the pest population targeted by this research, 2. tracking the movement and spread of biocontrol agents over time, and 3. the change in farmers' pest control practices and/or productivity outcomes as a result of the introduction of biocontrol agents. The PI-UIUC-1 project has collected data towards the first two types of information that will be useful to conduct an ex post impact assessment of this CRSP project in the future. As part of this project's workplan, in FY12, baseline household-level data were collected towards the third type of information needed to assess the impact of biocontrol research after several years of cumulative efforts by the UIUC-INERA team in Burkina Faso. The following activities have been carried out to date towards this study.

Meeting with collaborators during the DGP CRSP Global Meeting in Rwanda, February 2012 The UIUC-INERA PII-UIUC-1 and PIII-MSU-4 project teams held the first meeting to coordinate activities to be carried out under this project. The PIII-MSU-4 team developed two questionnaires for use during baseline data collection. The PII-UIUC-1 team provided feedback on these questionnaires and the final versions were translated into French.

The village-level questionnaire included questions about the general characteristics of the villages, location-specific characteristics, access to basic services, and agriculture-related information at the time of the survey. The household-level questionnaire included questions related to general information on the respondent, field characteristics, use of varieties and

cowpea production during the 2011 season, cowpea sales during the 2011 season, the use of labor and other inputs during the 2011 season, importance of the cowpea crop as a source of income and food security, pesticide sources of information, pesticide management and health effects due to pesticide use, infrastructure owned and access to services, and socioeconomic characteristics of the household (e.g., assets, composition).

Visit to Burkina Faso by Byron Reyes, March 2012. The main purpose of this visit was to participate in the training of the enumerators who were going to collect the baseline data, randomly select the villages to include in the study, and train the staff who were going to enter the data into Excel. Ten enumerators and two supervisors were trained.

The household-level instrument was pretested and a revised version was generated based on the pretesting experience. Four farmers were invited to INERA to participate in the pretesting activity; one of the four farmers was a woman.

Prior to this visit, the UIUC-INERA, PII-UIUC-1, and PIII-MSU-4 teams selected 10 provinces to include in this study, which were distributed in two parallel horizontal lines across the south and central regions of the country because the biocontrol agents will be released in the south region and are expected to move from south to north. This design allows us to evaluate the effects of these biocontrol agents in provinces closer to/in the release area (south region) and in provinces farther from the release area (central region). Within these provinces, 56 villages were randomly selected. Further, within each village, 10 households were randomly selected for interview; it was assumed that all farmers within each village produced cowpea in 2011, due to the provinces' high cowpea production.

Baseline data collection, tabulation, data cleaning, and selected descriptive statistics. A total of 560 households were interviewed across 56 villages in March–April 2012. The analysis is in progress.

From the sample, approximately 95 percent of respondents were the head of the household (HHH), about 3 percent were the spouse of the HHH, and two percent were related differently to the HHH. Further, 98 percent of households were male headed and, on average, respondents indicated that they had lived in the village for 43 years. The average household size was almost 12 members. On average, each household planted 1.3 cowpea fields and the number of cowpea fields per household ranged from one (74 percent) to five fields (0.2 percent). Approximately 95 percent of the households planted between one and two cowpea fields.

The use of inputs varied by type. While 54 percent of households applied chemical fertilizer to at least one of its cowpea fields, only 40 percent of households applied organic fertilizer (e.g., manure) to at least one of its cowpea fields. In contrast, 78 percent of households applied chemical or organic insecticides and 65 percent of households applied fungicides to their cowpea fields. Further, almost 43 percent of households purchased cowpea seed and spent an average of CFAs 3,866 on these seeds.

As expected, the cowpea crop is an important source of income and food security. Sixty-two percent of farmers reported that grain sales represented 50 percent or less of their HH income. Additionally, for most households (34 percent), their own production satisfied between one-third and two-thirds of their annual cowpea consumption.

When farmers ($N=452$) were asked about their knowledge of the existence of beneficial insects and viruses, only 7.3 percent of respondents knew about beneficial insects and 2.6 percent about beneficial viruses. This suggests that efforts will be needed to make farmers aware of the existence of these organisms so they can implement practices to favor their populations. This is confirmed by the fact that only 14.6 percent of respondents said that they or someone in their household have received training on integrated pest management (IPM) in the past. Since most farmers (95 percent) consider that pesticides are toxic for their health, it was no surprise that only a small share (6.6 percent) of farmers re-use pesticide containers after they are empty.

2b. Impact evaluation to test the effectiveness and impacts of methods of extension to disseminate materials for IPM of cowpea pests

This is a joint activity of the UIUC-INERA, PII-UIUC-1, and PIII-MSU-4 project teams. The team developed a randomized field experiment with the goal of collecting all the data before the end of December 2012.

Field experiment. The field experiment planned in Burkina Faso will address the research impact questions:

1. How effective is the animated educational video in inducing learning about the postharvest cowpea drying and storing technologies among low-literate farmers?
2. Does learning induce the adoption of technology if availability is not a constraint?

The field experiment plans to test the effectiveness of two animated videos in inducing learning among cowpea farmers in Burkina Faso. The videos describe a solarization technique for killing cowpea bruchids before the seeds are stored and the triple bagging technique to store grain without bruchid damage. Both videos can be downloaded from the Internet and are available in French and many local languages spoken in West Africa.

The advantages of these two techniques are that they are a) low-cost, simple, and quick; b) effective when properly used; c) easy to explain and to disseminate; and d) can reuse materials. Additional benefits of triple bagging include a) no use of pesticides; b) grains ready to be consumed when the bags are opened; c) good for storage of small and large quantities; and d) bags can be stored in homes.

Experimental design. As part of the collaborative CRSP research project, UIUC and INERA plan to pilot test the deployment of these two animated videos in selected villages in Burkina Faso using the government extension system. The experiment will address the research questions: 1. How effective is the animated educational video in inducing learning about postharvest cowpea drying and storing technologies among low-literate farmers and 2. Does learning induce the adoption of technology

if availability is not a constraint? (Note that for the second research question, the focus is only on the triple bag technology.)

Questionnaires/forms for data collection. The PIII-MSU-4 team developed the questionnaires/forms for use during training of extension agents and farmers, and during data collection. The final versions of 11 of the 12 questionnaires have been translated into French.

To measure the treatment effects in terms of indicators that measure effectiveness in inducing learning and adoption of the two technologies being promoted, farmer-level data will be collected using standard instruments. Baseline data on the pretreatment prior knowledge about the storage techniques will be collected from 20 randomly selected participants. A follow-up impact evaluation survey will be conducted for a subset of 15 farmers per village from the list of 20 farmers who attended the training/demonstration sessions and completed the pretreatment knowledge module.

Training of extension agents. The PII-UIUC-1 collaborator provided training to the extension agents in October 2012. The following activities will be carried out in the coming months:

- Visit to INERA by Byron Reyes in November 2012 to train the enumerators who will collect the village and household data and to train the data entry staff.
- Revise and translate the household-level questionnaire into French.
- Develop Excel/STATA templates for data entry for all questionnaires/forms.
- Data collection, tabulation, and cleaning.
- Project report and development of a research paper based on the results

Objective 2c. Benefit/Cost (B/C) analysis of the bean-based nutrition intervention in Tanzania

Meeting with collaborators during the DGP CRSP Global Meeting in Rwanda, February 2012. The PIII-MSU-3/SUA and PIII-MSU-4 project team members met to explore the feasibility of addressing the policy-relevant question of cost-effectiveness of the proposed food-based approach being implemented in Tanzania and to gauge mutual interest in conducting this study jointly. A follow-up meeting between MSU-3 and MSU-4 teams was held in April 2012 to lay out the next steps of the project and to develop a concept note as a guideline for SUA to follow in terms of the collection of appropriate data to conduct this analysis.

Background on the intervention, experimental design, and sample selection.

The research conducted by PIII-MSU-3 team involves testing three dietary supplements to improve the immune status of children and adolescents (2–15 years old) infected with HIV in rural Tanzania: (1) bean-maize, (2) cowpea-maize, and (3) fish-maize. This study includes 540 individuals receiving one of the dietary supplements but not receiving ARV (antiretroviral) drug treatments at the time of enrollment. Once the individuals get too sick and need to be given the ARV drug, they are excluded from the study. (Although because of ethical reasons

the study does not include a true control group, i.e., individuals receiving no treatment, it is possible to obtain indicators of health for years prior to the study for individuals who currently do not receive HIV treatment and use this information as a control.)

The individuals included in the study were randomly selected from a Government of Tanzania list of HIV-infected individuals in two regions. Participating individuals were randomly assigned to one of the three dietary groups; siblings infected with HIV who belonged to the same family were assigned to the same treatment (i.e., dietary supplement).

The following hypotheses are being tested by this feeding trial:

1. The bean–maize supplement will be more effective and cheaper than the other alternatives in increasing the indicators of health and nutrition among HIV-infected children.
2. The supplements in general will delay the time when HIV-infected children and adolescents reach the threshold level after which they are automatically given the ARV drug.

Although it would be ideal to estimate the overall lifespan of children consuming each of the dietary supplements, this will not be feasible since the study will last only 30 months. Thus, the main objective is to demonstrate the cost-effectiveness of different diets in preventing/delaying the use of ARV drugs among HIV-infected children and adolescents.

The three dietary supplements are prepared by SUA staff from supplies procured from the market. The process involves extrusion and addition of minerals and vitamins to meet daily nutritional requirements. The end product is a precooked flour (like an instant porridge mix), distributed in 130 g bags. Each bag/dosage represents one serving per day, which should be consumed as a mid-afternoon snack so other meals are not displaced. Health workers visit the households of recruited subjects every month. During these visits, each infected individual is given enough dosages for one month (30 dosages) and the adults in the household receive instructions on how to feed the supplement to children.

Although the supplements are prepared following food safety regulations and have a one-year shelf life, once prepared there is no further monitoring of the quality of the supplements. Thus, controlling for potential variations in the quality of the supplements is not possible.

Data collection plan. Data about the following indicators of health are collected: CD4, CD8, CD3, viral and total lymphocyte counts (from blood samples), stunting and wasting (based on height and weight), and other physical growth indicators (e.g., upper arm circumference). Blood tests and physical growth indicators (e.g., anthropometric measures) are assessed every three months, except for children under five years of age, for whom these tests/measurements are performed monthly. Because there is an inverse relationship between viral counts and CD4 counts, it is expected that if the supplements have a positive nutritional impact, CD4 counts will increase while viral counts will decrease.

Food consumption data for the individuals who are the subjects of this study are also being collected to get a picture of the quantity and quality of the overall diet. Because hygiene has a direct effect on an individual's health, hygiene-related information, such as the WASH (water, sanitation, and hygiene) indicators are collected on at least some of the indicators during the trimonthly visit.

Design of cost-effectiveness analysis. Once the data collection is completed by the team, cost-effectiveness (C/E) ratios will be calculated for each of the three dietary supplements. Cost-effectiveness analysis will be used rather than benefit-cost analysis because the benefits of the dietary supplements cannot be adequately expressed in monetary terms. However, costs can be determined and impacts can be expressed by physical indicators.

The two parts of the C/E ratio will be determined as follows:

1. Costs will include buying, preparing, and delivering the supplements:
 - a. Costs of raw materials at the time of purchase. For items such as grain whose prices fluctuate, the price should be recorded at the time of each purchase or at critical times during the year to allow estimating an average price. If the supplement will be prepared at home, the cost of purchasing raw materials by household members needs to be collected.
 - b. Cost of preparing the supplements (inputs, labor, electricity)
 - c. Transportation costs (for the inputs used in producing the supplements and for transporting the supplements)
 - d. Cost of the usual diet in the household
 - e. Cost of preparing the supplement directly in the household
 - f. Cost of ARV drug treatment and any other medical treatments required by children who get sick due to their HIV status
2. Based on the statistical analysis of data from the dietary supplement trials, the effectiveness (impact) of the treatments will be evaluated using the following:
 - a. Extension of time during which children remain healthy, i.e., below the threshold at which they must be treated with ARV drugs
 - b. CD4, CD8, CD3 levels
 - c. Viral and total lymphocyte counts
 - d. Stunting and wasting measures
 - e. Upper arm circumference measurements

Potential analytical challenges. Once C/E ratios have been calculated, they must be evaluated, which involves several potential complications:

1. For cost-effectiveness analysis, the best option is the one that is most cost-effective, i.e., has the lowest C/E ratio; however, it is generally important to evaluate whether the best option is

acceptable in terms of absolute level of cost relative to the impacts achieved. (If impacts could be expressed in monetary terms, one could simply determine whether monetary benefits exceed monetary costs.) This can be done by reference to literature on other similar interventions, or by comparing the cost of the three dietary supplements with the cost of the typical household's diet in the zone studied, or to the cost of treating HIV-infected children with ARV drugs.



the governance mechanism in which the CSBs operate and the demographics of CSB members were also captured in the survey. The surveys were completed and the database was developed in August 2012. A total of 153 CSBs participated in the survey. The preliminary analysis of this survey data indicates that the average CSB was formed by seven members and had 21 percent female participation. Insight was also gained into the various types of CSBs. In 29 communities, a single farmer acted as the CSB by multiplying and disseminating seed to his or her community. In another region, a group of seven experienced seed producers formed a centralized seed multiplication and dissemination effort for six neighboring communities.

In August 2012, 480 Nicaraguan farmers were surveyed who were the beneficiaries of the seed distribution efforts of the Bean Technology Dissemination (BTD) project in 2011. The survey focused on the perceptions of the operations of the CSB as a seed multiplication and dissemination source in their community. The survey also asked about the quality of seed produced by the CSBs. The databases from the Nicaraguan farmer survey are in the final revision stage.

During the *primera* planting season 2012, seed production costs were recorded for each Nicaraguan CSB. The record keeping data will be used to estimate the cost of a CSB operation and, in combination with the other two surveys, will contribute towards a benefit/cost analysis of the CSB model.

CSBs plant high quality, registered seed (produced by INTA) to multiply bean seed for dissemination. Registered seed is the same seed used to multiply commercial certified seed but does not incur the cost of certification. Forthcoming comparison of CSB production costs and certified seed production costs vis-à-vis their benefits in terms of seed quality will reveal the relative net benefits gained from the CSB model in Nicaragua.

Objective 3: Build institutional capacity and develop human resources in the area of impact assessment research.

Although this project does not include a host country partner as in other CRSP projects, it does address the objective of institutional capacity building and human resource development through the following methods:

1. Field activities under objective 2 were conducted in collaboration with HC PIs and partners.
2. Activities under objectives 1 and 3 are conducted in close collaboration with the U.S. and HC PIs from existing CRSP projects.
3. The activities planned under this project involved four graduate students in the planning and conduct of field research. These students were recruited from within the Department of Agricultural, Food and Resource Economics at MSU as research assistants.
4. The project hired Byron Reyes as a postdoctoral fellow from January 2, 2012, to help execute some of the field activities and data analysis of this project.

2. The use of multiple measures of effectiveness means that multiple C/E ratios will be calculated for each dietary supplement. It is possible, therefore, that no single dietary supplement will be most cost-effective in terms of all impact indicators being analyzed. In that case, to select the best supplement, an index that encompasses impacts with respect to all indicators would need to be calculated or the trade-offs among the three dietary supplements assessed subjectively.
3. If the dietary supplements do not differ substantially in cost, it may be worth using a somewhat more expensive supplement than the one with the lowest C/E ratio, if the supplement with the next highest C/E ratio gives a significantly greater impact.
4. It will be important to assess and report qualitative or intangible factors that may differentiate the three dietary supplements, so that they may be considered along with the results of the quantitative C/E analysis.

Next steps. The plan is to continue the remaining data collection and analysis in the next phase of the CRSP project by including this activity in the impact assessment project.

Objective 2d. Case study of the bean seed multiplication and distribution system in Central America

Under this activity, the project team has completed three surveys:

1. A survey of 153 Community Seed Banks (CSBs) in Nicaragua
2. A survey of 480 Nicaraguan farmers who received bean seed in 2011
3. The cost of production record keeping by the Nicaraguan CSBs during the *primera* planting season 2012

The survey of Community Seed Banks in Nicaragua was designed to gain insight into the CSB formation, how they are structured, and what the design elements are that define them as a community seed bank. The socioeconomic conditions and



Networking and Linkages with Stakeholders

Mywish Maredia and George Norton (Impact Assessment PI for the IPM CRSP) were the co-organizers of a symposium entitled “Assessing the Impacts of Agricultural Research and Development in a Global Bio-Economy” at the 28th International Conference of Agricultural Economists in Brazil, August 2012, which included speakers representing five CRSPs. The symposium presented case studies on how investments in agricultural research by the U.S. Agency for International Development (USAID) through the Collaborative Research Support Programs (CRSPs) have improved the productivity, profitability, and sustainability of global agriculture. It also highlighted examples of methods and results of assessing the impacts of public goods science in improving human well-being.

Mywish Maredia was invited to participate in the development meeting of the CGIAR Research Program (CRP) 3.5 focused on legume crops organized by ICRISAT and held in Dubai in May, which was attended by representatives from four CGIAR centers and two CRSP directors, U.S. universities, consultants, and the Bill and Melinda Gates Foundation.

Leveraged Funds

Dr. Maredia and her collaborators successfully leveraged \$12,000 from MSU’s College of Agriculture and Natural Resources to support a Ph.D. student, in part due to the assistance received from the Dry Grain Pulses CRSP.

Publications

Reyes, B. 2012. *The Economic Impact of Improved Bean Varieties and Determinants of Market Participation: Evidence from Latin America and Angola*. Ph.D. Dissertation. Michigan State University. 203 pp.

Impact Briefs

Based on the research conducted by this project and past ex post impact studies, the project team developed and published three *Impact Briefs* in 2012:

Reyes, B.; Maredia, M.; and Bernsten, R. Improved bean varieties in Central America and Ecuador generate economic benefits to farmers. *Impact Assessment Research Brief* No. 1, July 2012.

Reyes, B.; Bernsten, R.; and Maredia, M. Sustaining a steady flow of high yielding, improved bean varieties through the bean research network in Central America. *Impact Assessment Research Brief* No. 2, July 2012.

Moussa, B.; Lowenberg-DeBoer, J.; Fulton, J.; and Boys, K. Farmers in West and Central Africa obtain economic benefits from enhanced cowpea storage technologies. *Impact Assessment Research Brief* No. 3, July 2012.



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-MSⁿ. 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*. Heritability of cowpea polyphenols was found to be strongly associated with the expression of seed coat color as a phenotypic trait. We confirmed that light brown cowpea lines high in tannins were the most effective oxidative stress inhibitors, whereas the black and red lines high in flavonols and anthocyanins were most effective against various anti-inflammatory markers. We also discovered that the chemical nature of the major tannin compounds specific to cowpea are such that they are less likely to interfere with bioavailability of essential micronutrients like zinc and iron than bioactive compounds common in other food plants. We also found that compounds in cowpeas can prevent processes relevant to intestinal inflammation. Thus cowpea has tremendous potential as a dual purpose food crop that provides essential nutrition and promotes health and disease prevention. Our findings lay the foundation for additional investigations that will uncover the specific health benefits of cowpea in human intervention studies. We believe the crop will feature prominently as a strategically important component of nutrition and health initiatives in Sub-Saharan Africa.

Project Justification and Objectives

Poor families in sub-Saharan Africa suffer high rates of malnutrition, especially the children, while diet-related chronic diseases have become a common phenomenon among urban African populations. For example, 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 is linked to depressed immunity and may lead to increased risk of chronic diseases, e.g., cancer in adulthood. In fact nutrition-related chronic diseases are becoming increasingly common in Africa, especially in urban areas, thus 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 the 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 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 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 healthy diet. This will lead to increased demand for cowpea and improvement in economic well-being of producers and overall health of consumers.

Objectives

1. Identify cowpea lines with 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 inheritance (heritability) of select bioactive traits in cowpea and genetic association between physical and bioactive traits.

Results, Achievements, and Outputs of Research

Objective 1: Identify cowpea lines with 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.

Gross phenolic composition was used to confirm heritability of composition in progeny. The following analyses were used for the screening: gross phenol content, anthocyanin pigments, and tannins content. Ground samples were extracted in 0.12 mol/L HCl in methanol. Anthocyanin pigment content was measured by pH differential method, which is based on measuring absorbance in pH 1.0 and pH 4.5 buffers at I_{\max} using a scanning UV-Vis spectrophotometer. The Folin-Ciocalteu method was used to estimate gross phenols content, by measuring reactant absorbance at 600 nm using gallic acid as the standard. The vanillin-HCl method was used for condensed tannin assay; reactant absorbance (with blank subtraction to correct for nontannin pigments) was measured at 500 nm, catechin was used as standard. In addition, we established a new rapid method for screening breeding populations for gross phenolic content using FT-NIR technology.

Flavonoids profiling. Sample extracts were washed through a C-18 column to remove sugars and other nonflavonoid constituents. Flavonoids were eluted using 70 percent acidified methanol, rotoevaporated and reconstituted in 10 percent methanol containing 10 mL/L formic acid and filtered through a 0.45 mm membrane before analysis. A reversed phase C-18 column was used for separation; and an Agilent 1200 HPLC system was used for characterization. MS analysis was performed using a Thermo-Finnigan TSQ7000 triple-quadrupole mass spectrometer equipped with an API2 source and an Electrospray Ionization (ESI) interface.

Phenolic acid and phenolate esters. Free phenolic acids were measured in methanol extract whereas alkaline hydrolysis of residue was used to measure esterified phenolic acids. Reversed phase HPLC separation, with appropriate standards, was used to identify the compounds; LC-MS was used for structural determination when needed.

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 initially cooked by boiling in water for 30 to 75 minutes (until soft) and then drying at 45 to 50°C. Protein content was measured using the combustion method. The complete amino acid and lysine profile was measured. In vitro protein digestibility was determined.

Results

Flavonoid composition of cowpea

The emphasis this year was to confirm previous year's data and identify major flavonoid compounds previously not identified.

1. *Anthocyanins.* We confirmed that black and green cowpea varieties accumulate eight major monomeric anthocyanin compounds. The relative proportions of these compounds did not change regardless of growth environment, indicating that genetics is the major factor controlling anthocyanin synthesis in cowpea.

We also identified the same anthocyanin compounds in a grey speckled cowpea variety, Mounge, not previously reported. The only difference between Mounge and the other cowpea varieties with anthocyanins was that in Mounge, malvidin-3-*O*-glucoside and petunidin-3-*O*-glucoside (both *O*-methylated anthocyanins) were the dominant compounds as opposed to the nonmethylated anthocyanins that dominate the black and green varieties. *O*-Methyl substitution can significantly impact bioavailability and bioactivity of phenolic compounds, and thus the nutritional implications of such compositional differences should be explored further.

2. *Flavonols.* The findings confirmed previous year's data that indicated major variability in flavonol composition of cowpea. Glycosides of quercetin dominated the flavonol profile of all cowpea lines tested, accounting for about 80 percent of flavonols in red cowpea varieties, and more than 90 percent in most of the other phenotypes. Myricetin and kaempferol were minor components. These proportions did not change regardless of growth environment, again confirming that genetics play a bigger role in determining flavonoid composition in cowpea. In general, we confirmed that the red cowpea lines contain the most flavonols, followed by golden brown lines. The white cowpea lines had the lowest flavonol content.

3. *Flavan-3-ols.* Due to the major impact tannins are known to produce on nutrient digestibility and micronutrient bioavailability, we were keen to understand the polymeric profile of the cowpea tannins we identified the previous year. Among the key factors that determine the biological effects of tannins are their molecular weight distribution and structure. Catechin and (epi)afzelechin were the major flavan-3-ol units that made up the tannin polymers of cowpea. Unusual composition was observed in all cowpea phenotypes with significant degrees of glycosylation in the monomers and dimers. Monomeric flavan-3-ols were the largest group of tannins (36 – 69 percent) in cowpea with catechin-7-*O*-glucoside accounting for most (about 88 percent) of the monomers. The oligomers with degree of polymerization (DP) 2 – 4 ranged from 0.41 – 1.3 mg/g (15 to 20 percent), whereas DP>10 polymers accounted for only 13.5 percent of the tannins. By contrast, in most other food crops, including legumes, oligomeric and polymeric tannins usually account for more than 97 percent of tannins. Given that the high molecular weight tannins are the ones that bind most strongly to protein and other nutrients, thus reducing their bioavailability, the composition of cowpea tannins is advantageous from a nutrient bioavailability perspective. As reported in the previous year, light brown cowpea lines had the highest tannin content regardless of growth environment. Follow-up studies using animals and humans will be necessary to demonstrate if the unique tannin composition of cowpeas translates to beneficial effects *in vivo*.

Gross phenolic

Regardless of origin, the light brown cowpea varieties once more had the highest phenol content on average, likely related to their high tannin content previously reported. For example, 09FCV-CC27M and Lutembwe, grown in Texas and Zambia, respectively, had phenol contents of 14.9 and 17.2 mg GAE/g, respectively). By comparison, white Early Acre variety had phenol contents of 2.6 mg GAE/g. In general, the phenol content of cowpea varieties analyzed were confirmed to have the following trend: light brown > black > red > golden brown > green > white, confirming that cowpea phenolic composition is highly influenced by seed coat color.

From the results, it is apparent that seed coat color has a major influence on the type of phenolic compounds accumulated by cowpea. Once more we confirmed that 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)

Darker-colored grains have been shown to contain higher levels of phenolic compounds than lighter-colored grains; however, for cowpeas, seed coat color is a good indicator of the type of phenolic compounds present but not their content.

Protein content and quality

In general, protein content of cowpeas was very similar across varieties and environments. The major cowpea lines in Zambia, M'sandile, Bubebe, and Lutembwe had protein contents of 23.5 – 25.6 percent. South African lines, Agrinawa, Agrigold, and Glenda had protein contents of 23.4 – 24.4 percent. The IITA lines maintained at Texas A&M and University of California, Riverside, tended to have higher protein content than East and Southern African lines. Lysine content and available lysine were also higher for the IITA lines compared to the East and Southern African lines. The values are for cooked cowpea. The average protein digestibility of cooked cowpea is 79 percent; the micronization process, which significantly reduces cooking time, had no deleterious effects on *in vitro* protein digestibility of cooked cowpeas.

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

The goal was to establish how the phytochemical profiles affect the ability of cowpeas to influence metabolic, cardiovascular, and chemoprotective health predictors *in vitro*. To support results reported in the previous year, enzymic digestion (simulating gastrointestinal conditions) of cooked cowpea samples was performed to better mimic what would happen in humans. The enzyme digests were compared to extracts obtained using standard procedures based on organic solvents. Additional pathways for anti-inflammatory properties of these compounds were also explored.

Hydroxyl/free radical scavenging properties. Protection against oxidative stress is an important component of chronic disease prevention. Antioxidant capacity of cowpeas and their fractions was measured by two widely accepted methods that involve hydrogen atom transfer (HAT) and single electron transfer (SET) that have been shown to correlate with biological oxidative status measures. The Trolox Equivalent Antioxidant Capacity (TEAC) was used for SET assay. Samples were reacted with preformed ABTS^o free radical, and the ability of the sample to quench the free radical measured after 30 min by monitoring color at 734 nm. Trolox was used as standard in both assays.



Inhibition of low density lipoprotein (LDL) oxidation. Oxidation of LDL leads to impairment in the regulation of cholesterol uptake, which potentially leads to development of atherosclerosis and cardiovascular disease. The ability of extracts from the cowpea/bean varieties to inhibit LDL oxidation was determined by monitoring formation of conjugated dienes at 234 nm.

Glycemic properties. Procedures described by Goni were used to measure rate of *in vitro* starch hydrolysis in selected cowpea lines. Hydrolysis index and estimated glycemic index was calculated from area under curve, using fresh white bread as a control.

Cell culture assays

Two strategies were used to assess how cowpea compounds can protect against cancer and cardiovascular disease.

Anti-cancer effects

1. *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. We used the NAD(P)H:quinone oxidoreductase (NQO) inducer activity. Murine hepatoma cells were incubated with various concentrations of cowpea extracts and NQO enzyme activity and cytotoxicity were measured. Sulforaphane was used as a positive control.
2. *Anti-proliferation assays.* These methods measured how the various cowpea extracts affect growth of preformed cancer cells. We used the HT-29 and Caco-2 human colon carcinoma cells for this assay following the viable cell (MTT) and DNA (PicoGreen) procedures as recently modified. Various concentrations of the cowpea extracts were incubated with the cells for 48 hours, after which the MTT assay kit was

used to measure viable cell population by established protocols. Double stranded DNA was measured; Genistein was used as a positive control in both assays. *Apoptosis* was assessed by analyzing in cells by analyzing PARP-cleavage.

Cardiovascular Disease

To determine the in vitro effects of phenolic extracts and fractions from cowpea on biomarkers for antioxidant properties and inflammation using human colonic myofibroblasts (CCD-18co), we measured:

1. *Biomarkers for inflammation* nuclear factor kappa B (NFκ-B), interleukins IL-6, IL-8, tumor necrosis factor TNF-α and Nf-kB was determined by ELISA assays. These biomarkers are typically used to assess inflammation, and cowpeas extract is expected to decrease LPS-induced inflammation in these cells.
2. *Antioxidant biomarkers.* Cells were treated with different extract concentrations and antioxidant effects were determined after different incubation times with the ORAC assay and the generation of reactive oxygen species. Additionally, oxidative stress was induced with hydrogen peroxide and the mitigation of pro-oxidant potential by different concentrations of cowpea extract was assessed. Oxidative DNA damage was assessed in the same manner; after the induction of DNA-damage with H₂O₂, the alleviating effects of cowpea was assessed with the ApoAlert™ DNA Fragmentation Assay.

Results

1. *Antioxidant capacity.* The data obtained last year were largely confirmed, with gross phenol content strongly correlating with antioxidant activity measured by various methods. Thus the light brown cowpea varieties tended to produce the strongest antioxidant activity, followed by red and black varieties.

As reported in the previous year for other oxidative processes, cooking produced a modest decrease in the ability of cowpea extracts to inhibit low density lipoprotein (LDL) oxidation. However, micronization significantly reduced the antioxidant capacities of all cowpea types against LDL oxidation. This observation was somewhat unusual, given antioxidant activity measured by other methods was not impacted by micronization by this magnitude. Additional work is needed to confirm the data.

2. *Anti-inflammatory properties.* As a follow-up to previous years' positive findings, we investigated additional inflammatory pathways relevant to immune response in the gastrointestinal tract. Reduction of mRNA expression of inflammatory biomarkers was dose-dependent. In general, the ability of cowpea extracts to reduce expression of interleukine-8 was most dependent on flavonol content; varieties with the highest level of flavonols (red cowpea) were most effective at the lowest concentrations tested (2 mg/mL). Interleukine-8 is involved in initiation and amplification of the inflammatory process. Similar results were observed for the tumor necrosis factor (TNF), a cytokine involved in chronic inflammation and proliferative cell signaling. Coupled with previous years' results, it is apparent that cowpea is a promising source of compounds that would be beneficial in controlling chronic inflammation and improving immune response, especially in the gastrointestinal tract.

3. *Antiproliferation and phase II enzyme activity.* The ability of cowpea extracts to inhibit colon cancer cell proliferation was compared to that of enzyme digests. Agrinawa (red) and Blackeye were used for this investigation. In general, a dose response was observed for cancer cell growth inhibition for both samples. Cooking samples modestly reduced the potency of the extracts, especially from the red Agrinawa sample, against cancer cell proliferation. This may be related to reduction in polyphenol content due to cooking as reported last year. The red cowpea extracts and digest were more effective against cancer cell proliferation than the blackeye variety. Enzyme digest more effectively inhibited cancer cell proliferation than organic solvent extract when compared based on the original cowpea sample, indicating that the standard organic solvent methods used to estimate bioactive properties of grains may be underestimating potential health benefits.

For phase II enzyme activity, cooked Agrinawa (red cowpea) produced the strongest induction of phase II enzymes; this may be related to the relatively high levels of flavonols in the red cowpeas. However, the overall effect was still modest compared to other known phase II enzyme inducing grains.

From this work, we have successfully identified important cowpea phenotypes that should be targeted in follow up in vivo studies using humans and animal models to confirm how the flavonoid composition of cowpea influences specific health benefits.

Objective 3: Elucidate the mode of inheritance (heritability) of select bioactive traits in cowpea and genetic association between physical and bioactive traits.

Greenhouse experiments were conducted to study the inheritance of antioxidant activity in cowpea. As indicated below, a number of crosses were made between 10 different parents selected based on their polyphenol content/antioxidant activity as low parent with high parent. These were:

1. Parents with low antioxidant activity:
 - Early acre, (white with thin black hilum line)
 - TX 2028-1-3-1 (green with black eye)
 - IT98K-205-8 (white with small black eye)
 - GEC (white with small brown eye)
 - Bambe 21 (white)
 - CB-27 (white with large blackeye)
2. Parents with high antioxidant activity
 - IT82D-889 (red self color)
 - IT97K-1042-3 (red self color)
 - IT97K-556-4 (light brown self color)
 - TVu 7778 (light brown self color)
3. The successful crosses made were:
 - Early Acre (low antioxidant) x IT82D-889 (high antioxidant)
 - TX 2028-1-3-1 (low antioxidant) x 1042-3 (high antioxidant)

- IT98K-205-8 (low antioxidant) x IT97K-1042-3 (high antioxidant)
- GEC (low antioxidant) x IT97K-1042-3 (high antioxidant)
- Bambey- 21 (low antioxidant) x IT97K-556-4 (high antioxidant)
- CB-27 (low antioxidant) x IT97K-556-4 (high antioxidant)
- Bambey -21 (low antioxidant) x TVu 7777-8 (high antioxidant)

The resulting F1 seeds were grown in the greenhouse and F2 populations were generated for most of the crosses. In view of the significant association between seed color and antioxidant content, notes were also taken on seed phenotypic traits in F1 and subsequent generations. Various combinations of seed colors were observed in F2 and F3 seeds, including complementary gene action for seed color in some crosses.

Analysis of total polyphenols and condensed tannins using Fourier transform near infrared spectrophotometer (FT-NIR)

A nondestructive method was developed to estimate total polyphenols and total condensed tannins in seeds of crosses. A standard curve for total polyphenols, total condensed tannins, and antioxidant activities was established using 60 diverse varieties analyzed for these values using wet chemistry. Originally these 60 samples were analyzed in triplicates using the UV spectrometric method. The values from wet chemistry were plugged in equations of FTNIR scans to establish correlations. High correlations were observed between the FTNIR data and wet chemistry methods for polyphenols and tannins, respectively. Antioxidant activity did not correlate with the scans. A set of 28 more samples was validated against the correlations to determine the performance index, which was found to be higher than 65, an indication of a good validation. This method is currently being used to scan seeds from the above-mentioned crosses to determine the inheritance of polyphenols and tannins in cowpea.

HPLC method. Select samples were characterized by HPLC using methods described under objective 1 to confirm association of seed coat color with flavonoid composition.

Results

The analysis of the genetic materials is in progress. Some interesting preliminary observations are:

Analysis of flavonoids and anthocyanins

The F1 plants from the cross involving Bambey 21 (white selfed) and IT97K- 556-4 (light brown selfed) produced black seeds showing a complementary gene action for seed coat color.

The results showed complete absence of anthocyanins in both parents but very high levels in the hybrid, indicating gene complementation for anthocyanin accumulation. In addition, hybrid vigor in flavonol content was observed in the hybrid, where accumulation of quercetin derivatives increased compared to either parent.

As previously reported, these results indicate that the amount of bioactive compounds is significantly correlated with the seed color. Also, the genetic segregation for seed color seems to determine the genetic segregation for the bioactive compounds. Based on the current data, there may be only two to three pairs of genes controlling these traits. Further analysis of more F2 and backcross populations are in progress and the results would provide further insight into the exact nature of inheritance.

Thus, even though it is apparent that heritability of seed coat color in cowpea is complex, the fact that the seed coat color itself is a good indicator of the type of flavonoid compounds accumulated by the seed may be more important. This is because our findings demonstrate that the type of flavonoids present in cowpea seed coat correlate with indicators of specific bioactive properties. Further studies involving the F2 and F3 populations are in progress.

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.

Pulse Value Chain Initiative— Zambia (PVCI-Z)

KSU-1

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Abstract of Research Achievements and Impacts

The final year of operation was focused on completing data collection and analyzing data, testing hypotheses, and envisaging implications of the results from the study toward the Feed the Future goals of reduced poverty and enhanced nutrition. This report provides an overview of the project's activities over the past three years. The major accomplishments are that the number of people trained under the institutional capacity building initiative was exceeded due to stewardship. Additionally, the project built enduring relationships with local institutions, such as Zambia National Farmers Union (ZNFU) and the Southern Africa Bean Research Network (SABREN) to work on improving understanding and operation of the bean and cowpea value chain. Finally, the governance experiments, started with a small group of producers, have begun yielding enduring results—reducing operations costs and increasing incomes.

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 of the 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, probably because they are more drought tolerant than the traditional maize crop.

Despite their importance in the country, knowledge about bean and cowpea value chains is overwhelmingly anecdotal. It is particularly unclear 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 overall profitability of production activities. Securing more and 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 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. determining the most efficacious value chains given producer and partner characteristics
3. working 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 the distribution of new value created is done in an equitable and fair manner.

The foregoing is in line with the overall Pulse CRSP 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.

Objectives

1. 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.
2. Identify and estimate the effects of stakeholder characteristics on producers' supply chain participation decisions.
3. Describe and estimate the pecuniary and nonpecuniary value for different supply chain participants.
4. Identify the institutional and policy issues influencing value creation and determine if any differences exist by crop, location, gender, and stage of the chain.
5. Pilot at least one governance system to identify the factors and characteristics of participants who influence performance.
6. Based on the results from the foregoing, develop and deliver education and outreach programs targeting specific stakeholders and provide policy recommendations to facilitate solutions.

Results, Achievements and Outputs of Research

Primary data is used to achieve the objectives of the first category objectives described above. This study was conducted in three districts: Kalomo in Southern Province, Lundazi in Eastern Province, and Mbala in Northern Province. These districts were selected on the basis of their relatively high levels of bean (Mbala and Lundazi) and cowpea (Kalomo and Lundazi) production. Production estimates used in this decision were determined using data from the 2008 rural income and livelihood survey (RILS) by the Central Statistical Office (CSO), the Ministry of Agriculture and Cooperatives (MACO), and the Food Security Research Project (FSRP).

A two-stage cluster sampling strategy was used to select sample households from the three districts.

The survey was conducted between November 2011 and February 2012 and resulted in a total of 1,050 households being interviewed. After stringent data cleaning, a total of 1,000 usable responses (95.2 percent) of the original dataset remained. Their distribution shows that the majority of respondents (435) were in SEA 2 while SEA 1 and SEA 3 accounted for 295 and 269 respondents each; however, the distribution across the three districts used in the study was as follows: Lundazi, representing

Eastern Province (297); Mbala, representing Northern Province (349); and Kalomo, representing Southern Province (354). The figure shows that the cleaned data results in an underestimation of the optimal 350 sample size for Lundazi District by about 15 percent and overestimating Kalomo by about 1 percent. Mbala District was the only one statistically equal to the estimated optimal size. Because the appropriate sampling weights were applied in all the analyses, and because the sampling focused on centers of production concentration in the identified crops, confidence in the inferential power of the dataset is preserved despite these slight deviations from the optimal sample sizes per stratum.



Another preparation of the data was to identify extreme outliers with the possibility of unduly skewing the results. Given that the majority of the respondents were smallholder farmers, total household cropland was determined to explain the extremeness of the outliers responses that were three or more standard deviations from the mean; however, because the study was interested in smallholders of all sizes, the screening rule focused only on those respondents whose cropland holdings were three or more standard deviations higher than the estimated mean. This reduced the cleaned sample from 1,000 to 940, indicating that about 2.6 percent of the original usable dataset was large enough to possibly distort results. This small group of large producers have been grouped into a different category and not used in any of the analyses in this report.

The summary weighted demographic statistics for the respondents shows that the nearly all respondents (90.2 percent) indicated farming as their primary occupation. About 14.0 percent of the respondents were female and 86.5 percent were married, with nearly 68 percent of respondents growing mixed beans compared to 52.5 percent cowpea; approximately 97 percent produced maize. A little more than 27.0 percent of the respondents were located in Eastern Province, and Southern Province, respectively, while 45.2 percent resided in Northern Province.

The weighted sample average age was approximately 42 years, with a standard deviation of about 15 years. The average formal education was 6.2 years, with a standard deviation of about 3.4

years, implying that a typical respondent had only completed primary school. Weighted average cropland was about 8.3 ha with a standard deviation of 6.7 ha and ranged from 0.3 ha to 37.0 ha. This is higher than the less than 4 ha estimated average cropland holdings for smallholders in sub-Saharan Africa. The average annual income of the sample was ZMK 3.1 million with a standard deviation of ZMK 6.4 million and had a range of almost ZMK 72 million. (The Zambian Kwacha [ZMK] exchanges at about ZMK 5,000 to US \$1.00.) The World Bank estimated gross national income for capita (purchasing power parity approach) for 2011 was US \$1,490, equivalent to ZMK 7.45 million. This would suggest that the average income of the sample is significantly lower than the national average income, an observation that is not surprising given that most rural people's incomes are below the national average. This measure also provides a benchmark for designing policy as the opportunities embedded in mixed beans and cowpeas are explored to enhance producer incomes and reduce poverty.

It is known that smallholder farmers often undertake multiple cropping; this was true in Zambia. The results from the survey show that 189 households planted maize, mixed beans, and cowpeas together but only three households planted both mixed beans and cowpeas. While only three households planted only cowpeas and maize, 402 households planted both beans and maize, while 322 planted both cowpeas and maize. The correlation between maize and mixed beans acreage was positive (0.18) and significant at the one percent level, while the correlation between maize and cowpeas was not significant. The correlation between mixed beans and cowpeas was, as expected, negative (-0.12) and statistically significant at the one percent level. The correlation between available cropland and the acreages of these three crops was positive and statistically significant at the one percent level.

The issue of gender is important in development conversations because of overwhelming research showing the critical role women play in the nutrition status of their families, the education of children, and agricultural production; however, research shows that women tend to control less production resources in most communities, including smallholder agricultural communities. Therefore, the gender and regional differences in these summary statistics are evaluated to determine gender and regional disparities in the sample. The results show that there were statistical difference between men and women in five characteristics: marital status, mixed beans, education, age, and income. There were about equal proportions of men and women in the different provinces and their cropland ownership was also about the same.

The weighted summary statistics of all crop survey respondents indicated planting in 2010 was as follows: The largest average crop area was maize, which was unsurprising given the place of maize in Zambia's food profile. It received an average of 2.6 ha and ranged from about 0.2 ha to 22 ha. Mixed beans and cowpeas were the second and third crops with the largest areas, with mean areas of approximately 2.3 ha each. Eleven other crops had an average area of 2 ha or more but only ten crops had more than 100 producers reporting. For example, burley

tobacco had an average area of 3.7 ha but only eight producers reported its production. Similar, the average area for millet was about 2.1 ha but only 68 producers reported producing the crop.

Thus, taking the crops that are planted by more than 100 producers, it is observed that mixed beans and cowpeas were in the top five crops based on the average land allocation; however, ranking by number of participating producers, these two are the second and third most important crops to the producers, more important than cash crops, such as groundnuts and seed cotton.

The weighted statistics of households' output for the various crops indicates that maize output was the highest among the food crops, with a weighted average in excess of 3,000 kg per household. For nonfood crops, burley tobacco had the highest output, averaging 3,350 kg per household; however, unlike maize, which had 914 households, burley tobacco involved only eight households. The average output per household for mixed beans and cowpeas was about 257 kg and 85 kg, respectively.

Female rankings of production for maize, mixed beans, and cowpeas were the same as for the total population, albeit with lower averages. For example, average maize output by women was 2,169.7 kg, about 39 percent lower than the estimated mean for the total average output for maize. Mixed beans average output was 141.3 kg, again lower than that of the average mixed beans output, but with a smaller standard deviation of about 187 kg.

The importance of mixed beans and cowpeas cannot be judged solely by their yield indicators. For example, they do not feature among the top 10 crops; however, if evaluated by number of households producing the crop, then mixed beans and cowpeas take the second and third positions, respectively, as found in the allocation of cropland.

The cropland productivity for maize, mixed beans, and cowpeas is presented in this section. Weighted average yield for maize was 1,660.6 kg/ha while yield for mixed beans and cowpeas was 234.9 kg/ha and 147.7 kg/ha, respectively. On-farm cowpea yields in Africa have been estimated at about 240 kg/ha—significantly higher than the average estimated for the most intensive cowpea-producing regions in Zambia. The principal rationale for this low yield may be because cowpeas are not as important in Zambia as they are in Nigeria and other West African countries. The average yield for mixed beans was estimated at 235.3 kg/ha. Similarly, the average mixed beans yield for the 2007 to 2010 period in neighboring Malawi and Zimbabwe was estimated at 568.8 kg/ha and 494.2 kg/ha, respectively. Average maize yield for the three provinces was lower than the national average of approximately 2,587.1 kg/ha, which is not surprising given that the locations were selected for their mixed bean and cowpea production concentration.

Evaluating the average yields by gender shows that there are statistically significant differences between men and women for mixed beans and maize but not cowpeas. Average yield for women was 73 percent that of men for maize, 63 percent for cowpeas, and 58 percent for beans. The difference in performance between the genders was highest for mixed beans; this difference was significant at the one percent level. The 95 percent confidence

interval for this difference ranged from 39.4 kg/ha to approximately 169.3 kg/ha across the three provinces. Like mixed beans, the difference between men and women for maize yield was significant at the one percent level, with a 95 percent confidence interval ranging from 134.7 kg/ha to more than 808.0 kg/ha.

Objective 1: 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.

Market participation has been identified as critical to enhancing the performance of smallholder agricultural producers in developing countries. Certain nonmarket factors, such as institutional factors, influence market participation more than prices; yet the extent of the influence of market and nonmarket factors, including transaction costs, pivots the focus point of the market participation decision on potential marketed surplus and the household's economic situation. For example, the household's cash needs balanced by its food needs would determine the extent of market participation for certain food crops, especially staples.



The distribution of responding households by the channels they were involved in started with identifying the intensity of market participation in the dataset. The data revealed that of the 538 households that produced dry mixed bean grains, and 468 produced dry cowpea grains. Of the total number of households producing mixed beans, about 66 percent of them participated in some manner in the market, i.e., sold part or all of their production. The remaining 34 percent did not sell any of their production. This implies that for this group of 208 households, mixed beans production was solely for nonmarket use—home consumption, gifts, seed, and in-kind payment for goods and services. About 32.7 percent of cowpea-producing households participated in the market, representing about 150 households. The remaining 67.3 percent used their production for non-market exchanges—home consumption, gifts, seed, and in-kind payment for good and services. Market participation intensity for cowpea producers was 32.7 percent, representing 150 of the 468 households that produced the crop.

Evaluating the foregoing distributions on a gender basis shows that there were not a lot of proportional differences between the sexes with respect to market participation intensity. While nearly 70 percent of both men and women produced but did not sell dry grain cowpeas, only about 38 percent of men and 41.3 percent of women produced but did not sell dry grain mixed beans. The figure shows that the market participation intensity for men producing dry grain beans was slightly higher than for women, but there was virtually no difference between the market participation intensity for male and female cowpea-producing households.

The nodes in the supply chain are defined to encompass consumers, retailers, traders, and institutions. Traders include both local and foreign traders while institutions encompass hotels and restaurants, processors, brokers, NGOs, cooperatives and trade associations, schools, universities, hospitals, and government agencies.

Seventy-eight mixed bean market participants sold directly to consumers, while only 58 and 124 sold directly to retailers and traders. About 129 mixed bean market participants sold directly to institutions, equivalent to about 39 percent of all mixed bean market participants. Five of these participants sold to both consumers and retailers while nine sold to both consumers and institutions. While 122 sold to traders and not retailers, two sold to both.

Fifty-seven cowpea market participants sold directly to consumers, while only 22 and 67 sold directly to retailers and traders. The proportion of cowpea market participants selling directly to traders was estimated at about 45.6 percent. About 14 cowpea market participants sold directly to institutions. Only one of these participants sold to both consumers and retailers and to both traders and institutions. No one selling cowpeas directly to retailers sold to institutions.

The total sample for this research has 86.1 percent men and 13.9 percent women. This provides a framework for assessing the gender effect on market participation. Mixed bean market participation by gender was slightly off from the distribution of population by gender. The proportion of mixed bean market participants who were male was about 88.5 percent compared to 11.5 percent female. Of all mixed bean market participants, about 18.6 percent sold directly consumers compared to 39 percent selling directly to institutions. Of the number selling to consumers, 89.7 percent were men and 10.3 percent women. Women were most active at the institutional node in the mixed bean market, accounting for nearly 5.6 percent of all market participants and almost 15 percent of those selling directly to institutions.

The proportion of male cowpea market participants was about 87.7 percent compared to 12.3 percent female. Of all mixed bean market participants, about 18.6 percent sold directly to consumers compared to 39 percent selling to institutions.

Objective 2: Identify and estimate the effects of stakeholder characteristics on producers' supply chain participation decisions.

It has been shown that upon deciding whether to participate in the market, decision makers choose the supply chain nodes that they want to use. Do the characteristics of the producers influence their choice of supply chain node used to directly participate in the market, and if so, which ones are statistically significant in explaining choices among supply chain node?

A logistic regression model is used to determine the probability of different household characteristics influencing choice of market channels selected. The generic model is expressed as follows:

$$\theta_{kj} = \begin{cases} 0 & \text{if } j=0 \\ 1 & \text{if } j=1 \end{cases} = \frac{\exp(\alpha + \sum \beta x_i)}{1 + \exp(\alpha + \sum \beta x_i)} \quad (1)$$

where a = the constant of the model equation, β = the coefficient of the predictor variables, and j is the node choice where $j=1$ implies the node is selected by the decision maker and is zero if it is not. The alternative nodes, k , are consumer, retailer, traders, and institutions. The independent variables of interest are gender, education, location (province), cropland (as an indicator of household wealth), and the proportion of that cropland allocated to the crop of interest. The presented results show percent change in the probability of choosing a particular supply chain node as a result of a percent change in any of the explanatory variables. This offers a response indicator akin to the traditional elasticity measure, making it a lot easier to explain the effects of these producer characteristics' effect on producers' binary choice at each supply chain node.

The results of the question, *What is the percentage change in the probability of choosing to participate at a selected node when there is a percentage change in the explanatory variable of interest?* show that there is a 29 percent higher probability of selling mixed beans to consumers if the market participant is located in the Northern Province instead of the Eastern Province. Similarly, a percent increase in the proportion of cropland allocated to mixed bean production increases the probability of selling to consumers by 2.3 percent. On the other hand, a percent increase in the total cropland owned by household leads to a 5.3 percent decrease in the probability of participating at the consumer node in the supply chain. There seem to be no statistical difference between market participants selling to retailers and those not selling to retailers for all variables, with the exception of location in the Southern Province and education. The percentage increase in the number of years of formal education decreases the probability of selling to retailers by 4.2 percent. Incidentally, this is the only node at which education proved to be statistically significant in explaining choice.

Gender is statistically significant at five percent in explaining the selling to traders and shows that the probability of a female mixed bean market participant selling to traders is about 13.1 percent lower than men selling directly to the same supply chain node. Also, there is a 113 percent lower probability of selling mixed beans to traders if the seller is located in the Southern

Province than in the Eastern Province. Finally, location in the Northern Province increases the probability of selling to the other category of traders by more than 88 percent, in comparison to the Eastern Province.

The situation for cowpea market participants is different in certain ways. Women have about a 23.4 percent lower probability of selling cowpeas to consumers; being located in the Southern Province increases the probability of selling to consumers by 14.4 percent while being located in the Northern Province decreased it by almost 54 percent relative to the Eastern Province. Education, cropland, and cowpea share were all not statistically significant in their explanation of whether a market participant sold cowpeas to a consumer; however, being located in the Northern Province decreased the probability of selling cowpeas to retailers by almost 116 percent and to traders by 177 percent, relative to the Eastern Province. Cowpea share of total cropland presented a positive effect on selling to secondary, tertiary, and higher level nodes in the cowpea supply chain. A percent increase in cropland holdings increased the probability of selling to this node category 0.5 percent.

Objective 3: Describe and estimate the pecuniary and nonpecuniary value for different supply chain participants.

Smallholder producers' business objectives are multifaceted, encompassing family food security and cash needs. The inclusion of multiple variables in smallholder producers' decision framework suggests a more comprehensive approach to assessing the value emanating from their activities. Food security considerations suggest that most smallholder producers focus on ensuring their families have enough to eat from the production they undertake.

To appreciate the total value of a crop requires an immersion and understanding of the socioeconomic activities of the producers. The complexity of capturing nonmarket products and the intangibility of the benefits emanating from these products can lead to significant variability in the value of production agriculture for subsistence crops. Within the context of these challenges, it is observed that beans and cowpeas are produced in Zambia for more than the dry grain that is often the focus of value measurement. For example, the bean's young leaves, green pods, and fresh bean grains are used as vegetables, alleviating food insecurity problems, which are often acute prior to harvest. These early foods are also good sources of iron and zinc micronutrients; the same is true of cowpeas. Little knowledge exists on the effect of leaf harvesting on grain yields and the profitability of cowpea (and similar) production systems. It is this potential interaction between the harvesting of other components of the crops that makes evaluating the full value of the different harvested parts of the crop so crucial in assessing its value to the producer. Some households may harvest the stover for animal feed or for fuel while the pods may be used as animal feed, spread on land to increase organic matter content, and/or burned as fuel. Estimating value of such crops solely on the dry grain yield may, thus, be underestimating their value to the household. Generally, the value of production is often

limited to the traded quantity of dry grain. The foregoing discussion suggests that more components of the crop should be brought into the calculation of production value. Focusing only on market surplus of dry grains leads to significant underestimation of value, which explains why small producers are often shown to generate negative net incomes from production yet continue to produce, leading to the erroneous conclusion that they are economically irrational.

The total value of production valuation model used in this research is defined as follows:

$$TV = NPV + PV \tag{2}$$

where TV is total value associated with each crop and NPV is the nonpecuniary value, while PV is the pecuniary value of each crop. Assuming each crop is harvested for leaves, fresh grain, green beans, and dry beans, denoted by the subscript i , and that each of these products may be consumed (c), given away as gift (g), or stored for use as seed (s), denoted by subscript j , then if the imputed price for each of the products from the crop is defined as p_j , then the total nonpecuniary value from the crop may be defined as:

$$NPV = \sum_j \sum_i p_i r_{ji} q_{ji} \tag{3}$$

where r_{ji} is the proportion of the quantity (q) of product i that is used for purpose j . The pecuniary value component is defined as the reported revenue received from the sale of the different products from each crop at different nodes of the supply chain. It is computed as follows:

$$PV = \sum_i R_i = \sum_i p_i Q_i \tag{4}$$

where R_i is the reported revenue from the sale of product i and Q_i is the quantity of product i sold while p_i is as defined above.

Recall from the summary statistics that production of dry mixed bean grain averaged 257.2 kg while dry cowpea grain averaged 85.1 kg for the weighted sample. Their standard deviations were respectively 397.2 kg and 173.3 kg. Mixed bean output ranged from a low 2.3 kg to 3,240 kg, while cowpea's ranged from 1.1 kg to 1,800 kg. Respondents were asked to estimate the quantity of dry grain that they did not sell and break it down into the quantity consumed, kept for seed, given away as gift, and lost due to pests, breakages, or other challenges.

The total dry grain not sold by households but used for consumption and/or seed was 75.2 kg; the average for men was 77.6 kg while that for women was 59.8 kg. A similar ranking is observed for dry cowpea grain, with the average of male-headed household being 51.5 kg and female-headed households 26.4 kg.

The distributions of the proportion of total dry grain crop not sold by gender and province shows that while the average dry mixed bean grain not sold for men and women was about equal at 64.7 percent and 64.2 percent in Eastern Province, it was about 47 percent and 61.4 percent in Northern Province. The average dry mixed bean grain not sold was highest in Southern

Province, implying that in Southern Province mixed beans are produced essentially for household consumption. Overall, it is observed that a larger proportion of dry cowpea grain is not sold in all provinces and by both genders.

Zambian smallholder farm households harvest more than dry grain from their production activities. The average dry mixed bean revenue was about ZMK 578,470. With 367 respondents providing information, dry bean sales revenue ranged from ZMK 7,500 to ZMK 8.9 million. Revenue from fresh grain was the second largest source of sales revenue.

As in the case of mixed beans, the largest single source of revenues for cowpea products was dry grain. Its weighted average revenue was approximately ZMK 172,000. Leaves, both fresh and dried, provided more revenues for cowpea producers on average than did fresh grain, about ZMK 35,000 and ZMK 41,000, respectively.

The summary weighted statistics of the total pecuniary value for the two crops have been measured by province and gender. On average, women's pecuniary value from mixed beans was 46 percent lower than their male counterparts. For cowpeas, the female average was nearly ZMK 117,000. The male average for cowpeas was ZMK 135,347. The female average pecuniary value from cowpea production was estimated to be only 14 percent lower than that for males in the sample. Given that the total weighted number of women in the sample was 124, the foregoing indicates that female market participation is just about 51 percent for mixed beans and 36.3 percent for cowpeas; male market participation for mixed beans and cowpeas was respectively 57.2 percent and 39.3 percent. The difference between the genders was statistically significant for mixed beans but not for cowpeas.

The total nonpecuniary value for mixed beans ranged from about ZMK 1,700 to ZMK 3.9 million, while that of cowpea ranged from just over ZMK 3,000 to ZMK 10.7 million. The average for women and men was respectively ZMK 365,422 and ZMK 326,214 for mixed beans and ZMK 125,816 and ZMK 242,345 for cowpeas. The minimum of total female

nonpecuniary value for mixed beans was about seven times that of men, while minimum for cowpeas was the same for both. The maximum nonpecuniary value for cowpeas for men was a lot higher, about 20 times, than that of women. For both crops, the total nonpecuniary value for men was higher in Eastern Province and Southern Province; however, in the Northern Province, weighted average mixed beans' nonpecuniary value was higher for women by more than ZMK 100,000.

Compared to the total average pecuniary value, the number of smallholder households reporting nonpecuniary values for both crops and both genders and in all regions was higher.

Finally, the summary statistics of total value to smallholder households producing cowpeas in the three major beans and cowpea producing provinces in Zambia indicate that mixed beans are a far more valuable crop across the three provinces than cowpeas, with a greater number of participants, too.

As expected, the average total value of producing these crops was higher for men, but the difference in average total value was not statistically different for mixed beans but statistically different for cowpeas. In short, men generally have equal to higher incomes than women for both crops in all regions.

On average, total nonpecuniary value accounted for about 58.3 percent and 75.7 percent of total value from mixed beans and cowpea production in the three Zambian provinces. It is, however, observed that nonpecuniary value accounted for 100 percent of total value in all situations considered—gender, crop, and province, but the range was as low as 0.4 percent for men growing mixed beans in Northern Province and 6.7 percent for men growing cowpeas in Eastern Province. As expected, the weighted average share of nonpecuniary value of cowpea production was above the overall average.

There was no statistical difference between the genders within crops within provinces, with the exception of cowpeas in Southern Province, suggesting that the share of total value from these crops to smallholder households in the three Zambian provinces did not differ between the genders.

The results indicate that the difference between the mean total value from mixed bean production in Northern and Southern Province was ZMK 346,150. Therefore, the hypothesis that mixed bean production is more valuable in Northern Province than in Southern Province cannot be rejected. The difference between Northern Province and Eastern Province was estimated at ZMK 507,494. Thus, as before, the hypothesis that mixed bean production is more valuable in Northern Province than in Eastern Province cannot be rejected. The mean total value of cowpea production for Southern Province was ZMK 182,910 compared to ZMK 346,296 and ZMK 183,852 for Eastern Province and Northern Province, respectively. The difference between Southern and Eastern Province was negative ZMK 163,386. Both hypotheses relating to cowpeas are rejected because (1) in the case of the comparison with Eastern Province, Eastern Province's total value from cowpeas was higher, not lower, and (2) in the case of Northern Province, the difference is also negative but not significantly different.



Objective 4: Identify the institutional and policy issues influencing value creation and determine if any differences exist by crop, location, gender, and stage of the chain.

Producers' perceptions of five policies were evaluated:

- seed and fertilizer subsidies
- extension services
- special training for producers
- readily available markets as offered through the food reserve agency's policies for maize
- producer price controls, such as seen for maize

The results show that the proportion of producers who perceived the use of extension services to support and/or encourage mixed bean production was the highest at 11.4 percent, followed by the availability of special training for producers at 5.25 percent. Availability of input subsidies was seen by 5.0 percent of mixed bean producers as a tool that the government has used to help expand and/or support their efforts. Market availability and price controls received the lowest perceptions—about 2 percent each. For cowpea producers, the highest proportion of producers was 4.9 percent, found under the use of subsidies, followed by 4.8 percent for extension; the remaining policies received 3 percent each. There were no statistical differences between men and women in these perceptions for both crops.

Objective 5: Pilot at least one governance system to identify the factors and characteristics of participants who influence performance.

The pilot governance initiative was conducted at only one stage in the supply chain: traders. Eleven traders were organized into a purchasing group focused on reducing transaction costs in the procurement process. They were trained in understanding the components of their procurement costs and organized into an informal buying cooperative. The research group tracked their purchasing and sales information; the results show that their transaction costs declined in the short period after the project started.

Objective 6: Based on the results from the foregoing, develop and deliver education and outreach programs targeting specific stakeholders and provide policy recommendations to facilitate solutions.

The following policy recommendations are suggested for intervening in the bean and cowpea markets to help smallholder producers:

1. Provide education and training through the extension system and other programs to support the appreciation of potential food security value embedded in the production of these crops.
2. Women were underendowed in the primary production resource: land. This affected their productivity and the production. Yet, women tend to have the primary responsibility for ensuring adequate nutrition for their families. Policies aimed at ameliorating these resource gaps among women will

be very helpful in facilitating the achievement of the nutrition objective of the Feed the Future Initiative.

3. The governance experiments with traders proved that providing business training can lead to significant improvement in well-being in very short periods.
4. Market expansion could contribute to production increases.

Networking and Linkages with Stakeholders

- Maintained operational communication with collaborating institutions: ZNFU, Central Farmers Association, and Central Statistics Office
- Maintained briefing and exchange meetings with USAID Mission staff
- Worked with SABREN in collecting primary farm-level data during survey instrument development and sampling process
- Worked with CIAT through SABREN to collect data on traders' activities

Leveraged Funds

SABREN provided \$6,000 and personnel support to help with the data collection as well as evaluation of survey instruments and testing of the instruments.

Contribution of Project to Target USAID Performance Indicators

The number of individuals enrolled in degree programs was 14. Of these, eight were women and six men. There were eight students in undergraduate programs and the remaining in postgraduate programs at the University of Zambia and Kansas State University.

We proposed to conduct one policy study and completed seven under the project. These were in the form of specific student theses that focused on both understanding the pulse value chain and providing policy direction on how to address identified challenges.

Contribution to Gender Equity Goal

Of the nine undergraduate students supported by the project, six are women; of the five graduate students, two are women.

Dry Grain Pulses CRSP

Institutional Capacity Building and Human Resource Development FY 2012 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 nondegree training, and the purchase of equipment or other special funding 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 2012 activities for the entire Pulse CRSP program.

A. Degree Training

The Dry Grain Pulses CRSP is continuing to make human resource development and institutional capacity building a priority objective for all projects awarded. All Pulse CRSP projects include resources for institutional capacity building for our partner/host countries and each project supports a minimum of two to three degree candidates—and our Host Countries nationals focus on this as a key contribution to their research systems.

By design, Pulse CRSP degree training is closely linked to research activities and aligned with CRSP project research and outreach objectives. Researchers work to integrate graduate students into the research and outreach activities; dissertation research is focused on problems that are relevant to the Host Country context in addition to contributing 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 of the socioeconomic, 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 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.

Annex 1 provides data on all the degree trainees financially supported by the Dry Grain Pulses CRSP from October 1, 2007, through September 30, 2012. A total of 81 students were either fully or partially supported in graduate or undergraduate degree programs in FY 2012, with 45 currently active students. Table 1 provides the summary statistics on the formal degree training investments.

	No. of Trainees
Training Status	
▪ Active (during FY 12):	80
▪ New trainees	8
▪ Delayed/Pending	0
▪ Discontinued/cancelled:	1
▪ Training Completed:	34
Profile of “Active” trainees (45)	
<u>Gender</u>	
▪ Male	19
▪ Female	26
<u>Region of Origin</u>	
▪ East Africa	12
▪ Southern Africa	15
▪ West Africa	11
▪ Latin America/Caribbean	3
▪ United States	2
<u>Degree program</u>	
▪ M.S.	27
▪ Ph.D.	16
• B.S.	10
<u>Training Location</u>	
▪ 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, 2012.

There were nine graduate students at U.S. universities in 2012 that were indirectly or only partially supported by the Dry Grain Pulses CRSP. Leveraging resources enables us to ensure formal training for a larger number of young professionals. These are students with research assistantships or other stipends who are conducting their research in the host countries in collaboration with the HC PIs. CRSP funds 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. While these graduate level degree students are not participant trainees, they are still included in the Pulse CRSP Trainee database, given the support that they do have and the importance of their contributions (Annex 1). Under the Pulse CRSP, subcontracted U.S. universities supporting graduate students on research assistantships are providing 25 percent 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 also noteworthy that 29 of the total 45 degree students supported by the Pulse CRSP in FY 2012 were enrolled in universities either in Host Countries or in academically advanced institutions in other countries (e.g., South Africa) than the United States. 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 lectures as well as serve on the guidance committees of graduate students. Finally, economics are

achieved by supporting the training of USAID sponsored students at universities in countries in Africa and Latin America. The Pulse CRSP remains committed to finding opportunities in regional universities that provide the high quality training expected for CRSP research.

B. NonDegree and Short-term Training

Nondegree training and short-term training are also considered 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 (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 nondegree training is integrated with research activities and is incorporated into the annual research workplans of each research project.

In FY 2012, an estimated 11,868 individuals benefitted from short-term training subcontracted through Phase II and III projects in the Dry Grain Pulses CRSP. Beneficiaries included farmers, researchers, students, government analysts and others. Of these short-term trainees, more than 49 percent were female. Table 2 presents a listing of selected short-term training activities completed in FY 2012. Experience has shown that short-term training is an effective strategy to build the capacity of technical staff at NARS and agricultural universities. Often, 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. Short-term training also fits the needs of farmers and extension agents when specific technologies are available. The Pulse CRSP funding of short-term training enables host institutions to work with CRSP PIs and colleagues to develop training materials that can be used repeatedly, with longer-term gains and a larger number of beneficiaries. Thus short-term training is an attractive option for HC institutions.

Table 2. Examples of FY 2012 Short-term Training Activities Supported by the Pulse CRSP

Pulse CRSP Project: PII-MSU-3

Type of Training: Food safety — Hazard Analysis Critical Control Points

When did the Short-Term Training Activity occur?: December 2011

Location of Short-Term Training: Michigan State University

Who benefitted from this Short-Term Training Activity? Faculty of Sokoine University of Agriculture

Number of Beneficiaries by Gender: Male – 2; Female – 0; Total – 2

Pulse CRSP Project: PII-TAMU-1

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, discussions on way forward.

When did the Short-term Training Activity occur? June 1, 2012

Location of Short-term Training: Lusaka, Zambia

Who benefitted from this Short-Term Training Activity?

Government officials, NGO representatives, academic and extension staff of ZARI, and UNZA

Number of Beneficiaries by Gender: Male – 10; FeMale – 11; Total – 21

Additional training provided to technicians at University of Zambia and local cowpea producers in Siavonga, Zambia: 1 female and 6 male

Pulse CRSP Project: PII-TAMU-1

Type of Training: Short-term hands on laboratory training and discussions on research strategies

Description of Training Activity: Explaining preliminary findings and what they mean to cowpea promotion strategy, discussions on way forward.

When did the Short-term Training Activity occur? June 8-9, 2012

Location of Short-term Training: Nairobi & Katumani, Kenya

Who benefitted from this Short-term Training Activity?

Academic and field staff of KARI-Katumani and Egerton University

Number of Beneficiaries by Gender: Male – 1; FeMale – 2; Total – 3

Pulse CRSP Project: PII-ISU-1

Type of Training: A series of short duration training sessions on production and postharvest handling

Description of Training Activity: Training with farmers and extension agents included bean crop management practices and technologies, including varieties, postharvest handling, and culinary aspects.

When did the Short-term Training Activity occur? March – September, 2012

Location of Short-term Training: Kamuli District, Uganda

Who benefitted from this Short-term Training Activity?

Farmers in the Kamuli Region and extension agents with VEDCO

Number of Beneficiaries by Gender: Male – 9; FeMale – 58; Total – 67

Pulse CRSP Project: PII-MSU-2

Type of Training: Training on Collection and Analysis of Market Prices

Description of Training Activity: This training discussed the role of prices, price discovery and analytical challenges with prices as times series data, including a computer-based session

on price analysis. During the training, it was clear that analytical skills and computer skills were both lacking to be able to conduct thorough price analysis, but attendees were able to develop simple graphs and estimations of real prices as a result.

When did the Short-term Training Activity occur?

Dec 12-13, 2011

Location of Short-term Training: Faculdade de Ciencias Agrarias, Chianga, Huambo, Angola

Who benefitted from this Short-term Training Activity? A combination of students of FCA, NGO staff members for World Vision, ADRA and others, staff members from the Ministry of Commerce, Agricultural Research Institute, and Agricultural Development Agency

Number of Beneficiaries by Gender: Male – 13; FeMale – 8; Total – 21

Pulse CRSP Project: PII-UIUC-1

Type of Training: Internship

Description of Training Activity: Biocontrol of cowpea pests, biopesticides

When did the Short-term Training Activity occur? Aug–Nov 2012 (ongoing)

Location of Short-term Training: IITA Benin

Who benefitted from this Short-term Training Activity? Technicians, students

Number of Beneficiaries by Gender: Male: 3; Female: 2; Total: 5

C. Equipment for Host Country Capacity Building

As has been done in the previous years, the Management Office of the Dry Grain Pulses CRSP budgets and competitively awards funds to Host Country institutions for capacity building. This funding assists National Agriculture Research Systems (NARS) and agriculture universities in acquiring and maintaining cutting edge research and extension capacity to effectively address the challenges facing the pulse (bean, cowpea and related edible legume crops) sectors and contributes to economic growth and food and nutritional security in their respective countries. These grants are designed to meet investment needs for human resource development, scientific equipment, laboratory and field facilities, computer technology, and infrastructure.

Host Country (HC) collaborators submit proposals to the MO for funding that exceeds the budgetary limits of the current projects or respond to needs of agricultural research institutions in USAID priority countries that may be future collaborators.

In FY 2012, the Management Office (MO), in consultation with the Technical Management Advisory Committee (TMAC), approved the award of five supplemental activities totaling \$113,911 that would enhance the capacity of host country institutions in strategic areas (e.g., development of seed production and storage capacity, training on MAS for breeding, 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).

Project	Last name	Given name	Country of citizenship	Gender	Training institute	Degree	Discipline	Training status as of Oct/12	Start month	Start year	Anticipated or realized completion month	Anticipated or realized completion year	Type of CRSP support
PI-CU-1	Chaves	Estevo	Angola	M	University Federal Vicosa, Brazil	M.S.	Agricultural Economics	Completed	4	2009	7	2011	Full
PI-CU-1	Diaz	Jorge	Peru	M	EAP-Zamorano	B.S.	Crop Science	Completed	1	2011	12	2011	Partial
PI-CU-1	Juma	Roselyne	Kenya	F	Moi University	M.S.	Plant Breeding/Evaluation	Completed		2010	3	2011	Full
PI-CU-1	Marealle	Rosemary	Tanzania	M	Sokoine U. of Agriculture	M.S.	Nutrition	Completed	8	2009	11	2011	Full
PI-CU-1	Ngoma	Edna	Zambian	F	University of Zambia	B.S.	Agriculture Economics	Completed					Partial
PI-CU-1	Njeru	Crispus Mugambi	Kenya	M	Moi University	M.S.	Soil Science	Completed	2	2008	2	2010	Full
PI-CU-1	Odundo	Silvester	Kenya	M	Moi University	M.S.	Soil Science	Completed		2009	2	2011	Full
PI-CU-1	Onyango	Eunice	Kenya	F	Moi University	M.S.	Applied Environmental & Social Science	Completed		2009	9	2010	Full
PI-ISU-1	Abwate	Martha	Uganda	F	Makerere University	M.S.	Soil Science	Active	9	2010	8	2012	Full
PI-ISU-1	Adom	Medetissi	Togo	M	IITA Benin	B.S.	Entomology/Biology	Active	5	2012	12	2012	Partial
PI-ISU-1	Dannon	Elie	Benin	M	IITA-Benin	Ph.D.	Entomology	Completed	9	2009	9	2011	Partial
PI-ISU-1	Jjagwe	George	Uganda	M	Makerere University	M.S.	Ag. Extension & Education	Active	8	2010	12	2012	Partial
PI-ISU-1	Ndagre	Catherine	Uganda	F	Makerere University	M.S.	Food Science and Technology	Active	8	2009	12	2012	Partial
PI-ISU-1	Somakpon	Hermann	Benin	M	IITA-Benin	M.S.	Entomology	Completed	7	2009	7	2011	Partial
PI-ISU-1	Ssenyonga	Peter	Uganda	M	Makerere University	M.S.	Soil Microbiology	Active	9	2010	8	2012	Full
PI-MSU-1	Sanou	Apoline	Burkina Faso	F	University of Ouagadougou	Ph.D.	Entomology	Active		2011	7	2013	Partial
PI-MSU-1	Sarr	Mame Penda	Senegal	F	University of Dakar	Ph.D.	Plant Breeding/Pathology	Active	10	2010	10	2013	Full
PI-MSU-2	Chimuka	Samboko	Zambian	M	University of Zambia	B.S.	Agriculture Economics	Completed					Partial
PI-MSU-2	Kayitesi	Eugene	Rwandan	F	University of Pretoria	Pd.D.	Food Science	Active	1	2010	12	2012	Indirect
PI-MSU-2	Quinhentos	Maria da Luz	Mozambique	F	Michigan State University	M.S.	Agricultural Economics	Active	09	2011	06	2013	Partial
PI-PSU-1	Camilo	Samuel	Mozambique	M	Penn State	M.S.	Agonomy	Active	5	2011	12	2012	Full
PI-PSU-1	David	Antonio	Angola	M	University of Puerto Rico	M.S.	Plant Breeding	Completed	8	2009	8	2011	Full
PI-ISU-1	Nyakuni	Geoffrey Arijole	Uganda	M	Iowa State University	Ph.D.	Food Science & Human Nutrition	Canceled					

Annex 1: Status of degree training planned and executed in FY 2012 (continued)

Project	Last name	Given name	Country of citizenship	Gender	Training Institute	Degree	Discipline	Training status as of Oct/12	Start month	Start year	Anticipated or realized completion month	Anticipated or realized completion year	Type of CRSP support
PI-ISU-1	Oloo	Caren	Kenya	F	University of Nairobi	M.S.	Plant Protection	Withdrew		2009			Full
PII-UPR-1	Chiona	Susan	Zambian	F	University of Zambia	M.S.	Food/Nutrition	Completed					Partial
PII-UPR-1	Komba	Charles	Tanzania	M	Sokoine U. of Agriculture	M.S.	Agronomy	Completed	9	2010	9	2012	Full
PII-UPR-1	Lege	Michael	USA	M	Washington State University	M.S.	Soil Microbiology/Biochemistry	Completed	9	2010	8	2012	Full
PII-UPR-1	Moncaño	Luis	Ecuador	M	EAP-Zamorano	B.S.	Crop Science	Completed	1	2011	12	2011	Partial
PII-UPR-1	Ndeve	Arsenio	Mozambique	M	University of California Riverside	Ph.D.	Plant breeding/Plant Pathology	Active	1	2012	10	2016	Partial
PII-UPR-1	Toffa	Joelle	Benin	F	IITA-Benin	Ph.D.	Entomology	Active	7	2010	7	2013	Partial
PII-UPR-1	Traore	Fousseni	Burkina Faso	M	University of Ouagadougou	Ph.D.	Entomology	Active		2009	3	2013	Partial
PII-UPR-1	Weya	Belinda Akinyi	Kenya	F	Egerton University	M.S.	Soil Science	Completed	8	2008	2	2011	Full
PII-UCR-1	Adebiyi	Kamarou	Benin	M	IITA Benin	M.S.	Entomology/Biology	Active	7	2012	7	2013	Partial
PII-UCR-1	Cando	Marcelino	Ecuador	M	University of Puerto Rico	M.S.	Plant Breeding and Genetics	Active	1	2010	12	2012	Partial
PII-UCR-1	Costa	Manuel	Angola	M	University of Puerto Rico	M.S.	Plant Breeding/Pathology	Canceled					
PII-UCR-1	Johnson	Sarah	Tanzania	M	Sokoine U. of Agriculture	M.S.	Food Science	Active	8	2009	11	2012	Full
PII-UCR-1	Khafa	Beata	Tanzania	F	Sokoine U. of Agriculture	M.S.	Plant Breeding	Completed	9	2010	9	2012	Full
PII-UCR-1	Nderitu	Alice	Kenya	F	University of Pretoria	Ph.D.	Food Science	Active	8	2010	6	2013	Full
PII-UTUC-1	Alatchao	Edinam	Togo	F	IITA Benin	B.S.	Entomology/Biology	Active	5	2012	12	2012	Partial
PII-UTUC-1	Docinvil	Ronald	Haiti	M	University of Puerto Rico	M.S.	Soil Sciences	Completed	8	2006	5	2009	Partial
PII-UTUC-1	Gawde	Archana	India	F	Texas A&M University	Ph.D.	Molecular/Env. Plant Sci	Active	1	2009	12	2012	Partial
PII-UTUC-1	Gungulo	Ana Lidia	Mozambique	F	University of Pretoria, South Africa	M.S.	Agricultural Economics	Active	2	2009	12	2012	Full
PII-UTUC-1	Hachibamba	Twambo	Zambia	F	University of Pretoria	Ph.D.	Food Science	Active	8	2010	6	2013	Full
PII-UTUC-1	Hooper	Sharon	Jamaica	F	Michigan State University	Ph.D.	Food Science	Active	8	2009	5	2013	Partial
PII-UTUC-1	Lusweti	Jane Francisca	Kenya	F	University of Nairobi	M.S.	Plant Protection	Completed	10	2007	10	2009	Partial
PII-UTUC-1	Mukeshimana	Gerardine	Rwanda	F	Michigan State University	Ph.D.	Plant Breeding and Genetics	Active	8	2008	5	2013	Partial

Annex 1: Status of degree training planned and executed in FY 2012 (continued)

Project	Last name	Given name	Country of citizenship	Gender	Training institute	Degree	Discipline	Training status as of Oct/12	Start month	Start year	Anticipated or realized completion month	Anticipated or realized completion year	Type of CRSP support
PII-UIUC-1	Ojwang	Leonard	Kenya	M	Texas A&M University	Ph.D.	Nutrition/Food Science	Completed	1	2010	12	2011	Partial
PII-UIUC-1	Pottorff	Marti	Philippines	F	U. California-Riverside	Ph.D.	Plant Breeding/Pathology	Active	10	2008	3	2012	Full
PII-UIUC-1	Sunga	Chalwe	Zambian	F	University of Zambia	B.S.	Agriculture Economics	Completed					Partial
PII-UIUC-1	Syanobe	Cyrille	Rwanda	M	Makerere University	M.S.	Food Science & Technology	Withdrawn	8	2008			
PII-UIUC-1	Valentinetti	Sara	Italy	F	EAP-Zamorano	B.S.	Crop Science	Active	1	2012	12	2012	Partial
PII-UIUC-1	Zanana	Karimou	Benin	M	ITTA Benin	MS	Entomology/Biology	Active	7	2012	7	2013	Partial
PIII-ISU-2	Diangar Moussa	Mouhammadou Moussa	Senegal	M	University of Ouagadougou	M.S.	Plant Breeding/Genetics	Active	10	2010	2	2013	Indirect
PIII-ISU-2	Kabahuma	Mercy	Uganda	F	Iowa State University	M.S.	Crop Production/Physiology	Active	8	2010	5	2013	Full
PIII-ISU-2	Kamfwa	Kelvin	Rwanda	M	Michigan State University	M.S.	Plant breeding/genetics	Active	9	2010	8	2013	Full
PIII-ISU-2	Mbui Martins	Monica	Angola	F	University of Puerto Rico	M.S.	Plant breeding	Completed	8	2009	8	2011	Full
PIII-ISU-2	Musaazi	Aisha Nakitto	Uganda	F	Makerere University	M.S.	Food Science & Technology	Completed	8	2008	12	2011	Partial
PIII-ISU-2	Onyango	Stanley	Kenya	M	University of Nairobi	M.S.	Food Technology & Nutrition	Completed		2010	4	2011	Full
PIII-ISU-2	Sebuwufu	Gerald	Uganda	M	Iowa State University	Ph.D.	Agronomy	Active	8	2008	12	2012	Partial
PIII-ISU-2	Zulu	Esther	Zambian	F	University of Zambia	B.S.	Agriculture Economics	Completed		2010	12	2011	Full
PIII-MSU-3	Amos	Nyangi	Tanzania	F	Sokoine U. of Agriculture	M.S.	Food Science	Active	9	2009	11	2012	Partial
PIII-MSU-3	Appoline	Sanou	Burkina Faso	F	University of Ouagadougou	Ph.D.	Entomology	Active		2010	7	2014	Partial
PIII-MSU-3	Jacob	Sacred	Tanzania	F	Sokoine University of Agriculture	M.S.	Nutrition	Active		2011	12	2012	Partial
PIII-MSU-3	Matambuka	Martin	Uganda	M	Iowa State University	Ph.D.	Food Science & Human Nutrition	Active	1	2009	12	2012	Partial
PIII-MSU-3	Myece	Agness	Zambian	F	University of Zambia	B.S.	Agriculture Economics	Completed		2010		2011	Full
PIII-TAMU-1	Biaou	Jeanne	Beninese	F	ITTA Benin	M.S.	Entomology	Active	9	2012	3	2013	Partial
PIII-TAMU-1	Gonzalez-Belez	Abiezer	United States	M	University of Puerto Rico	M.S.	Plant Breeding and Genetics	Completed	8	2010	5	2012	Full
PIII-TAMU-1	Iaacs	Krista	USA	F	Michigan State University	Ph.D.	Ecology and Nutrition	Active	8	2008		2013	Partial
PIII-TAMU-1	Kiprop	Billy	Kenya	M	Egerton University	M.S.	Biochemistry	Completed	1	2010	12	2011	Partial

Annex 1: Status of degree training planned and executed in FY 2012 (continued)

Project	Last name	Given name	Country of citizenship	Gender	Training institute	Degree	Discipline	Training status as of Oct/12	Start month	Start year	Anticipated or realized completion month	Anticipated or realized completion year	Type of CRSP support
PIII-TAMU-1	Laraiaz	Laura	Guatemalan	F	EAP-Zamorano	M.S.	Crop Science	Active	1	2012	12	2012	Indirect
PIII-TAMU-1	Nkundabombi	Marie Grace	Rwanda	F	Makerere University	M.S.	Food Science & Nutrition	Active	8	2011	8	2013	Partial
PIII-KSU-1	Chesale	Virginia	Malawi	F	Penn State	M.S.	Plant Nutrition	Completed		2009		2012	Partial
PIII-KSU-1	Chilala	Cornard	Zambian	M	University of Zambia	M.S.	Economics	Active		2011	5	2013	Partial
PIII-KSU-1	Chilundika	Natasha	Zambian	F	University of Zambia	B.S.	Agriculture Economics	Completed					Partial
PIII-KSU-1	Chishimba	Elizabeth	Zambian	F	University of Zambia	B.S.	Agriculture Economics	Active		2011	5	2013	Partial
PIII-KSU-1	Foussent	Traore	Burkina Faso	M	University of Ouagadougou	M.S.	Entomology	Completed	9	2008	8	2012	Full
PIII-KSU-1	Karimou	Laouali	Niger	M	University of Niamey	M.S.	Entomology	Completed	9	2010	8	2012	Partial
PIII-KSU-1	Mtsocha	Lydia	Zambian	F	Kansas State University	MAB	Masters in Agribusiness	Active		2011	5	2013	Full
PIII-KSU-1	Mwansa	Martin	Zambian	M	Kansas State University	MAB	Masters in Agribusiness	Active		2011	5	2013	Full
PIII-KSU-1	Mwansa	Sosthenes	Zambian	M	Kansas State University	MAB	Masters in Agribusiness	Active		2011	5	2013	Full
PIII-KSU-1	Nhlane	Robert	Zambian	M	University of Zambia	B.S.	Agriculture Economics	Active		2011	5	2013	Partial
PIII-KSU-1	Okitor	Simon	Uganda	M	Makerere University	M.S.	Agricultural Economics/Agriculture Economics	Completed	8	2008	12	2010	Partial
PIII-KSU-1	Sambo	Jairos	Zambian	M	University of Zambia	B.S.	Agriculture Economics	Active		2011	5	2013	Partial
PIII-KSU-1	Sanouchi	Adere	Niger	M	University of Niamey	M.S.	Entomology/Biology	Completed	9	2010	10	2012	Partial
PIII-KSU-1	Tuyiringire	Justin	Rwanda	M	Umutara Polytechnic University	BS	Agriculture	Completed			6	2012	Partial

Annex 2: Dry Grain Pulses CRSP FY 2012 Investments in Institutional Capacity Building

Total FY 2012 Investment by Dry Grain Pulses CRSP in Institutional Capacity Building — \$113,911 across five projects

PII-UCR-1: Modern Cowpea Breeding to Overcome Critical Production Constraints in Africa and the U.S.

Amount Awarded: \$10,000

CRSP cowpea breeders developed training for selected West African breeders and students in the development and application of DNA-based markers for Marker Assisted Selection (MAS) for use in their cowpea breeding programs. Two scientists were able to join other breeders for a training workshop at Riverside in FY2012. In conjunction with the Global Pulse Researchers meeting in February 2012 in Kigali, Rwanda, CRSP researchers conducted a two-day training workshop through joint interpretation of data sets and progeny selections as a hands-on MAS and MARS experience. Pulse CRSP common bean breeders were also invited and trained at the Kigali workshop. Training was also conducted at meetings in Addis-Ababa, Ethiopia, in May 2012, linked with CGIAR GCP-organized meetings.

PII-ISU-1: Enhancing Nutritional Value and Marketability of Beans through Research and Strengthening Key Value Chain Stakeholders in Uganda and Rwanda

Award Amount: \$20,000

The objectives of this proposal were to: (1) develop training materials on improved bean management practices, (2) evaluate training methods, and (3) build capacity of VEDCO staff in Uganda. The project team developed new training materials and media (print, video) for improved management practices and technologies that was utilized in training more than 800 farmers in 60 demonstration sites. Topics covered by print and posters included germination testing; plant spacing; soil nutrient amendments (manure application); pest and disease management; harvesting, threshing, drying, and moisture testing; solarization and triple bagging; sorting and seed selection; and processing and preparation of food products from beans. Three different training methods/media, all followed by field demonstrations, were used in training on planting and triple bagging: (1) interactive training, (2) animated video, and (3) farmer acted video. The animated video on triple bagging was produced by Dr. Barry Pittendrigh and colleagues at the University of Illinois.

A small battery-powered projector (3M MPRO 150 Pocket Projector) with supplemental Speaker Case (Insignia) was used to present the video at farmers' training facilities. Trainers on foot or bicycle can easily carry these portable devices to places where farmers live and work. The visual and audio qualities were adequate, depending on the size of group. Groups with less than 10 participants exhibited a relatively low level of interaction and there were few group discussions among farmers during the training. However, when the size of the training group was more than 40, training became messy, and farmers sitting at the back had problems hearing and watching the videos.

Research by an ISU-based graduate student in communication and sustainable agriculture (Ms. Tian Cai) explored the effectiveness of video to complement or replace existing lecture/demonstration training for small farmer groups in rural Uganda. Her master's thesis supervisor, Dr. Eric Abbott, is a development communications specialist. The study was conducted in four parishes in Butansi subcounty of Kamuli district during February–March, 2012. A total of 325 project farmers participated in the study in which quantitative and qualitative data were gathered through an experiment including a pretest and a posttest with a control group design. Results showed that video could effectively complement or, in some circumstances, replace conventional lecture/demonstration training methods in knowledge improvement, attitude, and intention to adopt improved management practices and technologies. Combining video, lecture, and demonstration is especially effective for groups with little or no prior knowledge of a topic. Moreover, video alone or video plus lecture/demonstration can be as effective as conventional training in decreasing gaps in knowledge of training topics among subjects of both genders and varying education levels and scale of bean planting.

Consistent capacity building activities in Kamuli were guided by Dr. Haroon Sseguya, Makerere University faculty member from the Department of Agricultural Extension and Innovation. VEDCO's staff and Community Based Trainers (CBTs) learned advanced methods of farmer learning facilitation, and numerous farmers learned improved practices. The supplemental funding also strengthened collaboration among VEDCO, Makerere University, and Iowa State University faculty and students. It helped scale up innovative management practices and technologies to wider populations in Uganda.

PII-UPR-1: Building Capacity for Enhancing Biological Nitrogen Fixation in Bean Production Systems in Central America, Haiti, Angola and Mozambique

Award Amount: \$25,300

Equipment and materials have been purchased to improve the plant pathology laboratory and repair greenhouses at the IIA Research Station in Huambo, Angola. Tim Porch and Jim Beaver visited Angola in December 2011 and reviewed progress in the establishment of the plant pathology facilities. Monica Mbui Martins has completed M.S. degree training at the UPR and has returned to Huambo, Angola. She plans to use both the plant pathology and greenhouse facilities for research related to the Dry Grain Pulses CRSP project. Other IIA staff members currently receiving graduate training in plant pathology in Spain are expected to use the laboratory on completion of their degrees. Threshers were purchased by the bean research programs in Haiti and Angola. This equipment will permit the harvest of larger quantities of bean and cowpea seed on a timely basis. The purchase of a 4WD vehicle for the National Seed Service in Haiti was approved by the BTD project.

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: \$40,000

The PII-UIUC-1 project is focused on the development and the deployment of practical technologies that can be used by smallholder farmers to optimize their cowpea production by minimizing insect attack, specifically to develop a delivery system for endophytic strains of *Beauveria bassiana* and *Metarhizium anisopliae* active against cowpea pod borer. The supplemental funds were also to develop sustainable virus production capacity at the farmer level in West Africa. Additional funds were included to collect and assess appropriate farm-level data to capture the effects of IPM extension messages and methods on changes in farmers' knowledge, perceptions and behavior in Burkina Faso.

A technology 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. A series of eight training and demonstration videos were produced locally in Benin. The first set of four videos illustrates the four major field pests of cowpea: aphids, thrips, pod borers, and pod sucking bugs. After a general introduction to cowpea, the videos describe the different life stages of the pest as well as their feeding habit, damage symptoms on the plant, and natural enemies in the field. The second set of videos is more of a technical nature and describes in details the steps of rearing *M. vitrata* and its parasitoid *A. taragamae* both on artificial diet and cowpea sprouts. The first version of the videos are in French; an English version is already being prepared while translation in the most important local languages (Hausa, Yoruba, Bambara, Mooré, Zarma, Dendi, etc.) is planned for early next year. An animated video on biocontrol for *Maruca* was created in collaboration with IITA and SAWBO (UIUC).

In response to feedback from host country collaborators on the outcomes of successful pest control strategies (based on cultural practices), we have developed a series of animations (in local languages), which can be and have been deployed using cell phones. We have developed videos for hermetic sealing of cowpeas for storage, solar treating of cowpeas, use of biocontrol agents to suppress pest populations, and proper preparation and use of Neem sprays. Three of these videos released in 2011 were translated in FY 2012 into multiple local languages (please see for an incomplete list of the total language videos for two of the videos <http://sawbo.illinois.edu/OnlineMaterials/VIDEO/AGR/Cowpea01/EN/> and <http://sawbo.illinois.edu/OnlineMaterials/VIDEO/AGR/NEEM01/EN/>) and can be found on the SusDeViKI system (<http://susdeviki.illinois.edu> - more than 100 entries into the system - with more than 10,000 downloads of these videos). The biocontrol video, developed

with CRSP funds, has been completed and is soon to be released. 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), which will also be producing videos for other development and socially related projects and programs (with other funding sources). A video explaining the overall program can be found at <http://www.youtube.com/watch?v=JjtOHFFJSpC>.

For our CRSP project, our videos have been shared with Drs. Robert Mazur and Cynthia Donovan so that they can use these materials in the countries they are working in (Rwanda and Mozambique). A first contact has been made with staff of the Ministry of Communication in Benin to promote this technology within their current strategy of introducing ICT innovations to the rural areas. A meeting has been scheduled in early November to discuss the details of this collaboration. Over FY12 SAWBO has developed links with more than 30 partner groups around the planet who are helping to create new videos, do voice overlays in local languages, and perform local deployment of these videos.

Between known field deployment (where we have feedback from organizations), views, and downloads from our online systems, we estimate an impact on well more than 100,000 people. In FY12 we have performed studies to address the potential for spreading these animations through cell phone networks in Burkina Faso and Niger. The results suggest that the videos do spread through Bluetooth® from cell phone to cell phone. This work continues to be developed in collaboration with the PIII-MSU-4 Impact Assessment project.

PII-PSU-1: Improving Bean Production in Drought-Prone, Low Fertility Soils of Africa and Latin America – An Integrated Approach

Award Amount: \$18,611

The supplemental funding was used to acquire an irrigation pump for field research and a refrigerator for bean seed storage, both at the Chokwe Research Center of IIAM in Mozambique. The Chokwe motor pump was installed and is being used for direct irrigation to the experimental fields. A cold room facility was rehabilitated in Chokwe to better preserve bean materials being evaluated and developed by the breeding component led by Celestina Jochua in Chokwe.