Feed the Future Innovation Lab for Collaborative Research on Grain Legumes
LEGUME INNOVATION LAB
2015 ANNUAL TECHNICAL PROGRESS REPORT
(October 1, 2014 – September 30, 2015)

Code and Title: SO1.A5 Genetic improvement of cowpea to overcome biotic stress and drought constraints to grain productivity

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I. Abstract of Research Achievements and Impacts

A panel of cowpea accessions was used with uniform test protocols for field and screenhouse testing to characterize resistance to aphids and to identify cowpea aphid biotypes in three African and one US locations. Cowpea populations segregating for insect resistance (pod bugs, Burkina Faso; flower thrips, Ghana and Senegal; aphids, Ghana and California) were advanced, phenotyped and genotyped for QTL discovery for use in marker-assisted breeding. Five large white-seeded CRSP cowpea varieties were formally released in Senegal under the names Lisard, Thieye, Leona, Kelle and Sam after multi-year on-farm demonstration trials, and Breeder and Foundation seed produced and distributed to farmers' organizations. In Burkina Faso 4 pre-release CRSP cowpea lines were selected from on-farm trials and on-station Breeder seed produced in anticipation of their release as improved varieties in 2016. In California, advanced lygus and disease resistant blackeye lines were on-farm and on-station performance tested. Five African students engaged in degree training programs (3 PhD, 2 MS) in the project. Capacity strengthening awards from the MSU management entity supported development of screenhouses, an irrigation system (Ghana and Burkina Faso) and cowpea seed storage (Senegal). Continuous short-term training occurred through iterative data analysis and interpretation cycles using the phenotype and genotype data from each Host Country. A training/planning workshop in 2015 at UCR for scientists from Ghana, Senegal, Burkina Faso, Nigeria, and Mozambique utilized molecular breeding modules.

II. Project Problem Statement and Justification:

The project focus is to 1) discover insect tolerance and resistance QTL for cowpea breeding; 2) increase African and US cowpea productivity by improved varieties with resistance to insect stresses, drought tolerance or disease resistance; 3) expand farmer marketing opportunities with improved cowpea varieties; and 4) provide training and capacity building in modern cowpea breeding. The project is aligned with FTF research strategic priorities 1) crop resistance to heat, drought, salinity and flood; 2) West African Sudano-Sahelian systems emphasizing insect-resistant cowpea; and 3) grain legume productivity. Strategically, our partner countries Ghana, Senegal and Burkina Faso represent primary agro-ecologies for cowpea production in the Sudano-Sahel.

The project uses genomics and modern breeding to improve cowpea yield by targeting insect tolerance and resistance. By leveraging genomic resources developed with CGIAR Generation Challenge Program and USAID Climate Resilient Cowpea Innovation Lab funding, we apply comprehensive modern breeding tools. Insect pests constrain cowpea productivity in West Africa; the project targets insects attacking early (aphids), mid-flowering and pod-set (flower thrips), and later pod-filling (pod-sucking bugs) cowpea stages. Discovery work through phenotyping, genetic mapping and QTL identification needs to be done for these insect pests, using high throughput SNP genotyping, genetic maps, and QTL discovery. The project breeding programs have early generation populations with target traits, providing valuable starting points for breeding.

Low productivity of agriculture 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 often five to ten times greater. Most of the loss in yield potential is due to drought, poor soil fertility, and insect pests. By targeting insect tolerance and combining with drought tolerance, cowpea productivity, food security and rural incomes can be increased. To increase marketing options, new cowpea varieties must have features desired by consumers - grain appearance, cooking and processing characteristics. Regionally adapted cowpea varieties with large white grain and large rough brown grain with resistance to pests would increase the marketing opportunities of cowpea farmers and traders in both West Africa and the US.

III. Technical Research Progress

Objective 1: Discover QTL for insect resistance and apply in molecular breeding for target regions in West Africa and the US

1.1 Aphid resistance: We are testing the genetic relatedness of five sources of cowpea aphid (*Aphis craccivora*) resistance. Field observations in Africa and California indicate differential effects of resistance sources on aphid populations from different cowpea production areas. Cowpea lines IT97K-556-6, KvX295-2-124-99, an IITA wild donor line (TVNu1158), UCR01-11-52/SARC1-57-2, and 58-77 representing a set of resistance donor genotypes plus known susceptible control lines were seed-multiplied in 2014 and again in 2015. The panel is shown in Table 1. Uniform screens in field locations across all project NARS (Burkina, Ghana, Senegal) and California were conducted in 2014 in field

plots or in screenhouses, with 4-fold replication, using standard resistance assessment scales across all test sites. The uniform test design and coordination planning for the aphid resistance assessment was developed by the project team in FY13 – FY14. Additional germplasm lines were included in the screening sites to search for more sources of resistance. This multi-site phenotype screening for resistance response was repeated in FY15, following additional seed increases in NARS and UCR, to provide a minimum of 2 years of data. The resistance donors and susceptible controls were SNP genotyped in FY14, coordinated by UCR. More seed of TVNu1158 was produced at UCR because of problems with this wild accession in the NARS. In 2015 in Senegal because of a wet season with heavy rain, the aphid population did not become established enough with uniform infestation in the field to discriminate between genotypes. However, enough seeds were produced for later experimentation. In Ghana, the seedling stage screening of the aphid resistance panel at SARI found IT97K-556-6, KvX-295-2-124-99, SARC-1-57-2, 58-77 and CB27 to be resistant to the cowpea aphids in northern Ghana (see Figure 1). In testing the mode of inheritance and the genetic relatedness of these lines, F1 populations have been developed between each of these lines with Apagbaala (aphid susceptible popular variety in Ghana) and in addition, each of these lines found to be resistant has also been crossed to each other. These populations are currently being advanced to the F2 at SARI. They will be genotyped and phenotyped to determine the mode of inheritance of the source of aphid resistance in each of the resistant lines and to determine the uniqueness of the aphid resistance gene(s) in each of these lines.

We are working with Dr. B. Pittendrigh and M. Tamo (Project SO1.B1) in the characterization (molecular fingerprinting) of the aphid isolates representing the different aphid populations at each location. This will be especially valuable if, as expected, aphid biotypes are delineated on the cowpea resistance sources. Samples of aphids were collected and stored for DNA extraction, with a view to developing a DNA sequence based fingerprint to distinguish the isolates. For example, in Burkina Faso, aphids were collected from Kamboinse, Pobe-Mengao and Farako-Ba representing three diverse cowpea production zones. New segregating populations and some existing ones between aphid resistant and susceptible parents will be used to phenotype screen for QTL discovery. Depending on the source, we are at different stages of QTL mapping. We finished a QTL discovery effort for aphid resistance in IT97K-556-6, identifying two resistance QTL. In Ghana we have an F7 population between a susceptible elite line and resistance donor KvX295-2-124-99. This population has been genotyped and phenotyped in FY15 for QTL mapping (see section 1.2). From the wild donor IITA line TVNu1158 a RIL population has been developed for mapping QTL and is currently being genotyped using the 60K SNP iSelect by UCR. This work is being conducted in collaboration with Drs. Fatokun and Boukar at IITA, Nigeria.

1.2 Flower thrips resistance: In recent work on QTL discovery, we identified and SNP-mapped loci (*Cft-1* and *Cft-2*) for flower thrips (*Megalurothrips sjostedti*) tolerance donated by Sanzi in the cross Sanzi x Vita 7, and these loci are promising for introduction and selection in breeding progenies but require better definition through phenotyping.

Additional sources of thrips tolerance are 58-77 (biparental RIL population from 58-77 x Yacine is available) and Tvx3236. In Senegal, the populations 58-77 x Yacine and Sanzi x Vita 7 were field-screened for flower thrips tolerance with two planting dates at Bambey. Because of insufficient seed quantity for multi-locations, the trials were conducted at Bambey only. Field-phenotyping for tolerance to flower Thrips uses the Jackai and Singh (1988) tolerance scale. Screens were designed as a 3-replication (Bambey) RCBD and included the parents, and run by entomologist Ibrahima Sarr. In Senegal the different tolerance sources in Sanzi, 58-77 and Tvx3236 were intercrossed in all combinations by Dr. Cisse in FY14 and each of these populations was advanced to the F3 in FY15. For breeding purposes, the F1 of Sanzi x 58-77 was crossed to Yacine and to the new large seeded varieties ISRA-3178 and ISRA-3217. Also M3 generations of Yacine generated through mutagenesis were evaluated under a no-spray nursery at Bambey for flower thrips reaction. Selections were made between and within families. At SARI, Ghana, Dr. Kusi received seed in FY15 of the two RIL populations, Sanzi x Vita7 and Yacine x 58-77 from Senegal to be phenotyped for QTL refinement for flower thrips tolerance. Given the limited seeds per line received, the populations were planted to increase the seeds and so the thrips phenotyping was not vigorously carried out. Each of the lines in the populations was flower-sampled at 7 days after insecticide spray to generate preliminary data to have a fair assessment of the lines for thrips tolerance. Field preparation is currently in progress to plant the populations under spray and non-spray conditions for vigorous thrips tolerance phenotyping. The aim is to combine the phenotyping data sets from Senegal and Ghana for improved QTL mapping of the thrips tolerance loci.

In Ghana, three Sanzi-derived F7 populations segregating for seed color (including white) and flower thrips resistance are being evaluated for QTL discovery and breeding. One parent is IT97K-499-35, now the popular Ghana variety ‘Songotra’, a high yielding black-eye resistant to Striga but thrips sensitive which can be improved for thrips tolerance via the F7 population. A second parent is SARC1-57-2, which carries aphid resistance. The SARI team is phenotyping these populations using the previously described experimental protocols. The 280 single-seed derived F7 families were leaf sampled and the samples sent to UCR where they were DNA-extracted and sent for SNP genotyping using the Illumina iSelect platform. The seeds produced from each of the single-seed descent plants were phenotyped for both flower thrips and Striga resistance. The populations have now been classified into individuals that recorded Striga emergence on the field and those that did not record Striga emergence. The individuals that did not record a single Striga emergence are currently been prepared for screening in pots infested with Striga seeds to validate the field results by washing the root to check if there was no attachment underground. The thrips phenotyping was not vigorously done because we also wanted to produce more seeds. Therefore, each family was flower-sampled at 7 days after insecticide spray to generate preliminary data on assessment of the lines with thrips resistance. Now with enough seeds available, the populations are being prepared to be planted under spray and non-spray conditions for vigorous thrips phenotyping at Tamale and Manga. These data will be used with the SNP

genotyping for QTL mapping. As part of the project effort to offer training opportunities to scientists and technicians, a post-graduate student from Kwame Nkrumah University of Science and Technology has been engaged on this work for his MS thesis.

1.3 Pod-sucking bug resistance: The Heteropteran Coreid pod-sucking bugs (*Clavigralla tomentosicollis* complex) are a major yield suppressor in Burkina Faso, Ghana and neighboring countries. We have not yet identified genes or QTL for resistance to pod-sucking bugs but resistant cowpea accessions are available. We started to use biparental resistant x susceptible segregating populations in FY14 to map QTL and initiate their selection as a new breeding target. This work is a focus of effort in Burkina Faso. A primary tolerance source is IT86D-716 (used in Burkina Faso); pods on F2 plants were phenotyped in FY15 to identify the underlying QTL, using standard screens of young pods in petri dishes to score bug viability and fecundity. The 2014 screening was not successful because of poor germination of the first set of F3 seeds. New recombinations were then made in 2015. Four different F2 and BC1F1 populations are available. The re-phenotyping is planned for February 2016. The phenotyping will be repeated by July 2016 to provide validated QTL mapping data. Additional potential tolerance donor lines were included in the initial phenotyping screens in FY14, including those in the pedigree of resistance donor IT86D-716, to broaden the knowledge base and potentially identify additional sources of tolerance. Two existing F2 populations generated from resistance donor IT86D-716 with parents Kvx771-10G (Nafi), Tiligre, Gourgou, and IT98K-205-8 enable combining Striga resistance with pod-sucking bug tolerance. The parents have been genotyped through LGC Genomics and the F2 and F3 populations will be phenotyped in FY16 for pod bug resistance in Burkina Faso, in collaboration with Dr. Dabire. Using leaf samples collected from phenotyped plants in Burkina Faso, single F2 plants and F3 family bulks consisting of a minimum of 12 individual plants will be genotyped. The phenotype and genotype data from the F2 and F3 generations will be used for QTL discovery with the ICI Mapping program, which will be conducted at UCR.

For the three insect groups (aphids, thrips, pod bugs), we collaborated with Dr. Pittendrigh and Dr. Tamo (Project SO1.B1) to utilize our project trial sites to collect insect samples for use in molecular characterization of the insect populations. Collections will be made at all test locations, thereby allowing a robust comparative profiling of insect populations. We have tested a protocol for insect DNA collection, in which insects are placed in plastic bags with silica gel packs; this dries the insect samples and preserves the DNA. Tests on aphid DNA with primers for the COX1 gene demonstrated excellent DNA integrity. In Burkina Faso, pod bugs were collected from Kamboinse, Pobe-Mengao and Farako-Ba.

Objective 2: Complete release and validation of advanced cowpea lines developed under the Pulse CRSP in Burkina Faso, Senegal, and US.

2.1. We continued to use our genotyping capability to advance the BT gene introgression for Muruca resistance with our SNP marker panel. Genotyping was

initiated in FY14 primarily focused on background selection with genome-wide markers in segregating progeny of backcross breeding populations in Burkina Faso and Ghana. The goal is to expedite the selection of lines with the highest percentage of elite recurrent parent content in each country (e.g., improvement of elite variety IT97K-499-35 in Ghana and several elite local varieties in Burkina Faso, including Moussa Local, Gourgou 3, 7 and 11, IT98K-205-8 and KVX 745-11P). In Burkina Faso BC3 were genotyped in FY14. In FY15, populations were advanced to the BC3F5 and BC5F3 stages and leaf samples were collected and are awaiting SNP genotyping. In FY15, trials were conducted at three locations for agronomic performance and also a single-site trial was conducted under insect net protection for resistance efficacy of the introgressed lines. The genotyping on sampled plants determined those carrying resistance with the highest level of recurrent parent genotype. Ghana BC2 progenies from FY14 were advanced in FY15 and leaf-sampled for SNP genotyping on the next generation of breeding lines. The phenotyping of the breeding lines for Maruca is being done in the host countries with funding from USAID through African Agricultural Technology Foundation (AATF). The Ghana and Burkina Faso breeders received extensive hands-on training at UCR in March 2014 and were trained further in March 2015 using their own datasets under this objective. The genotyping mostly followed the same general protocol as outlined under the Objective 1 work. Leaf samples from young screenhouse grown plants in the phenotyping and crossing blocks were used for DNA extraction in Burkina Faso and Ghana, and then SNP assayed by LGC Genomics (KASP). The genotype data were analyzed for molecular scores using Backcross Selector software.

2.2. We are capitalizing on the previous Pulse CRSP breeding effort by completing the release requirements of several advanced breeding lines that are in the final stages of performance testing in Burkina Faso, Senegal and California.

In Senegal, five large white grain type cowpeas (at least 25 g /100 grains) developed by Dr. Cisse were submitted as candidates for release by the national variety release committee in FY14. These were performance tested in 20 on-farm demonstration trials in main season FY13, and the data combined with performance data from 2011 and 2012 to support the formal release. The demonstration trials were conducted in the northern cowpea zone (Louga, Mekhe, Thilmakha). In 2015 the five new lines ISRA-3178, ISRA-3201, ISRA-3205, ISRA-3211 and ISRA-3217 were registered respectively as the new varieties Lisard, Thieye, Leona, Kelle and Sam. These are names of locations where the demonstration trials were conducted. Additional Breeder and Foundation seeds were produced during the 2015 off-season on 2000 m^2 for each variety; about 500 kg of seeds for each variety were obtained. Seeds were provided to several farmers' organizations (RESOPP, PAFAL, Millenium project, among others) and to the extension service (ANCAR) for large-scale demonstration to generate demand and also for Certified seed multiplication.

In Burkina Faso, 20 pre-release CRSP advanced lines developed by Dr. Drabo were on-farm performance tested in 2013, and a sub-set of the best nine lines were re-evaluated

in 2014. Multi-location tests were conducted at Saria, Pobe, and Kamboinse in Burkina Faso during the 2015 main rainy season. The four best performing of the nine lines plus two standard checks were used for testing and these will be re-evaluated in the off-season in FY16 (October, 2015 - April, 2016), emphasizing yield and grain quality, plus any disease susceptibility. Trial design was based on using 4-row plots, 5 m long and 4 reps arranged in a RCBD. The release petition to the national variety release committee that was scheduled for mid-FY15 had to be delayed, with a planned re-scheduling for FY16. Breeder seed of the best lines chosen for release submission based on main season 2014 and 2015 and off-season 2015 performance data was produced at Saria during the main season 2015 (June – October). About 20 kg of Breeder seeds of each of these lines is now available at the INERA Saria Station, and will be used to initiate Foundation seed production in the FY16 off-season.

In California, advanced breeding lines were field tested for release potential, based on performance data collected in previous on-station trials. These represent CRSP developed lines that carry a combination of lygus bug tolerance, and root-knot nematode and Fusarium wilt resistance. For the best advanced blackeyes from 2013, we conducted on-farm yield trials in a Tulare Co. farmer's field and on-station trials at the UC Kearney Station, Fresno Co., in main season 2014 (harvested in October-November 2014) to assess commercial yield performance. Seed size and yield data from the trials are presented in Tables 2 and 3, together with field assays conducted for resistance to three common root-knot nematode species and a greenhouse assay for resistance to Race 4 of Fusarium wilt. The eleven lines plus the standard variety CB46 were tested under insect-protected conditions (Table 2), while a no-insecticide unprotected versus insecticide protected split-plot lygus screening trial was conducted with three lines with lygus bug tolerance. The test design was a four-row 4-fold replicated RCBD or split-plot trials with the center two rows machine harvested. Yield weights, 100-seed weights and lygus damage to seed were assayed. All yield and performance data were analyzed by standard ANOVA. Lygus pressure was heavy but arrived late in 2014, resulting in grain yield loss of between 10% and 30% in comparisons between protected and unprotected conditions. The experimental lines had significantly higher protected yield than CB46 indicating they have high innate yield potential. However, the insecticide protection was unable to keep up with the late season lygus attack and this also contributed to the relatively lower yield of CB46 in protected plots. The unprotected yields were significantly higher than CB46 for all three advanced lines, indicating strong yield ability under lygus pressure. From the 2014 trials, we chose the most promising lines (combination of yield, seed quality and resistance) for performance testing in the 2015 main season. These trials were planted in May 2015 in Tulare Co. with four lines (CB46, N2, 10K-29, CB46Rk2) in large 0.5 acre field-length 6-row strips (harvested October 2015), and June 2015 at the Kearney station with five lines (CB46, N2, 10K-29, CB46Rk2, 07KN-74) in four-extended row 4-fold replicated RCBD. Harvesting, threshing and seed cleaning is underway at time of reporting.

The Senegal and Burkina Faso releases will represent tangible project outputs, and offer the opportunity for tracking along the impact pathway as new releases which will be entering the seed multiplication and distribution process in each country. During the 2015 main rainy season each of the five new releases were multiplied on 0.5 ha for additional Foundation seed production. The resulting products will be provided to Certified seed producers including new farmer organizations for increase and demonstration in 2016. Opportunities exist to initiate baseline data for the releases through the impact analyses under the LIL project led by Dr. M. Maredia.

Objective 3: Increase capacity of NARS in Burkina Faso, Ghana and Senegal to serve the cowpea sector.

Short-term Training: Molecular breeding for young trainee breeders and NARS scientists has been conducted. Continuous short-term training occured through iterative data analysis and interpretation cycles using the phenotyping and genotyping data generated by each of the three Host Country partner teams (about 12 participants). To provide periodic intensive training, we convened a training workshop in March 2015 at UCR, using training modules developed by the UC-R team and by the CGIAR GCP Integrated Breeding Platform program (IBP) Breeding Management System (BMS). The IBP-BMS is using our tropical legumes project cowpea breeding population data for the training modules development. We conducted our first breeding workshop in FY14 at UCR with great success, and used the same format for the workshop at UCR in FY15 (March 23-27, 2015). The molecular breeding approach is complex and requires a combination of hands-on experience with self-generated data sets, augmented with periodic intensive training workshops to improve knowledge, skills and problem-solving. The technologies underlying the genotyping capability are in a state of frequent enhancement and upgrade, requiring periodic training input. Thus both young breeder trainees new to the programs and experienced breeders from the HC NARS are in need of this training. Training materials and protocols used by the NARS breeders were also used to train the technical staff in the NARS programs after NARS breeders had been trained further on the standardized electronic field-book, leaf assay, and field phenotyping protocols.
Degree Training: We conducted degree training for two graduate students in the report period at UCR and three in Africa, one with each of the three HC PIs in Burkina Faso, Ghana and Senegal. The trainees are described in detail under Section VI 2.

IV. Major Achievements

Under Objective 1.1 -- Aphid resistance

A differential cowpea panel of aphid resistance sources and control lines was seed-multiplied and used in multi-location field screening and screenhouse seedling screening during FY15. Using a uniform test protocol for aphid biotype and resistance screening under field and greenhouse conditions, several aphid resistance sources effective against both US and West African aphid populations were identified. These protocols will enable direct comparisons of aphid populations from the West Africa and

U.S. target cowpea breeding areas. Sets of F1 and F2 populations were made from aphid resistant x drought tolerant line crosses at SARI, Ghana.

Advanced backcross progenies were developed by adding aphid resistance QTLs into recurrent parents CB27, CB46 and CB50 and field tested, to select for California blackeyes with aphid resistance for the US production system.

Under Objective 1.2 – Flower thrips resistance

Segregating populations were developed in Senegal and Ghana from mutagenesis or from natural crosses using three sources of thrips resistance. These are in various stages of phenotyping and genotyping for QTL mapping.

Under Objective 1.3 – Pod bug resistance

Four segregating populations were developed in Burkina Faso for use in QTL mapping for pod bug resistance.

Under Objective 2.2 – Variety releases

Formal release of five large white-seeded CRSP cowpea varieties was completed in Senegal following final performance testing in on-farm trials and Foundation seed of each variety was produced by ISRA and distributed to Farmers' organizations for Certified seed development. The varieties were released under the names Lisard, Thieye, Leona, Kelle and Sam, which represent the names of the main on-farm testing locations.

Nine pre-release CRSP advanced cowpea lines were evaluated in Burkina Faso in 20 on-farm trials in 2014 with farmers helping in Participatory Variety Selection (PVS). The four best-performing lines were re- evaluated in multi-location tests at Saria, Pobe, and Kamboinse during the 2015 main rainy season and are being re-tested in the off-season in FY16 (October, 2015 - April, 2016), emphasizing yield and grain quality, plus any disease susceptibility. The release petition to the national variety release committee has been re-scheduled for FY16. Breeder seeds of each of these lines was produced at the INERA Saria Station to initiate Foundation seed production in the FY16 off-season.

Five African students have engaged in degree training programs within the project, including three PhD and two MS students.

The project was awarded Capacity Strengthening awards from the MSU management entity, which were used for the development of screenhouses for SARI, Ghana and INERA, Burkina Faso, cowpea seed cold storage for ISRA, Senegal, and off-season field irrigation for INERA, Burkina Faso. These capacity projects were completed and in service by Summer 2015 (see Section V).

V. Research Capacity Strengthening

Approval through the LIL was granted to fund renovation of the 1960's cold room used for seed conservation at the ISRA Bambey research station, Senegal to insure adequate temperature and humidity required for cowpea germplasm

conservation. The necessary equipment and its installation were completed in FY15. Approval through the LIL was granted to fund INERA, Burkina Faso breeding activity enhancement at Kamboinse research station by developing an irrigated field for off-season activities (crosses, advancing lines, breeder seed production). This system was completed in FY15 and now a one-ha plot is being managed following implementation of the new drip-irrigation system. Approval through the LIL was granted to fund INERA, Burkina Faso to renovate one screenhouse at Kamboinse Research Station and a second screenhouse at Saria Research Station. The renovations were completed in FY15 and the screenhouses provide prevention of outcrossing during crossing and to advance breeding lines under protection from insect, rodent and rabbit damage. Approval through the LIL was granted to fund a screenhouse at SARI, Ghana to enhance successful crosses and multiplication of breeder seeds during the harmattan period. The facility includes a 16 m x 8 m screenhouse fitted with a 500-gallon poly-tank reservoir for supply of water, a metal frame covered with insect proof net and a polythene sheet for sealing the roof to prevent rain, and benches 80 cm to 1 m high for growth containers. The construction was completed during FY15 and has been in use since summer 2015.

VI. Human Resource and Institution Capacity Development

1. Short-Term Training

Short-term 1:

i. Purpose of Training: The molecular breeding approach is complex and requires a combination of hands-on experience with self-generated data sets, augmented with periodic intensive training workshops to improve knowledge, skills and problem–solving. The technologies underlying the genotyping capability are in a state of frequent enhancement and upgrade, requiring periodic training input. Thus both young breeder trainees new to the programs and experienced breeders from the HC NARS are in need of this training.

ii. Type of Training: Continuous short-term training occurred in FY15 through iterative data analysis and interpretation cycles using the phenotyping and genotyping data generated by each Host Country partner team. To provide periodic intensive training, we convened a training/planning workshop in March 2015, using molecular breeding training modules developed by the UC-R team, or developed by the CGIAR GCP Integrated Breeding Platform (IBP) Breeding Management System (BMS), which uses our tropical legumes project cowpea breeding population data for the training modules.

iii. Countries Benefiting: Ghana, Senegal, Burkina Faso, Nigeria, Mozambique

iv. Location and dates of training: March 23-27, 2015, UC-Riverside

v. Number receiving training (by gender): 12 African scientists/students (11 male; 1 female: a second Ghana female was denied a visa to attend).

vi. Home institutions: ISRA, SARI, INERA, Ahmadu Bello U., WACCI, IITA, UCR.

vii. Institution providing training or mechanism: UC Riverside

Short-term 2:

i. Purpose of Training: Cowpea production and seed storage techniques

ii. Type of Training: 2 days intensive field-based training
iii. Country Benefiting: Burkina Faso
iv. Location and dates of training: Saria 17-18 October 2014; Pobe 21-22 October 2014
v. Number receiving training (by gender): 45 ladies and 70 men
vi. Home institution(s) (if applicable): INERA
vii. Institution providing training or mechanism: INERA

2. Degree Training

Trainee 1:
i. Name of trainee: Arsenio Ndeve
ii. Country of Citizenship: Mozambique
iii. Gender: Male
iv. Host Country Institution Benefitting from Training: Eduardo Mondlane University
v. Institution providing training: University of California - Riverside
vi. Supervising LIL PI: Philip A. Roberts & Timothy Close
vii. Degree Program: PhD, Plant Pathology
viii. Field or Discipline: Plant pathology and genetics
ix. Research Project Title: Genomewide selection for disease and drought tolerance in SE African cowpeas
x. Start Date: January 2012
xi. Projected Completion Date: December 2016
xii. Is trainee USAID Participant Trainee and registered on TraiNet? No
xiii. Training status: Active

Trainee 2:
i. Name of trainee: Sassoum Lo
ii. Country of Citizenship: Senegal
iii. Gender: Female
iv. Host Country Institution Benefitting from Training: ISRA
v. Institution providing training: University of California - Riverside
vi. Supervising LIL PI: Philip A. Roberts & Timothy J. Close
vii. Degree Program: MS initially, now PhD, Plant Genetics
viii. Field or Discipline: Plant breeding and genetics
ix. Research Project Title: MABC for enhanced seed size in cowpea
x. Start Date: March 2014
xi. Projected Completion Date: June 2018 (projected)
xii. Is trainee a USAID Participant Trainee and registered on TraiNet? No
xiii. Training status: Active

Trainee 3:
i. Name of trainee: Mame Penda Sarr
ii. Country of Citizenship: Senegal
iii. Gender: Female

iv. Host Country Institution Benefitting from Training: ISRA
v. Institution providing training: University of Dakar (UCAD)
vi. Supervising LIL PI: Ndiaga Cisse
vii. Degree Program: PhD
viii. Field or Discipline: Plant Pathology
ix. Research Project Title (if applicable): Genetic diversity and temporal dynamics of *Macrophomina phaseolina.*
x. Start Date: 2010
xi. Projected Completion Date: December 2014 (completed)
xii. Is trainee a USAID Participant Trainee and registered on TraiNet? No
xiii. Training status: Completed

Trainee 4:
i. Name of trainee: Joel Lalsaga
ii. Country of Citizenship: Burkina Faso
iii. Gender: male
iv. Host Country Institution Benefitting from Training: Burkina Faso
v. Institution providing training: INERA
vi. Supervising CRSP PI: Issa Drabo and Joseph Batieno
vii. Degree Program: PhD program at the University of Ouagadougou
viii. Field or Discipline: Plant breeding
ix. Research Project Title (if applicable)
x. Start Date: 2014 (field research)
xi. Projected Completion Date: Dec. 2016
xii. Is trainee a USAID Participant Trainee and registered on TraiNet? No
xiii. Training status: Active

Trainee 5:
i. Name of trainee (First and Last Name): Emanuele Yaw Owusu
ii. Country of Citizenship: Ghana
iii. Gender: male
iv. Host Country Institution Benefitting from Training: Ghana
v. Institution providing training: SARI and UCR
vi. Supervising CRSP PI: Francis Kusi
vii. Degree Program: MS Plant Breeding
viii. Field or Discipline: Plant breeding
ix. Research Project Title: Combining early maturity, seed size and thrips resistance traits in cowpea
x. Start Date: 2015
xi. Projected Completion Date: Dec 2016
xii. Is trainee a USAID Participant Trainee and registered on TraiNet? No
xiii. Training status: Active

VII. Achievement of Gender Equity Goals

In Ghana, women farmer groups with a total of 480 women were trained by the SARI team during the reporting period. These were women groups from 15 communities who are in cowpea production and marketing. The project
team collaborated with CARE international Ghana, an international NGO operating in Ghana in reaching out to these groups. Current research to develop varieties resistant to insects and drought and integrated strategies to manage insect pests, diseases and drought were main topics in training. In Senegal, with the farmers' organization RESOPP training of its members on seed production and post-harvest operations was continued. More than 200 women producers were trained in FY15. In Burkina Faso, 190 women producers were trained on cowpea production and seed storage.

VIII. Achievement and Progress Along the Impact Pathway

Under Objective 1, the primary thrust of the impact pathway progress centers on identifying QTLs determining traits for insect tolerance and resistance. As described in the technical section under Objective 1, this involves a combination
of phenotype screening in the target areas (combination of greenhouse and field-based screens), together with high-throughput SNP genotyping with genomewide markers and followed by ICI-mapping to identify significant QTLs. The various populations for QTL discovery are at different stages of this process and will require additional years of data collection from the phenotyping trials.
Under Objective 2, the primary impact pathways are release of new cowpea varieties. As reported in the technical section, five all-white large seeded varieties (Lisard, Thieye, Leona, Kelle and Sam) have been formally released in Senegal during the reporting period, and have been launched into the seed development pipeline with Breeder and Foundation seed production. In Burkina Faso, a selected set of 9 white-seed advanced lines tested in on-farm multi-location field performance trials were reduced to four best-performing lines based on 2015 trials, and for which Breeder seed was produced by INERA. After unforeseen delay, these will be processed for national release in 2016. In California, likewise the advanced breeding lines are in different advanced stages of final testing, including on-farm strip trials, to determine which if any lines are suitable for release.

IX. Explanation for Changes

In Senegal, the aphid biotype identification trials were carried out, but were again not successful because the aphid pest infestations did not occur due to heavy rainfall. They were completed in the other countries, and they will be repeated in the coming year. The Ghana team started the flower thrips screening of the two RIL populations following seed increase at UCR. They used some of the planted plots with mild insecticide protection to develop more seed for the next test cycle, so the 2015 data are considered preliminary. These populations will be phenotyped fully by SARI in 2016.
In Burkina Faso, the pod-sucking bug phenotype screening is being prepared under direction of Dr. Dabire. Because of seed failures in 2014-15, additional segregating populations were generated and are being grown for phenotyping in February 2016, with a repeat test planned for summer 2016.

Genotyping of cowpea materials by UCR is in various stages of progress using both the KASP and iSelect cowpea SNP genotyping platforms depending on the specific objective, and will be completed to match with the phenotype data for insect resistance.
Funds are available to complete all the above activities.

X. Self-Evaluation and Lessons-Learned

Overall we have had a successful workplan period in 2014-15. The primary challenges to staying on timeline are ones familiar to us in conducting the collaborative cowpea improvement project. Three are worth highlighting: 1) Having enough seed of breeding lines or populations for genetic analysis is a limitation sometimes, because of failure of seed increases due to growing conditions or, in California, due to photoperiod sensitivity of African germplasm requiring short daylength for flowering. 2) Phenotyping for biotic stress resistance under field conditions is dependent on adequate, uniform infestations. This is especially difficult with insect screening, such as in Senegal for the last two years when aphid infestation was too low for data collection due to weather events. Multiple years and locations for testing are built into the planning to mitigate this problem. 3) Technical issues continue to arise occasionally with leaf or DNA sample shipments for SNP genotyping, due to delays, shipment loss, or spoilage of leaf samples from inadequate drying before shipping. Re-sampling is required to overcome these problems, and the US and HC team have got much better in handling this outsourcing process. Our team of U.S and Host Country partners works very well together, based on established relationships and the seamless integration of the new team from SARI, Ghana. Frequent communication is seen as a key in planning and execution of project activities. Of especial value this period has been the face-to-face meetings at UCR in March 2015, and in Ghana in September 2015.

XI. Scholarly Accomplishments

Huynh, B.L., Ehlers, J.D., Ndeve, A., Wanamaker, S., Lucas, M.R., Close, T.J., Roberts, P.A. 2015. Genetic mapping and legume synteny of aphid resistance in African cowpea (*Vigna unguiculata* L. Walp.) grown in California. Molecular Breeding 35:36 (1-9). DOI 10.1007/s11032-015-0254-0

Boukar, O., Fatokun, C.A., Roberts, P.A., Abberton, M., Huynh, B.L., Close, T.J., Kyei-Boahen, S., Higgins T.J.V., Ehlers, J.D. 2015. Cowpea. Pp. 219-250 *in* Grain Legumes. Editor: A.M. De Ron. Springer-Verlag, New York.

Lucas, M.R., Huynh, B.L., Roberts P.A., Close, T.J. 2015. Introgression of a rare haplotype from Southeastern Africa to breed California blackeyes with larger seeds. Frontiers in Plant Science 6:126. doi: 10.3389/fpls.2015.00126

Huynh, B.L., Matthews, W.C., Ehlers, J.D., Lucas, M.R., Santos, J.R.P., Ndeve, A., Close, T.J., Roberts, P.A. 2015. A major QTL corresponding to the *Rk* locus for resistance to root-knot nematodes in cowpea (*Vigna unguiculata* L. Walp.). Theoretical and Applied Genetics. In press.

Cisse, N. et al. 2015. Formal release of five new cowpea varieties in Senegal with large white seed-type and disease resistance. Variety name registrations Lisard, Thieye, Leona, Kelle and Sam.

XII. Data Management

As described in the Data Management Plan submitted in August 2015, data sets will be submitted to publicly accessible sites in project years 4 and 5. Data sets for phenotyping traits and genotyping with genomewide SNP markers are being generated for QTL mapping. These are being organized on standard excel spreadsheets designed to capture appropriate trait dictionary names and codes as used in the CGIAR-GCP databasing. Once datasets are analyzed to confirm their usefulness and validity relative to results, and those findings published, then the datasets will be made publicly available.

ANNEXES:

Table 1: Details of sources of resistance to the cowpea aphid for the differential panel for determining resistance uniqueness and aphid biotype differences.

Name	Type	Origin
58-77	Aphid resistant source	ISRA
INIA19	Aphid resistant source	MSU
IT97K-556-6	Aphid resistant source	IITA
KN1	Aphid resistant source	INERA
KvX-295-2-124-99	Aphid resistant source	INERA
SARC-1-57-2	Aphid resistant source	SARI
TVNu-1158	Aphid resistant source	IITA
APAGBAALA	Aphid susceptible check	SARI
BAMBEY21	Aphid susceptible check	ISRA
CB27	Aphid susceptible check	UCR
IT82E-18	Aphid susceptible check	IITA
VITA7	Aphid susceptible chzeck	IITA

Table 2. New blackeye breeding lines and checks tested at Kearney REC and Tulare in 2014: grain yield, 100-seed weight, galling ratings from 2014 field screening with root-knot nematodes *M. incognita, M. javanica,* and *M. incognita* Muller, and 2014 greenhouse screening with Fusarium Race 4.

Entry	**KREC Yield (lb/ac)**	**Tulare Yield (lb/ac)**	**KREC 100 seed wt (g)**	**Tulare 100 seed wt (g)**	**Galling M. incognita**	**Galling M. javanica**	**Galling M. incognita Muller**	**Fusarium Race 4 index**
N17	5212	4121	20.4	23.0	1.1	1.2	3.1	4.8

10K-77	5146	4172	22.6	25.3	0.9	1.7	3.7	5.0
N2	5106	4523	19.5	22.7	1.2	1.2	3.8	0.2
N5	4979	4132	19.5	22.4	0.8	1.5	3.5	0.8
N20	4974	4069	19.5	23.1	0.9	1.0	3.5	5.0
CB50	4589	3840	23.4	25.1	-	-	-	0.0
10K-29	4548	4072	22.2	23.3	2.4	2.9	4.2	0.0
N16	4499	3910	20.1	23.2	0.8	0.8	3.4	4.5
10K-19	4439	4144	20.8	25.0	1.3	2.9	4.1	5.0
10K-115	4433	3519	22.4	25.4	1.0	1.6	2.5	5.0
CB46Rk2	4288	4329	18.3	20.6	1.0	2.7	2.9	0.0
CB46	3789	4243	19.2	22.1	1.6	3.4	4.2	4.9
Mean	4667	4105	20.7	23.4				
CV(%)	11	9	5	3				
LSD(0.05)	754	530	1.4	1.0				

Kearney REC trial planted on May 29 and cut on October 6 (130 days).
Tulare trial planted on June 6 and hand-harvested on October 2 (118 days).
Root-galling score on scale of 0 (no galling) to 8 (severe galling).
Fusarium wilt disease index (0 to 5; where 0 = no wilt symptoms and 5 = plant death).

Table 3. Grain yield, 100-seed weight, and lygus grain damage of 3 advanced blackeye lines, CB46, CB50 and CB27 when grown under insect-protected and unprotected conditions at Kearney REC in 2014.

Line	Yield (lbs/ac)			100-seed weight (g)		*Lygus damage (%)
	Protected	Unprotected	Loss (%)	Protected	Unprotected	
07KN-74	3537	3083	13	20.2	21.6	17
09KLN-1-9	3359	2767	18	18.3	19.0	15
09KLN-2-30	3340	3015	10	18.8	18.8	13
CB27	3499	2764	21	22.0	22.6	14
CB46	2127	1549	27	19.8	20.0	16
CB50	2298	1612	30	23.0	24.4	14
Mean	3026	2465	19	20.4	21.1	15

CV(%)	9	15	80	6	5	28
LSD(0.05)	309	425	18	1.4	1.3	4.9

Kearney trial planted on May 29 and hand-harvested on September 2 (96 days). *Lygus damage on grain measured in the unprotected plots.

Figure 1. Examples of phenotypes of resistance panel entries infested with aphids at SARI, Ghana (resistant lines compared with susceptible cv. Apagbaala).